Emerging microbiological risks

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Let us begin with definitions

Definitions

According to the definition adopted by the Scientific Committee of EFSA in 2007, “an emerging risk to human, animal and/or plant health is understood as a risk resulting from a newly identified hazard to which a significant exposure may occur or from an unexpected new or increased significant exposure and/or susceptibility to a known hazard” (EFSA, 2007).

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Let us begin with an update

TECHNICAL REPORT

APPROVED: 9 November 2017

doi:10.2903/sp.efsa.2017.EN-1336

EFSA's Activities on Emerging Risks in 2016

European Food Safety Authority (EFSA),
Ana Afonso, Raquel García Matas, Angelo Maggiore, Caroline Merten, Tobin Robinson

Table 4: EREN survey on critical and emerging issues

Issues[9]	EREN survey results
Critical issues[10] related to food safety and quality, from present to the next 2–5 years	Invasive plant pests and diseases Invasive animal diseases Antimicrobial resistance Food supplements Emerging marine biotoxins Exposure to multiple chemicals Food fraud
Emerging issues[11] related to food safety and quality in the next 2–5 years	Circular economy New food production technologies Change of consumption pattern towards 'healthier' food choices Constraints on natural resources: water availability

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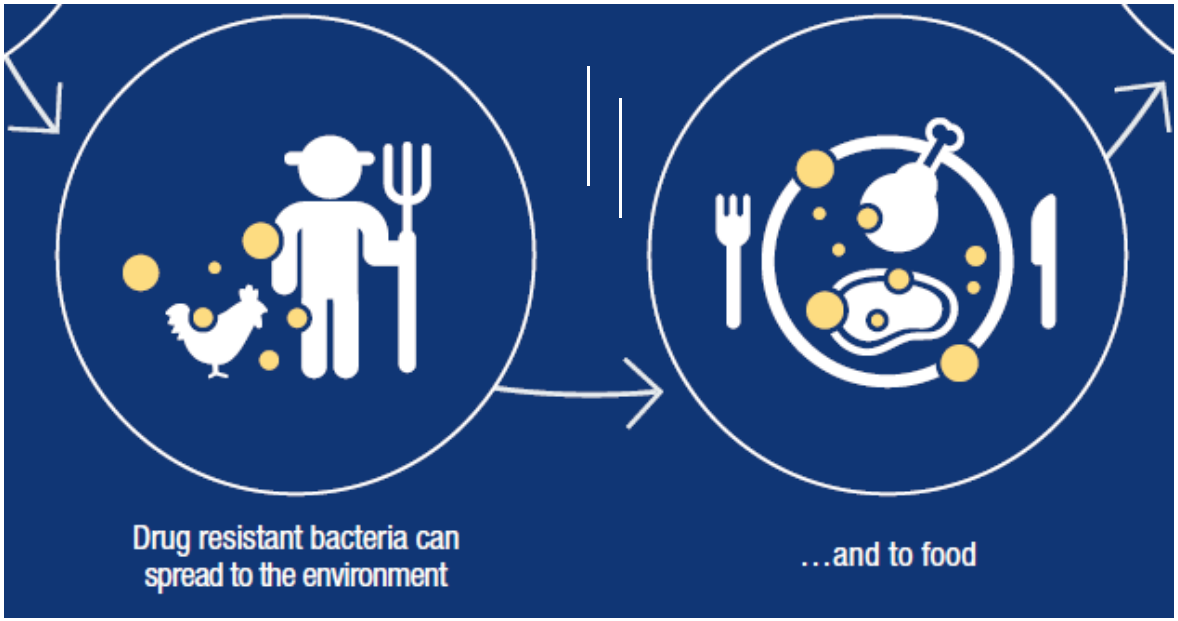
Let us begin with an update

WHO GAP AMR Newsletter No.32 November 2017

World Health Organization

IMPLEMENTATION OF THE GLOBAL ACTION PLAN ON ANTIMICROBIAL RESISTANCE



Antimicrobial resistance (AMR) is the ability of a microorganism (like bacteria, viruses, and some parasites) to stop an antimicrobial (such as antibiotics, antivirals and antimalarials) from working against it. As a result, standard treatments become ineffective, infections persist and may spread to others.

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Let us begin with an update

European Commission

AMR: a major European and Global challenge

What is AMR?

Antimicrobial Resistance (AMR) is the ability of microorganisms to resist antimicrobial treatments, especially antibiotics.

Excessive and inappropriate use of antimicrobial medicines and poor infection control practices have transformed AMR into a serious threat to public health worldwide.

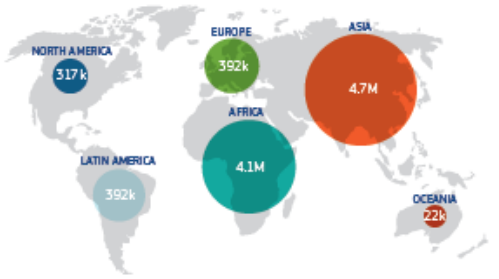
If trends continue we would revert to a world where simple infections are no longer treatable.

Why is AMR a serious threat to public health?

- **25 000 patients die annually in the EU** alone as a result of infections caused by resistant bacteria.
- **Globally this number could be as high as 700 000.**
- **10 million deaths per year are projected between 2015 and 2050** if current infection and resistance trends are not reversed. Only 0.7 million of these additional deaths would occur in North America or Europe, with the **largest numbers in Africa and Asia.**

What is the economic cost of AMR?

- **EUR 1.5 billion each year** – Extra healthcare costs and productivity losses due to multidrug-resistant bacteria in the EU.
- **USD 2.9 trillion by 2050** – Expected cumulative losses in OECD countries due to AMR.
- **USD 10 000 to 40 000 – Additional hospital costs per patient** in OECD countries. The associated impact of lost economic outputs due to increased mortality, prolonged sickness and reduced labour efficiency are **likely to double** this figure.
- **Losses to Trade and Agriculture** – For example, in 2015 chicken sales in Norway dropped by 20% (for some distributors) following the news that a resistant strain of Escherichia coli (E. coli) was found in chicken meat.



Number of deaths per year attributable to AMR by 2050 if current resistance rates increased by 40%

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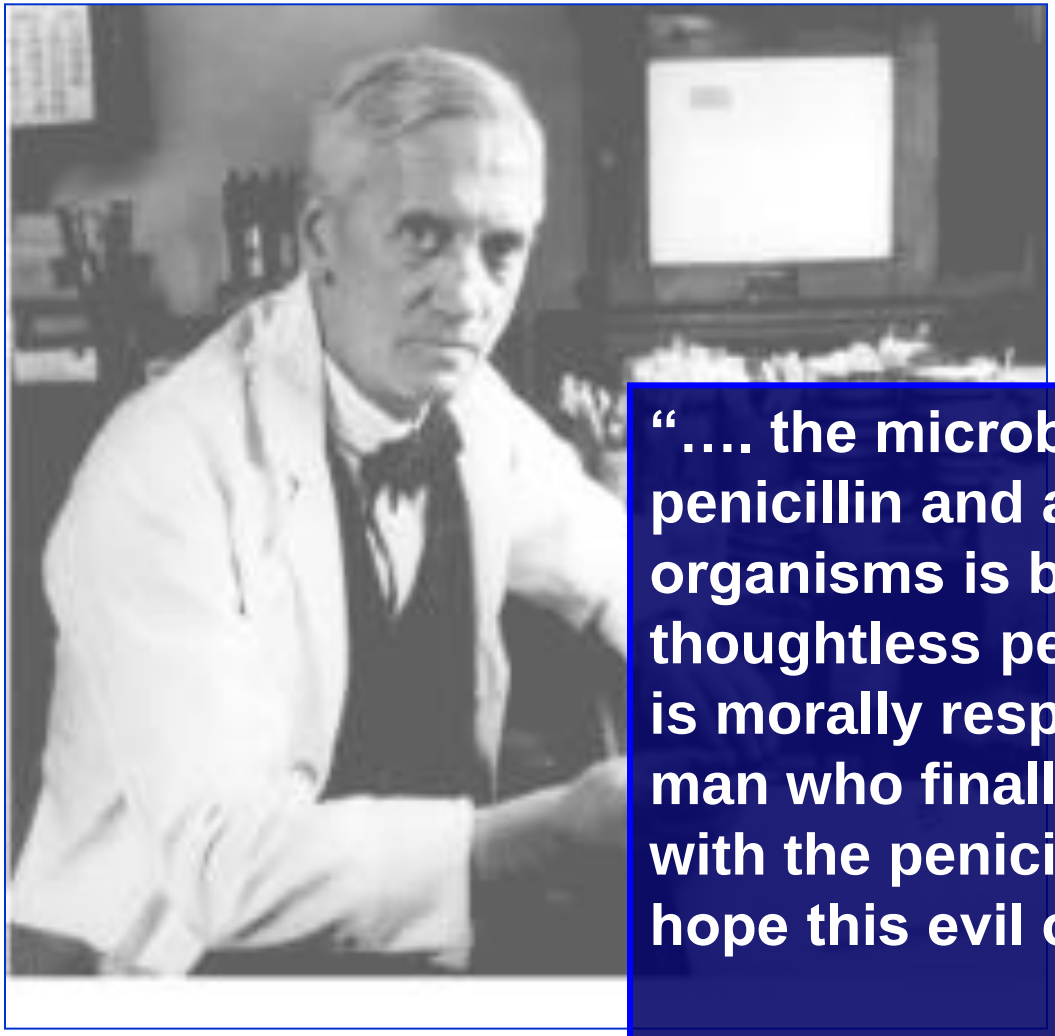
Not really unexpected



"…. the microbes are educated to resist penicillin and a host of penicillin-fast organisms is bred out… In such cases the thoughtless person playing with penicillin is morally responsible for the death of the man who finally succumbs to infection with the penicillin-resistant organism. I hope this evil can be averted."

- Sir Alexander Fleming, June 1945

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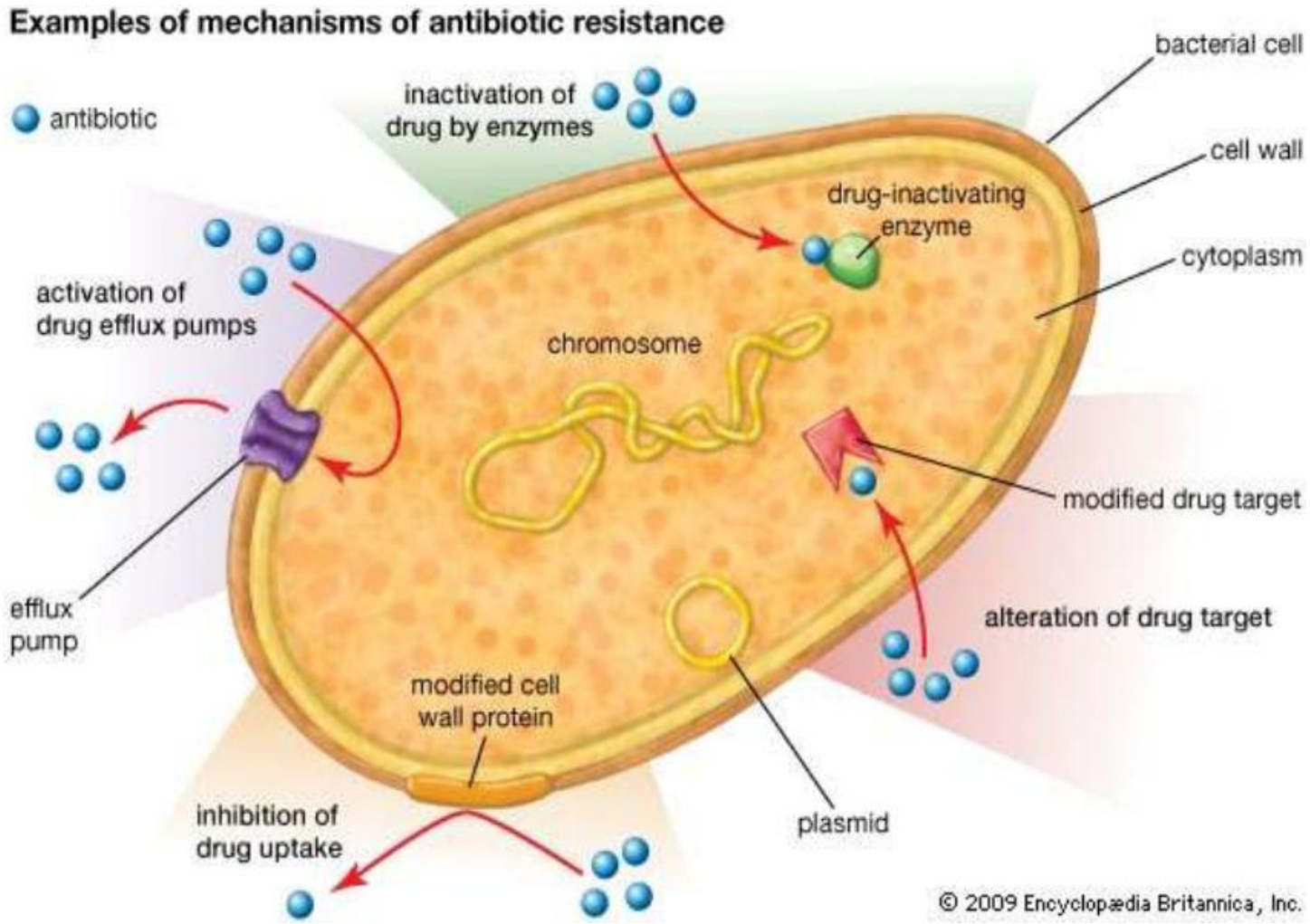
What AMRs are:



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What AMRs are:

Antimicrobials	Mechanisms	Effect
Chloramphenicol	Acetyltransferase enzyme Altered ribosomal target	Inactivation (Gram -ve→ constitutive, Gram +ve→ inducible) ↓ permeability & binding
Macrolides (Azithromycin, Erythromycin)	Esterases (Enterobactericeae) Altered ribosomal target (ermA, B, C gene) Efflux pumps (mefA, msrA, mefE gene)	Hydrolysis ↓ binding ↓ drug concentration
Tetracyclines	Efflux pumps (tetK gene) Altered ribosomal target (tetM gene) Drug modifying enzymes (tetX)	↓ drug concentration ↓ binding Inactivation

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What AMRs are:

Antimicrobials	Mechanisms	Effect
Lincosamide (Clindamycin)	Altered ribosomal target (erm gene)	↓ binding
Streptogramins (Quinupristin, Dalfopristin)	Altered ribosomal target (ermA, B, C gene) Lactonases (vgbB gene) Acetyltransferases (vatB, C, D, satA gene) Efflux pumps (msrA gene)	↓ binding Inactivation ↓ Drug concentration
Glycopeptides (Vancomycin)	Altered target (D-alanyl-D-alanine to D-alanyl-D-lactate/serine)	↓ binding
Sulphonamides	Point mutations in DHPS gene Altered metabolic pathway ↑ efflux Overproduction of PABA	↓ affinity & ↓ bacterial permeability ↓ inhibition of DHPS ↓ drug concentration ↓ drug effect

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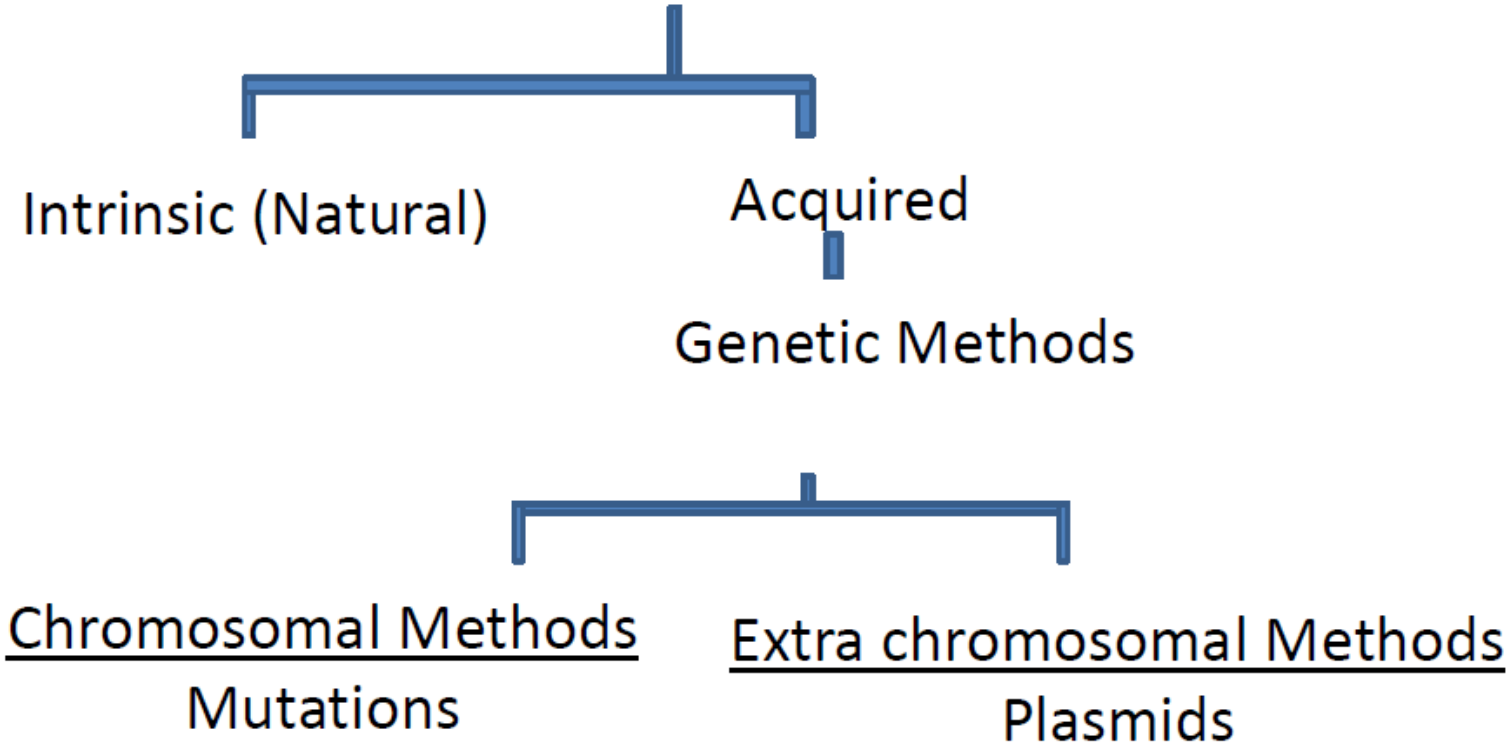
Mechanism Antibiotic Resistance



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Fake? or truth?

- Antibiotics select out the resistant strain
- Faulty use of antibiotics or widespread use of antibiotics increases the probability of such selection.
- Antibiotic resistant strains appear to be more virulent because we cannot kill them or stop their growth.

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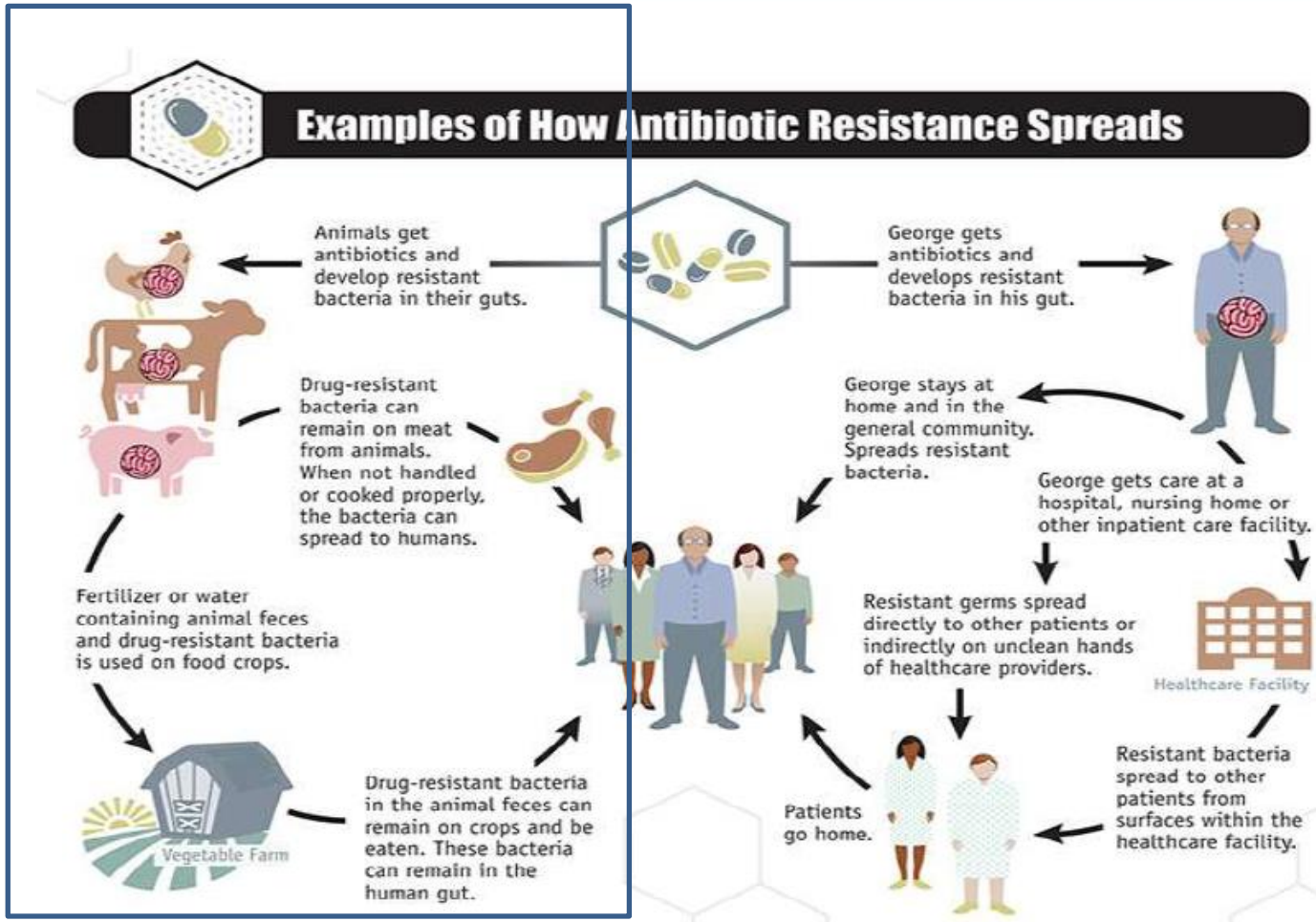
Turning to agri-food



Simply using antibiotics creates resistance. These drugs should only be used to treat infections.

The EU scenario

18.10.2003 EN Official Journal of the European Union L 268/29

REGULATION (EC) No 1831/2003 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL
of 22 September 2003
on additives for use in animal nutrition
(Text with EEA relevance)

3. The feed additive shall:

(a) favourably affect the characteristics of feed,

(b) favourably affect the characteristics of animal products,

(c) favourably affect the colour of ornamental fish and birds,

(d) satisfy the nutritional needs of animals,

(e) favourably affect the environmental consequences of animal production,

(f) favourably affect animal production, performance or welfare, particularly by affecting the gastro-intestinal flora or digestibility of feedingstuffs, or

(g) have a coccidiostatic or histomonostatic effect.

4. Antibiotics, other than coccidiostats or histomonostats, shall not be authorised as feed additives.

Article 11

Phasing out

1. With a view to a decision on the phasing out of the use of coccidiostats and histomonostats as feed additives by 31 December 2012, the Commission shall submit to the European Parliament and the Council before 1 January 2008 a report on the use of these substances as feed additives and available alternatives, accompanied, where appropriate, by legislative proposals.

2. By way of derogation from Article 10 and without prejudice to Article 13, antibiotics, other than coccidiostats and histomonostats, may be marketed and used as feed additives only until 31 December 2005; as from 1 January 2006, those substances shall be deleted from the Register.

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The EU scenario

The Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) identified the need to revise the SCAN Opinion taking into consideration the scientific developments and other data published after the adoption of the SCAN opinion. This resulted in the adoption of an opinion on the updating of the criteria used in the assessment of bacteria for resistance to antibiotics of human or veterinary importance (May 2005).[4] This opinion included a table laying down appropriate breakpoint values for 13 antibiotics, belonging to different groups of antibacterial compounds, which allow distinguishing strains harbouring acquired antimicrobial resistance from susceptible strains. Those microbiological breakpoints define a minimum inhibitory concentration (MIC) value which, if exceeded, triggers the need for a more extensive investigation to define the genetic basis of the observed resistance and to assess the risk for transfer of this resistance to other bacteria.

A considerable increase of available scientific data (e.g. ACE-ART project) justifies a second revision of the aforementioned microbiological breakpoints values. Consequently, the FEEDAP Panel proposes to revise its opinion on the updating of the criteria used in the assessment of bacteria for resistance to antibiotics of human or veterinary importance.

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Focus on non pathogenic, antibiotic resistant, food-borne bacteria

'ACE-ART and the assessment of drug resistance in non pathogenic, food related bacteria.'

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Focus on non pathogenic, antibiotic resistant, food-borne bacteria

The problem

- No official methods for phenotypic assessment of AR in non pathogenic, food related bacteria.
- No MIC, no microbiological breakpoints.
- EU asking to discriminate between intrinsic and acquired resistance; minimum of 50 strains per species are to be investigated.
- Nothing in place for human consumption; how to assess the AR in bacteria used as starters for food fermentation and as probiotics?

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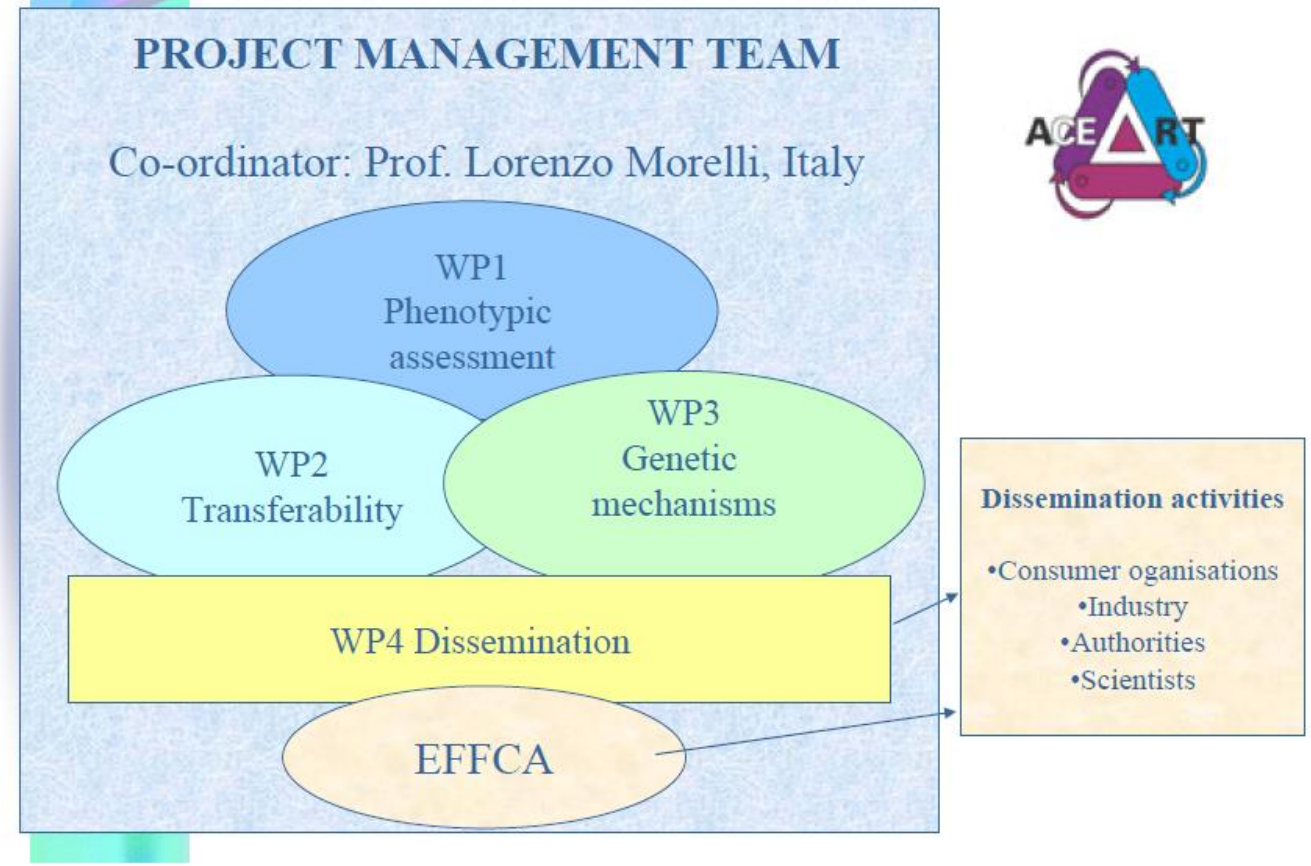
Focus on non pathogenic, antibiotic resistant, food-borne bacteria

Project structure and management



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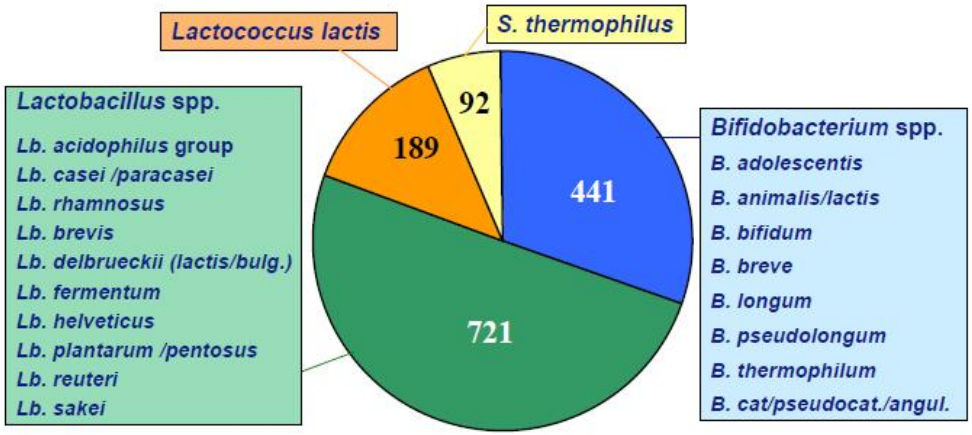
The research approach

Strain collection



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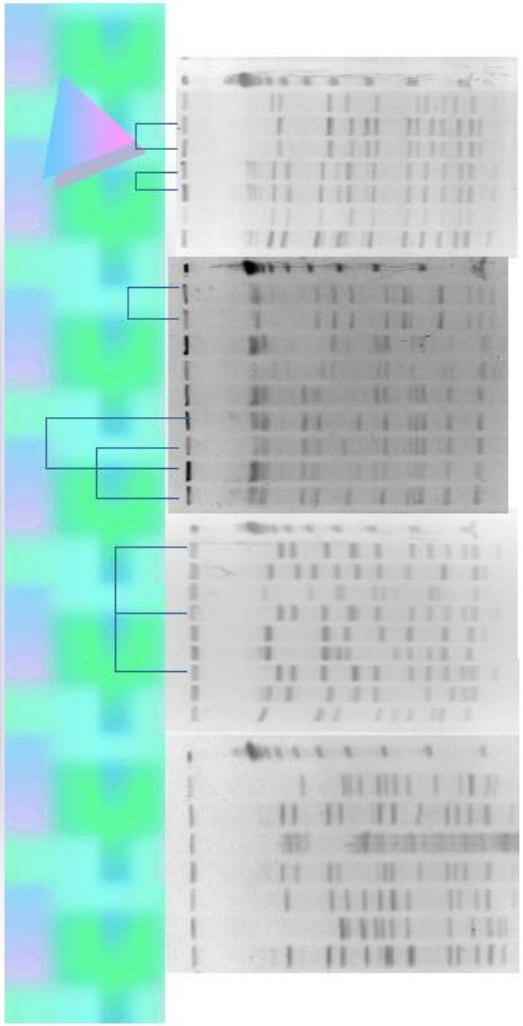
The research approach



PFGE analysis of *S. thermophilus*

PFGE typing was performed for the 52 isolate not coming from Int. Culture Collections. PFGE profiles allowed differentiation of 42 different genotypes

One representative per PFGE type was selected for further work leading to a reduction from 74 (52 UCSC isolates + 22 Culture collections strains) to 64 strains to be phenotypically tested .

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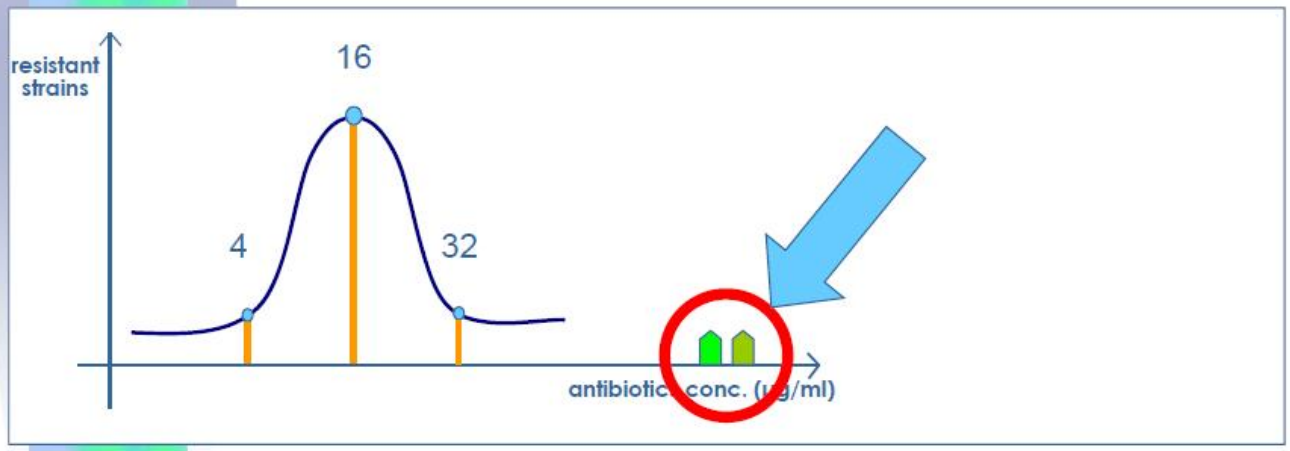
The research approach

The phenotypic approach was planned in order to provide "ecological breakpoints"

The genetic approach was used to confirm the presence of known drug resistant genes in "phenotypically atypical"strains but also to confirm the absence of these genes in strains falling down the "bell" distribution-



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The results

Antibiotic Species/group (total n)	Concentration of the antibiotic (µg/ml)														
	≤0.016	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	128	≥256
Tetracycline															
B. adolescentis gr. (53)		3	4	4	15	21	2					4			
B. longum gr. (84)				3	17	35	12	1	1		3	6	2	3	1
B. bifidum (24)				4	2	15	1					1	1		
B. catenulatum gr. (23)					4	17	1				1				
B. animalis (19)						1	1		6	6	1			1	3
B. Pseudolongum (100)						2	30	20	3	2		3	18	4	

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The results

- Results for species in which it was possible to collect > 50 strains

- 1. *S.thermophilus*
- 2. *Lc.lactis*
- 3. *L.plantarum*
- 4. *L.reuteri*
- 5. *L.fermentum*
- 6. *L.delbrueckii*
- 7. *L.heleveticus*
- 8. *L.sakei*
- 9. *L.paracasei*
- 10. *L.rhamnosus*
- 11. *B. pseudolongum*
- 12. *B.thermophilum*
- 13. *B.adolescentis*
- 14. *B.longum*

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The results

Ace-art: what we have learned

Methodology is of paramount relevance (medium, inoculum size, incubation time)

Breakpoints must be obtained at species level (or closely related species).

50 strains seems to be the adequate number of strains to be checked

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A major outcome

ISO 10932:2010(E)
IDF 223:2010(E)

Introduction

There are several reports on minimal inhibitory concentration (MIC) determination of lactic acid bacteria according to various methods. However, the MIC value obtained depends on the determination used and the strain cultivation technique. For example, MIC determined by different quantitative methods are not always equivalent. Also some media components are antagonistic to certain antibiotics.

Consequently, a standardized MIC determination which employs a suitable growth medium having little or no antagonistic effects towards the antibiotics studied is necessary.

Two EU projects (PROSAFE and ACE-ART) were launched to tackle these issues, and propose appropriate media and method to measure MIC. This International Standard is based on the SOP (standard operating procedure) proposed by ACE-ART.

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So, everything solved?

From the final report

The genetic basis of the detected resistances as well as transmission mechanisms were extensively investigated in WP3 by means of a full range of molecular biology tools, from PCR and nucleotide sequence analysis up to DNA microarray. In this final reporting period additional data were obtained, useful to draw two different scenarios for tetracycline and erythromycin resistances in lactic acid bacteria: a complex scenario for the first, where some phenotypic resistances are probably support by genes still undescribed, while the latter is probably the result of a recent horizontal acquisition from other sources.

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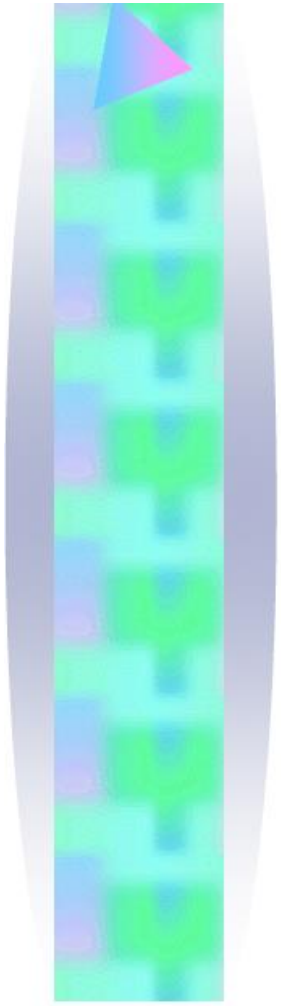
A new challenge: non specific AMR



	Type	Location	Substrate specificity	References
Lactococcus lactis				
LmrA	ABC	Chromosome	Antibiotics, drugs, metals	32, 33, 44
LmrP	Secondary	Chromosome	Antibiotics, drugs, detergents	4, 31, 33
LmrCD	ABC	Chromosome	Drugs	3, 18, 19
Mdt(A)	Secondary	Plasmid	Antibiotics	26
Lactobacillus brevis				
HorA	ABC	Plasmid	Hop compounds, drugs	35, 36
Lactobacillus lindneri HorC	Secondary	Plasmid	Hop compounds, drugs	42
Oenococcus oeni				
OmrA	ABC	Chromosome	Metals, antibiotics, drugs	1, 2, 5
Bifidobacterium longum				
Ctr		Chromosome	Bile, antibiotics, drugs	30
Bifidobacterium breve				
BbmR	Secondary	Chromosome	Antibiotics	21
BbmAB	ABC	Chromosome	Antibiotics, drugs	20

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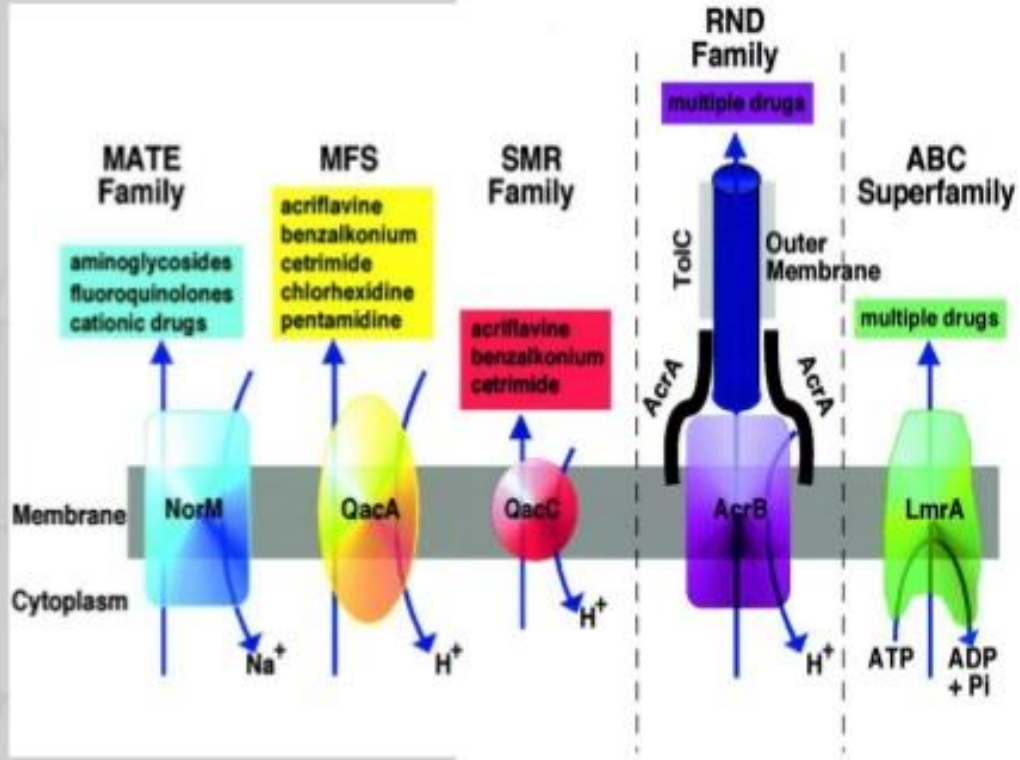
Multi functions genes

Efflux pumps

- Cytoplasmic membrane transport proteins



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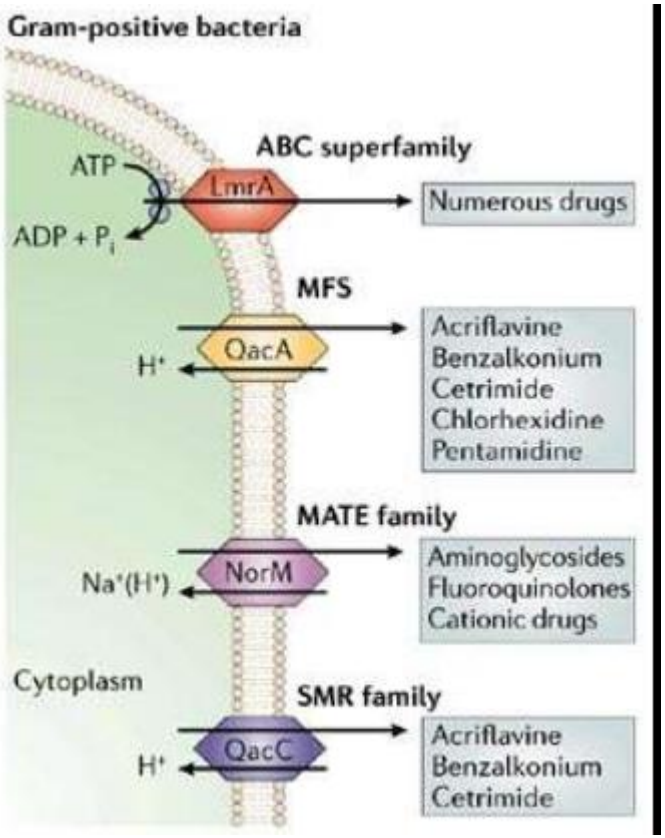
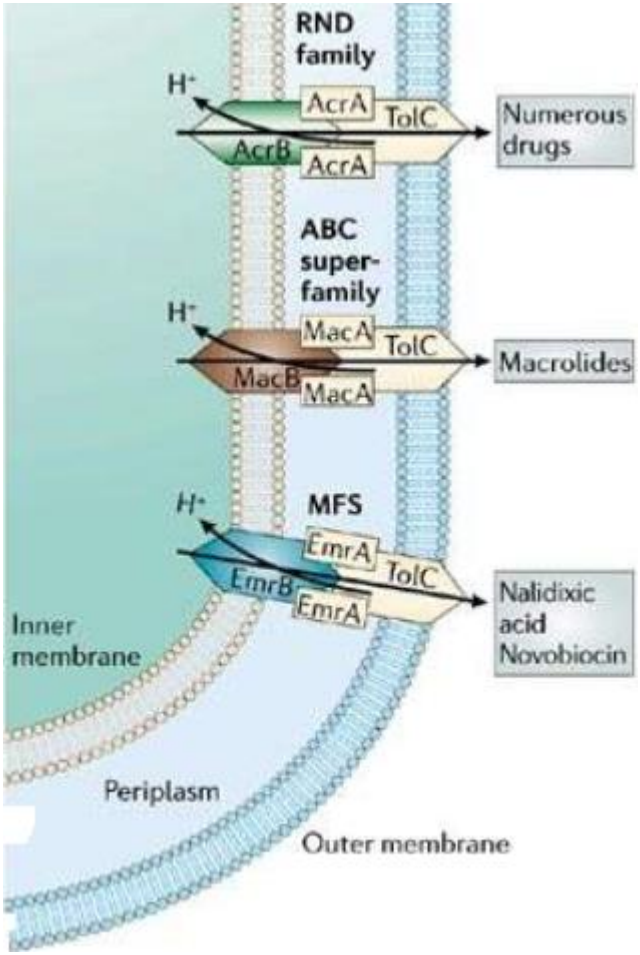
Multi functions genes





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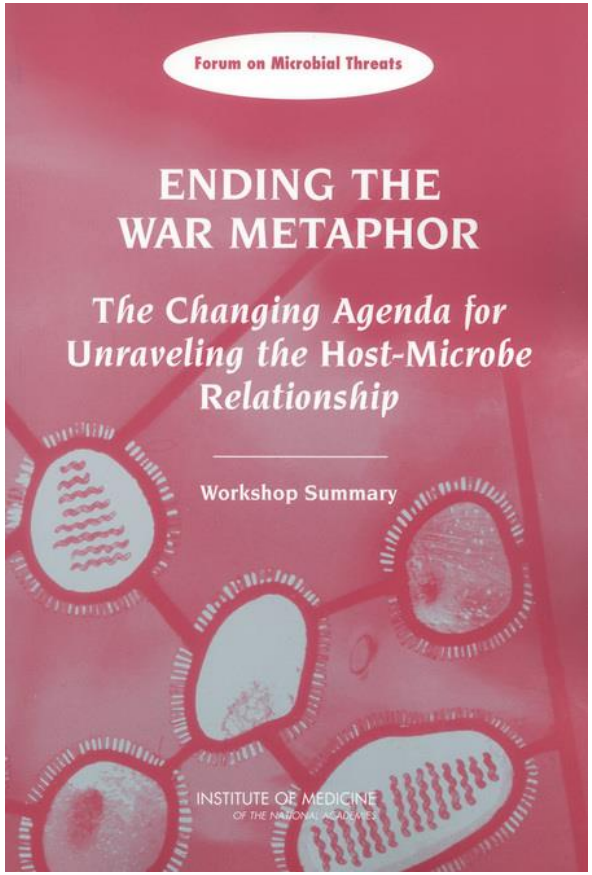
END OF A METAPHOR



It is not only clear that guidelines and regulations governing probiotics must be revised to reflect recent research findings, but also that this goal is a fast-moving target. In the final contribution to this chapter, workshop presenter Lorenzo Morelli and coworkers describe the process and considerations that produced the recent FAO/WHO guidelines for the evaluation of probiotics in food and raise a variety of cutting-edge issues that need to be addressed in subsequent guidelines, including:

- new genomic techniques that allow enhanced characterization of the gut microbiota,
- evaluation of emerging methods that allow assessment of bacteria-epithelial interactions,

FROM RESEARCH IN MICROBIOLOGY TO GUIDELINES

Lorenzo Morelli and Elena Bessi[18]

New Aspects of the War Metaphor: Good Bacteria as Potential Allies

From the point of view of a microbial ecologist the war metaphor describing the fight between humans and bacterial pathogens could still be useful if widened to include not only the human body and bacterial pathogens, but also potential bacterial allies already inhabiting, or parachuted into, the battleground to ensure support to the body.

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WAR AGAINST AMR BACTERIA IS A NEVER ENDING STORY

BE HAPPY

THIS IS THE END OF THE MY PRESENTATION
THANKS FOR YOUR ATTENTION