CARRERA DE ESPECIALIZACIÓN EN ESTERILIZACIÓN

ASIGNATURA: MICROBIOLOGÍA APLICADA

Doctor Roberto Ricardo Grau

Universidad Nacional de Rosario – Facultad de Ciencias Bioquímicas y Farmacéuticas
Pew Latin American Fellow – Philadelphia- EE.UU.
Fulbright International Scholar – Washignton – EE.UU.
CONICET – Argentina
Teléfono: 54-341-4353377 /4804592 (INTERNO 286)
WWW.MICROBIOLOGIAROSARIO.ORG / WWW.MICROBIOLOGYROSARIO.ORG

Clases 4 y 5

1 de Junio 2018

Rosario

THE BIOFILM LIFESTYLE

J.W. COSTERTON
ZBIGNIEW LEWANDOWSKI

Center for Biofilm Engineering
Montana State University
409 Cobleigh Hall
Bozeman, Montana 59717

Adv Dent Res 11(2):192-195, April, 1997

in certain medical, industrial, or environmental systems. Our objective was to take the same approach as the organisms themselves, which show no obvious regard for anthrocentric points of view and simply make themselves as safe and as comfortable as possible by adhering to available surfaces and forming biofilms in virtually all aquatic systems. Similarly, we have sought to understand the basic advantages of the "biofilm lifestyle" for bacteria growing in any and all aquatic ecosystems.

THE STRUCTURE OF BIOFILMS

It is a distinct pleasure to address the dental research community on the subject of microbial biofilms because it was this same community, more years ago than I care to admit, that inspired many of our initial thoughts in this area. Gibbons and van Houte, of the Forsyth Dental Center, had already addressed that most obvious and troublesome biofilm—dental plaque—in direct observations and elegant experiments, before we began our own oddessey, and our early 1978 article in Scientific American (Costerton *et al.*, 1978) made liberal use of their ideas. Their successors

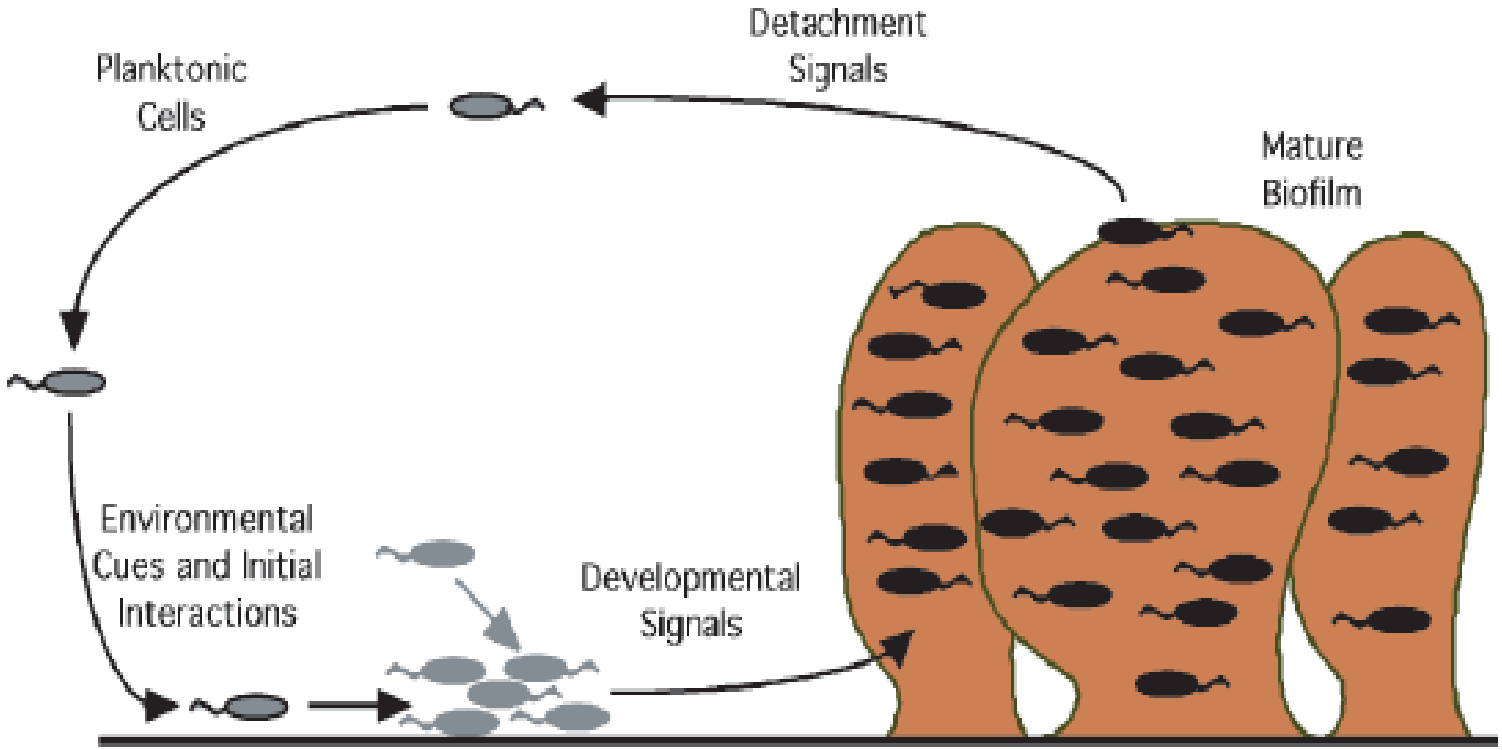
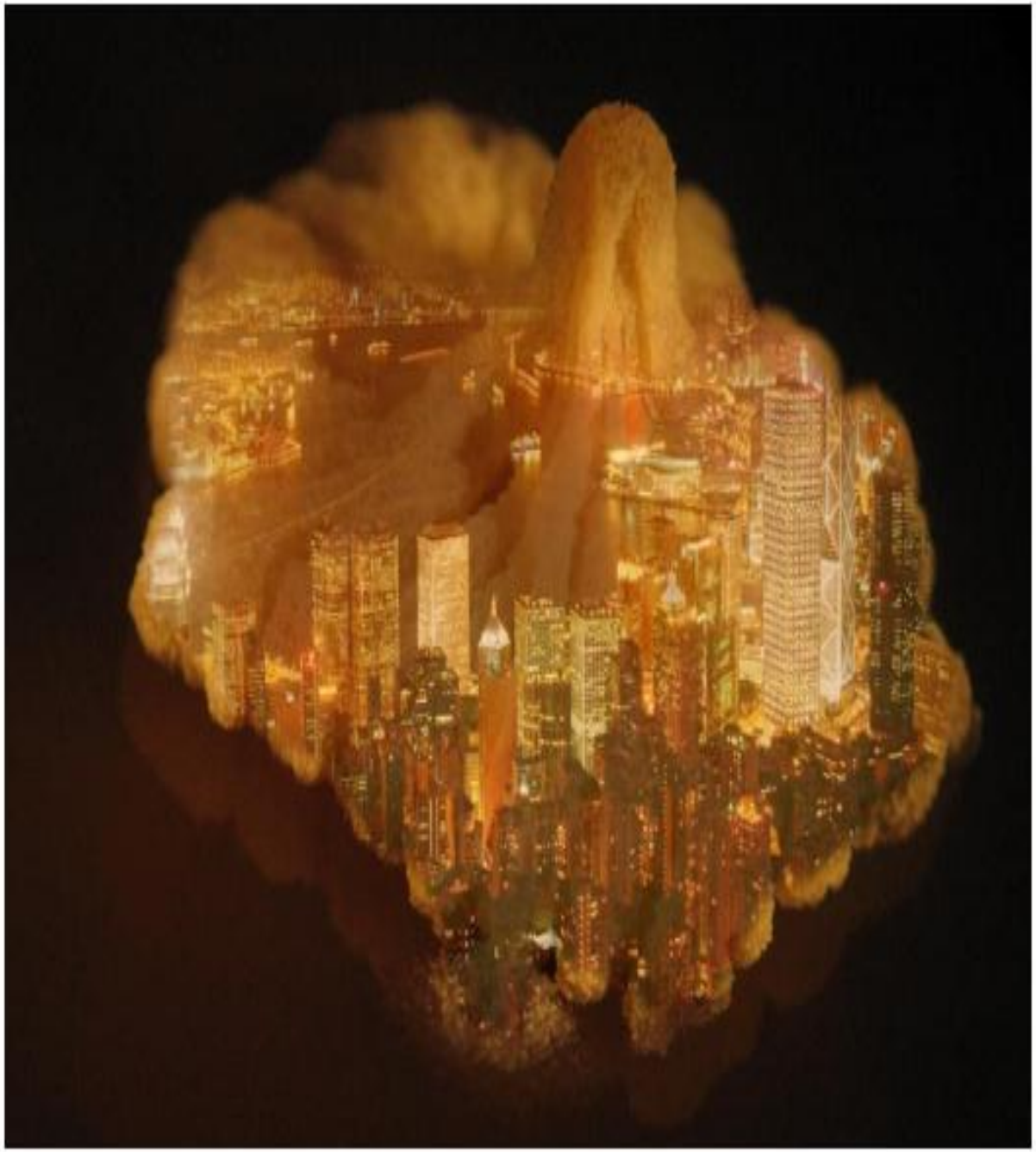
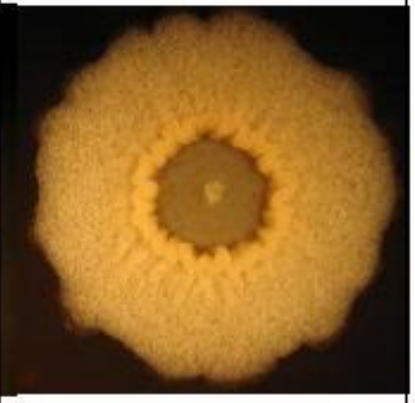


Figure 1 Model of biofilm development. Individual planktonic cells can form cell-to-surface and cel-to-cell contacts resulting in the formation of microcolonies. The hallmark architecture of the biofilms form in an acylhomoserine lactone-dependent process. Cells in the biofilm can return to a planktonic lifestyle to complete the cycle of biofilm development.



BIOFILMS DESARROLLADOS

	JH642	HS	PR2
Tubo			
Microplaca			
Colonia			

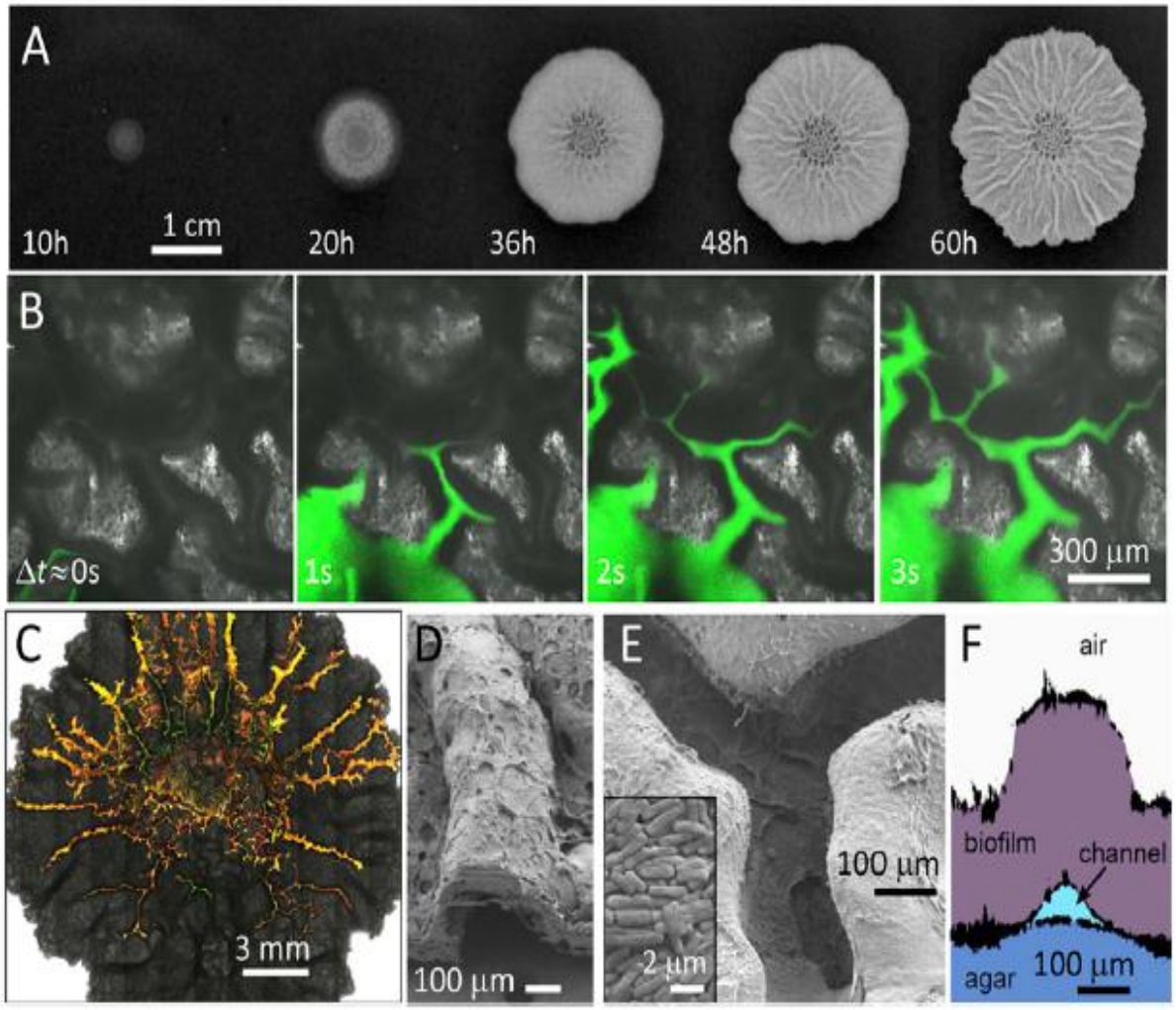


Fig. 1. Characterization of channels within *B. subtilis* biofilms. (*A*) Biofilm growing on the surface of an agar gel containing water and nutrients. The biofilm increases in height to hundreds of micrometers, spreads to reach a diameter of several centimeters, and forms macroscopic wrinkles. (*B*) Series of microscopy images of a region near the center of the biofilm. Injection of an aqueous dye reveals a network of channels beneath the wrinkles. (*C*) Microscopy image of a biofilm after injection of an aqueous solution containing a mixture of fluorescent beads reveals the connectivity of the channels. (*D*) SEM image of a wrinkle cross-section. (*E*) SEM image of the underside of a biofilm reveals well-defined channels. (*Inset*) SEM image of the microstructure of the biofilm. (*F*) Side view of a biofilm wrinkle reconstructed from profiles of plastic molds of the upper and lower surfaces of the biofilm and the surface of the agar.

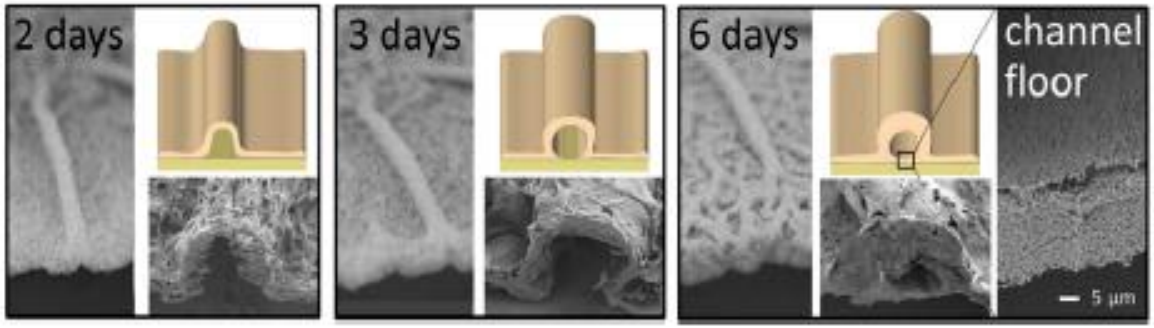
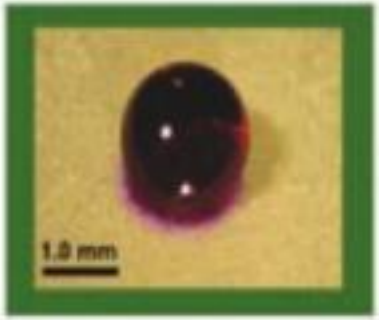


Fig. 4. Structural evolution of the channels. Photographs, SEM images, and illustrations depict the structural evolution of a channel over time. By 6 d the biofilm has spread to cover the floor of the channel.

Hidrofobicidad
repelencia al agua

NCIB3610 (◆)
(wt)



RG4365 (●)
(wt)

bslA (■)

Hidrofabicidad celular

Reparto

	NCIB36110	RG4365	*bslA*
Reparto$_{hexano}$	1.2 ± 0.02	2.2 ± 0.02	< 0.02
Reparto$_{octano}$	1.45 ± 0.02	3.87 ± 0.02	< 0.02

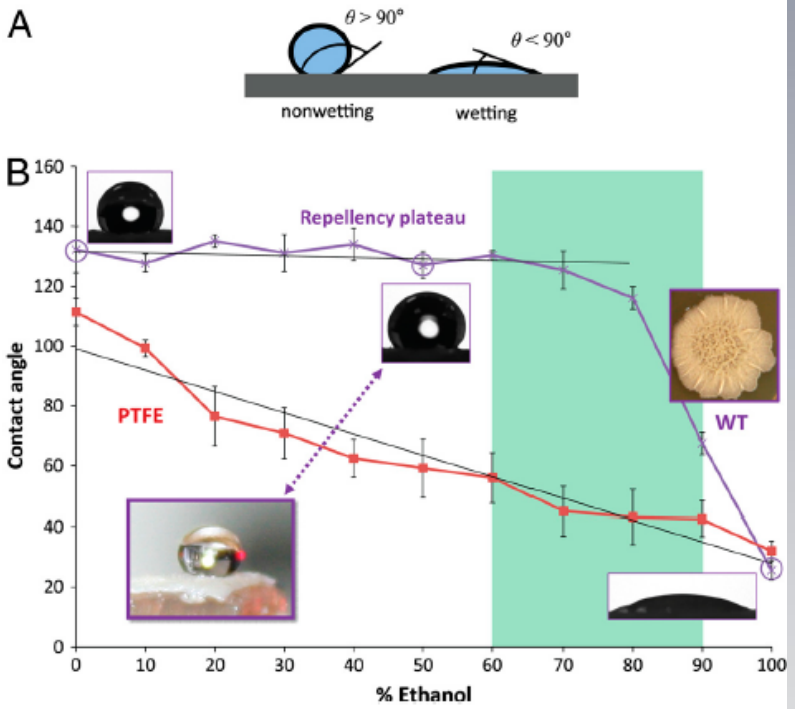


Fig. 1. Bacterial biofilm wetting characterization by contact angle analysis. (*A*) Schematic of the contact angle θ: low (high) surface tension liquids generally wet (do not wet) surfaces and have small (large) contact angles. (*B*) Contact angle of water droplets on a WT *B. subtilis* biofilm and a Teflon block as a function of ethanol concentration. A plateau of ~135–145° is seen for the biofilm up to ~80% ethanol, when it transitions to wetting. In contrast, Teflon displays a roughly linear decrease in contact angle. Liquid drop profiles used for determining the contact angle are inset for wild-type biofilm at 0, 50, and 100% ethanol. Antimicrobial activity of alcohols is believed to be optimal in the 60 to 90% range, denoted as the green region, where the biofilm is largely nonwetting, suggesting that ethanol-based bactericides may not wick into the biofilm. Error bars are SD, $n = 7$. (*Insets*) The architecture of the wild-type biofilm (*Right*) and a nonwetting droplet of 50% ethanol on the biofilm surface (*Left*).

Table 2. Commercial biocides on *B. subtilis* WT biofilms

Test liquid	Contact angle (°)
Clorox bleach	45.9 ± 9.4
Lysol Professional	121.9 ± 6.3
Hibiclens	130.8 ± 10.2
Drain opener (10 s)	123.0 ± 13.7
Drain opener (5 min)	47.0 ± 0.52

Error = standard deviation; $n = 9$

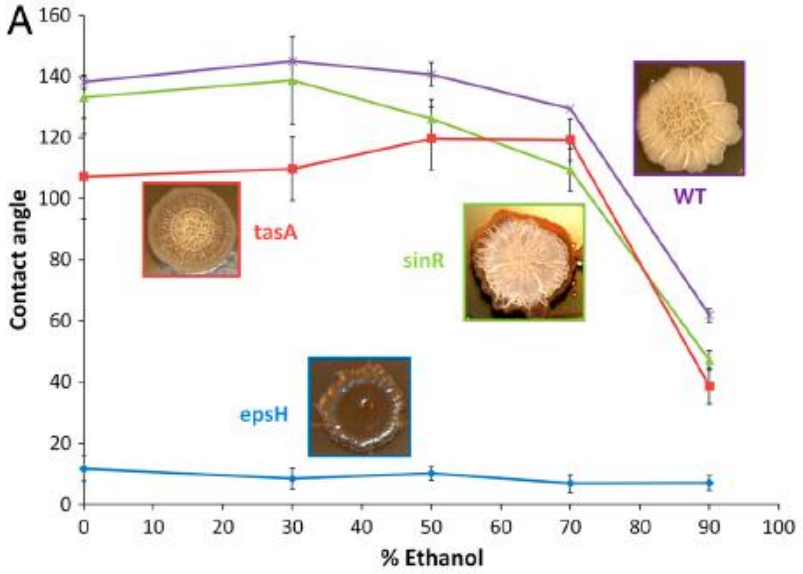
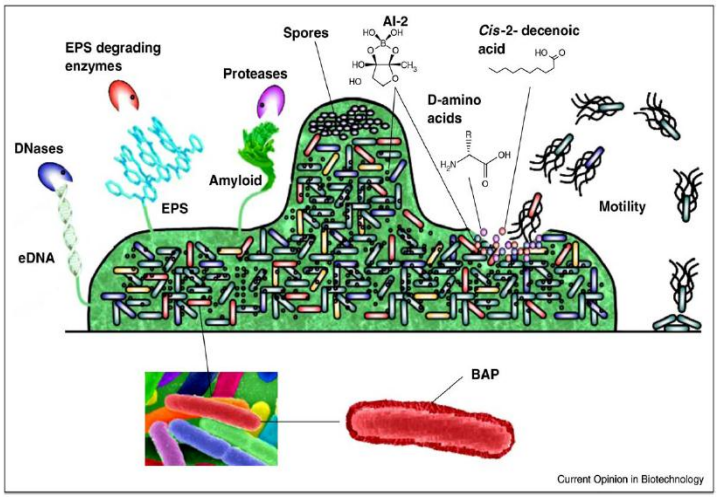


Fig. 4. Characterization of liquid repellency mechanisms using genetic mutants of *B. subtilis* biofilms lacking either the carbohydrate-rich epsH or protein tasA, or sinR. (*A*) The phenotypes are inset adjacent to their respective contact angle curves. Highly wrinkled sinR biofilm, with excess tasA protein and epsH, exhibits slightly decreased repellency relative to wild type, possibly related to suboptimal topography. Error bars are SD, $n = 7$ for WT and Teflon, $n = 8+$ for tasA, 8+ for epsH, and 12+ for sinR. A standard Wilcoxon two-sided test was performed to test statistical significance in contact angle differences (1% and 5% significance level). The contact angle for epsH is statistically different from any other strain; WT is statistically different from tasA at all ethanol concentrations, and from sinR at ethanol concentrations ≥50%; tasA and sinR are statistically different except at 50% and 90% ethanol concentration (and 70% at significance level 1%). (*B*) Corresponding magnification SEM images showing the surface features of the critical point dried WT biofilm (*Left*) and the sinR mutant (*Right*).

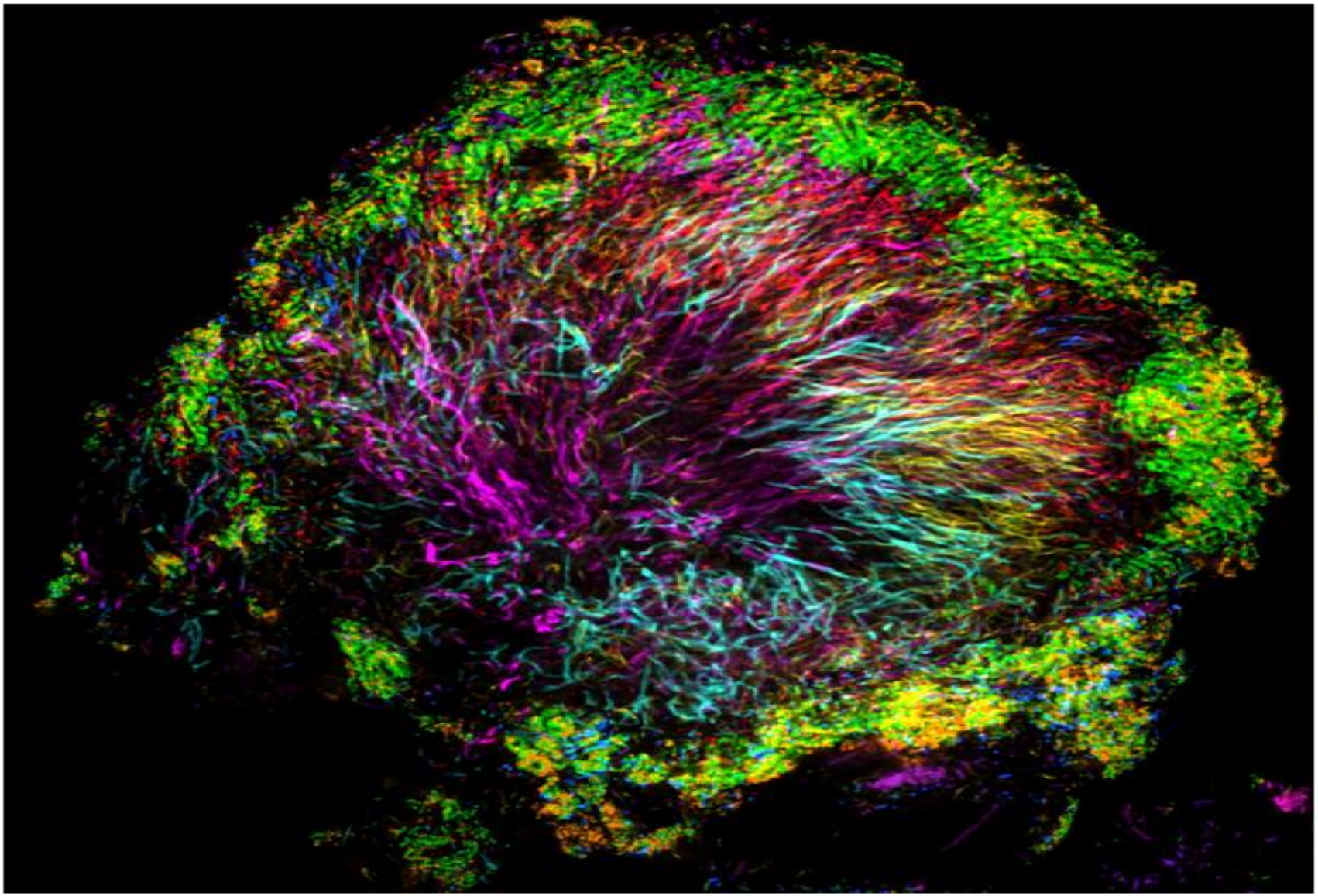


Schematic presentation showing mechanisms and components involved in biofilm formation and dispersal. Biofilms can contain various extracellular biopolymers like extracellular DNA (eDNA), extracellular polysaccharides, amyloid fibers, and biofilm-associated proteins (BAP). These matrix components might be good targets for (combinations of) putative enzymes such as DNases, proteases, and extracellular polysaccharide degrading enzymes to prevent formation of biofilms or to stimulate dispersal of already formed biofilms. Communication between cells during biofilm formation and dispersal of biofilms is dependent on quorum sensing systems and molecules like autoinducer 2 (AI-2), D-amino acids, and *cis*-2-decenoic acid. Furthermore, motility is an important factor in the establishment of new biofilms and the dispersal of cells from mature biofilms. Also, aerial structures of the biofilm serve as specific sites for the generation of spores (see text for details and corresponding references).

Table 1. Contact angles of aqueous solutions of organic solvents on *B. subtilis* biofilms

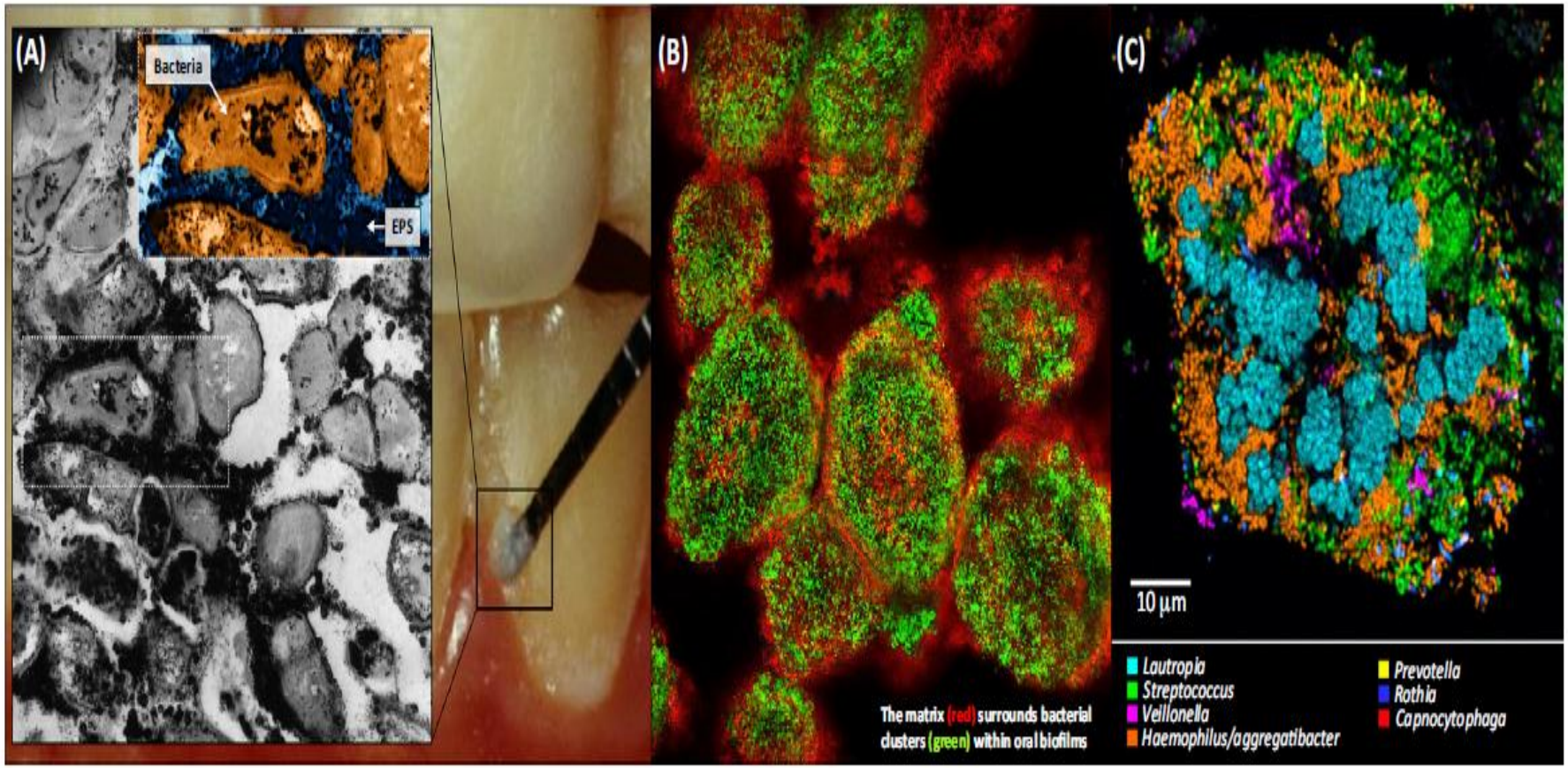
	WT	epsH	tasA	sinR
50% Ethanol	139.0 ± 3.9	10.2 ± 2.2	119.7 ± 10.3	128.9 ± 6.3
50% Isopropanol	125.3 ± 2.6	11 ± 1.5	110.9 ± 6.6	112.6 ± 2.1
50% Methanol	137.9 ± 4.0	8.4 ± 1.1	119.3 ± 8.3	115 ± 7.2
50% Acetone	139.7 ± 3.5	7.7 ± 3.0	117.2 ± 9.8	119.8 ± 3.6

Error = standard deviation; $n = 7$ for WT, 8+ for tasA, 8+ for epsH, 12+ for sinR.



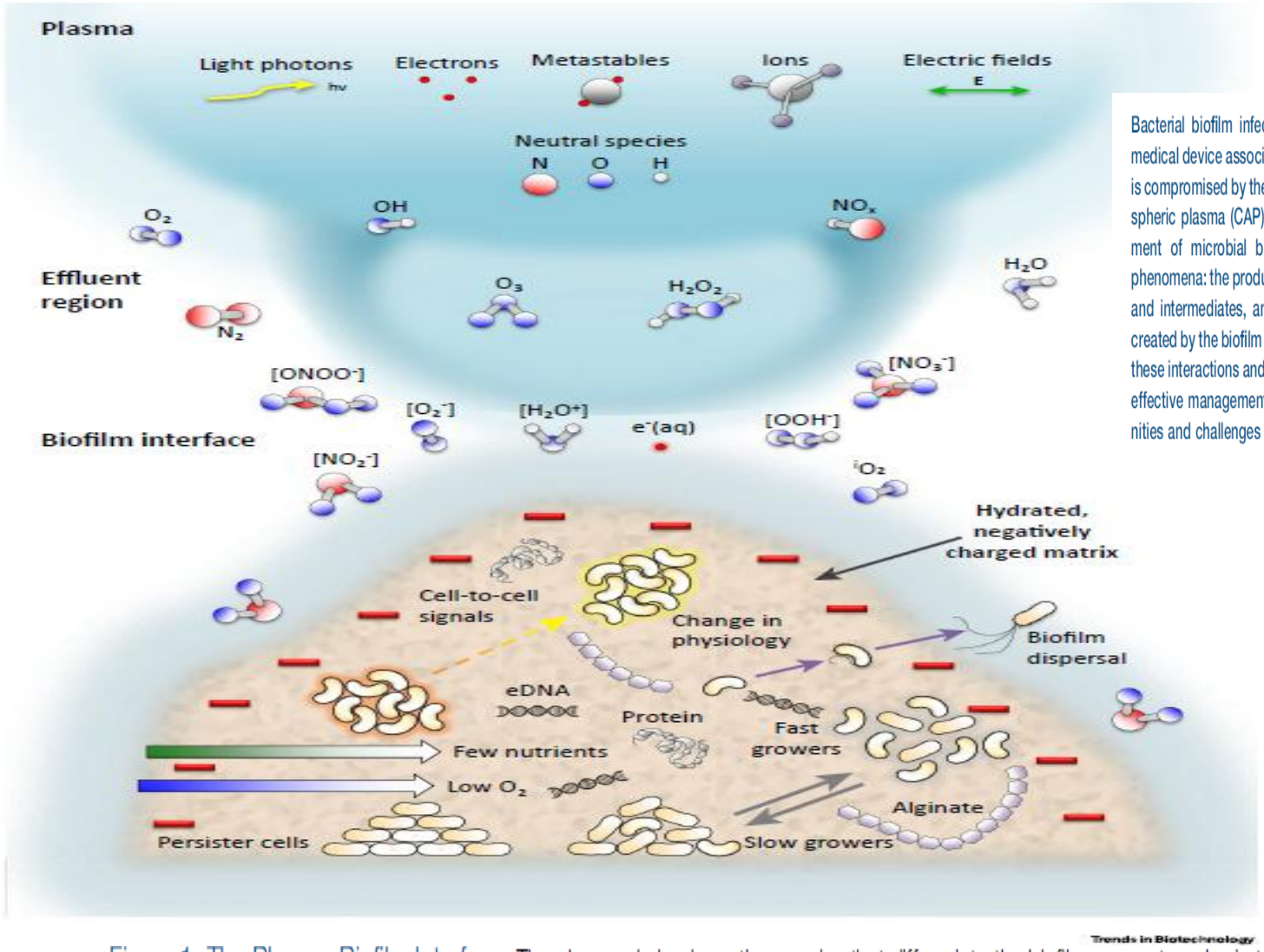
Trends in Microbiology

Figure 1. Image of the Oral Biofilm as seen by the CLASI-FISH Technique. Different colours indicate different bacteria present in a human dental plaque sample, with the outstanding presence of long filaments of *Corynebacterium* (labeled in magenta). Image courtesy Gary G. Borisy, The Forsyth Institute, and Jessica L. Mark Welch, Marine Biological Laboratory.



Trends in Microbiology

Figure 1. Dental Plaque Architecture: The EPS Matrix, Spatial Organization, and Polymicrobial Composition. (A) Plaque biofilm from a caries-active subject (photo courtesy of Dr Jaime A. Cury): microscopic image (inset) of plaque-biofilm showing a selected area containing bacterial cells (highlighted in orange) enmeshed in EPS (in dark blue); the image was pseudo-colored using Adobe Photoshop software for visualization purposes (adapted from [19]). (B) Bacterial clusters (green) surrounded by EPS matrix (red) detected in mature mixed-species oral biofilms formed in sucrose (adapted from [5]). (C) Spatial organization of human dental plaque showing multiple clusters of varying sizes containing different microbial species (adapted from [31]). Abbreviation: EPS, extracellular polymeric substances.



Bacterial biofilm infections account for a major proportion of chronic and medical device associated infections in humans, yet our ability to control them is compromised by their inherent tolerance to antimicrobial agents. Cold atmospheric plasma (CAP) represents a promising therapeutic option. CAP treatment of microbial biofilms represents the convergence of two complex phenomena: the production of a chemically diverse mixture of reactive species and intermediates, and their interaction with a heterogeneous 3D interface created by the biofilm extracellular polymeric matrix. Therefore, understanding these interactions and physiological responses to CAP exposure are central to effective management of infectious biofilms. We review the unique opportunities and challenges for translating CAP to the management of biofilms.

Figure 1. The Plasma–Biofilm Interface. The plasma-derived reactive species that diffuse into the biofilm encounter a hydrated, cationic extracellular polymeric matrix which may sequester RONS and attenuate plasma cidal efficacy and maintains a 3D architecture supporting heterogeneous microenvironments that in turn support multispecies microcolonies. Growth rate may be reduced due to nutrient and O_2 limitations within the biofilm, leading to elevated tolerance and persister formation. Quorum sensing, leading to alterations in microbial physiology may also affect microbial tolerance to plasma-derived RONS. Finally, RONS-mediated dispersal of microbes from the biofilm may reverse plasma tolerance. Adapted from [7,87]. Abbreviations: eDNA, extracellular DNA; RONS, reactive oxygen and nitrogen species.

Adhesion and removal kinetics of *Bacillus cereus* biofilms on Ni-PTFE modified stainless steel

Kang Huang, Lynne A. McLandsborough and Julie M. Goddard

Department of Food Science, University of Massachusetts, Amherst, MA, USA

ABSTRACT

Biofilm control remains a challenge to food safety. A well-studied non-fouling coating involves codeposition of polytetrafluoroethylene (PTFE) during electroless plating. This coating has been reported to reduce foulant build-up during pasteurization, but opportunities remain in demonstrating its efficacy in inhibiting biofilm formation. Herein, the initial adhesion, biofilm formation, and removal kinetics of *Bacillus cereus* on Ni-PTFE-modified stainless steel (SS) are characterized. Coatings lowered the surface energy of SS and reduced biofilm formation by > 2 log CFU cm^{-2}. Characterization of the kinetics of biofilm removal during cleaning demonstrated improved cleanability on the Ni-PTFE coated steel. There was no evidence of biofilm after cleaning by either solution on the Ni-PTFE coated steel, whereas more than 3 log and 1 log CFU cm^{-2} of bacteria remained on the native steel after cleaning with water and an alkaline cleaner, respectively. This work demonstrates the potential application of Ni-PTFE non-fouling coatings on SS to improve food safety by reducing biofilm formation and improving the cleaning efficiency of food processing equipment.

ARTICLE HISTORY
Received 13 October 2015
Accepted 25 February 2016

KEYWORDS
Nickel polytetrafluoroethylene (Ni-PTFE); non-fouling stainless steel; biofilm; *Bacillus cereus*; fouling release coatings; biofouling

Table 1. Identification of native stainless steel (SS) surface and 4 commercial coating technologies evaluated in this work

Coating abbreviation	Description	Manufacturer
Lectrofluor 641	Fluoro polymer-based coating on SS	General Magnaplate Corporation, Linden, NJ
AMC 18	Anti-stiction coating available commercially	Advanced Materials Components Express, Lemont, PA
Ni-P-PTFE[1]	Electroless deposition of nickel followed by co-deposition of PTFE particles	Avtec Finishing Systems, New Hope, MN
Dursan	Composed of carboxy silicon material inter-diffused with SS	SilcoTek Corporation, Bellefonte, PA
Native SS316	SS containing molybdenum imparting anticorrosive properties	AGC Heat Transfer, Portland, OR

[1]PTFE = polytetrafluoroethylene.

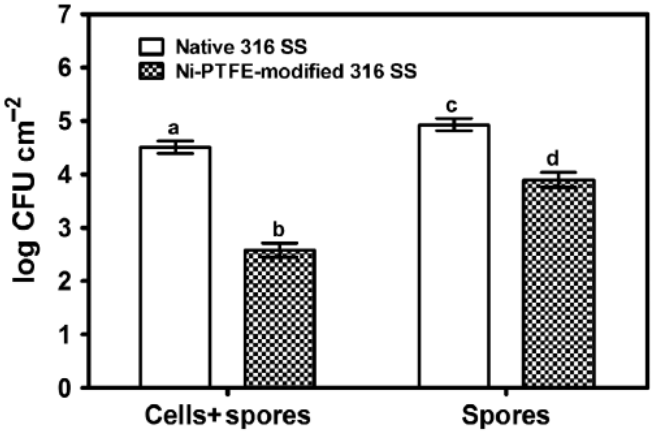


Figure 3. Initial adhesion behavior of vegetative cells and spores after 2 h exposure to native and Ni-PTFE-modified SS surfaces (n = 12). Treatments with different letters are significantly different ($p < 0.05$).

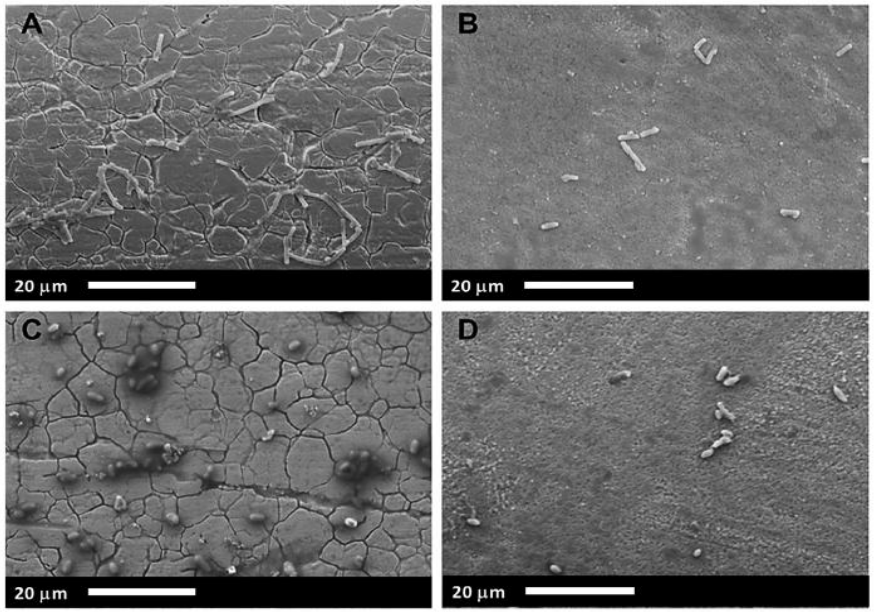


Figure 4. Representative SEM images of *B. cereus* vegetative cells and spores on native and Ni-PTFE-modified SS surfaces 2 h after an initial adhesion assay: (A) adherent vegetative cells on native 316 SS; (B) adherent vegetative cells on Ni-PTFE-modified 316 SS; (C) adherent spores on native 316 SS; (D) adherent spores on Ni-PTFE-modified 316 SS.

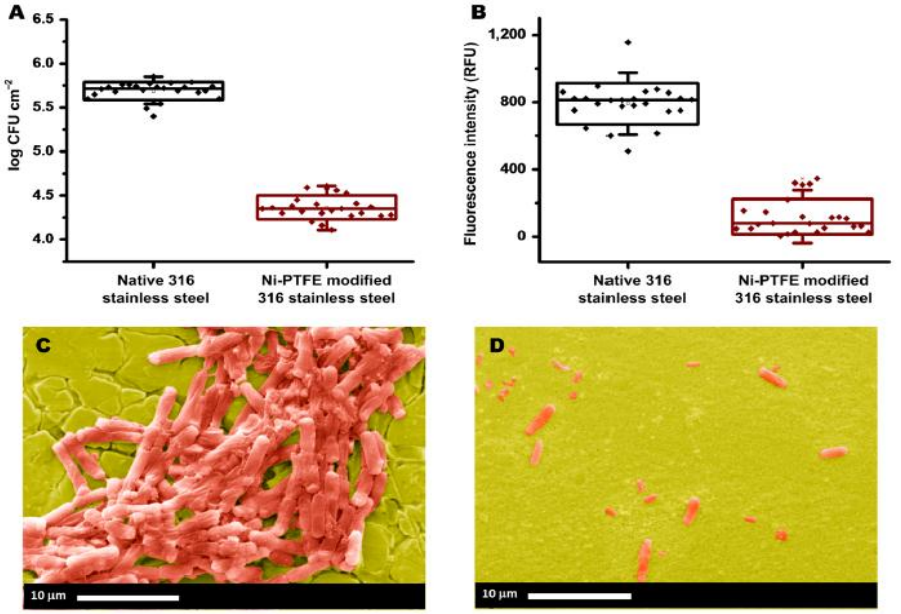
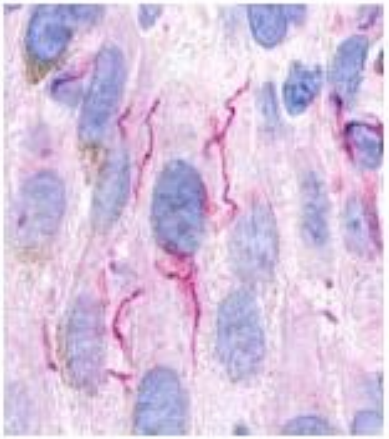


Figure 5. Characterization of biofilms formed on native and Ni-PTFE-modified SS surfaces after adhesion for 24 h and continuous growth for 48 h in LB broth. (A) Bacterial enumeration (log CFU cm^{-2}); (B) fluorescence intensity (RFU); (C) SEM micrograph of biofilm on native 316 SS; (D) SEM micrograph of biofilm on Ni-PTFE-modified 316 SS.

Antibióticos

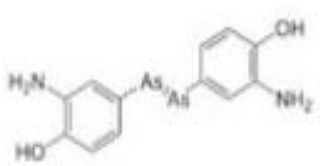
- **Qué son, cuál es su origen, cómo se clasifican?**
- **Modo de acción selectivo**
- **Quiénes los producen**
- **Origen y desarrollo de resistencia a antibióticos**
- **Necesidad de nuevos antibióticos**

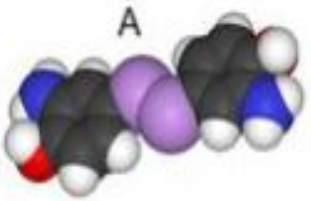
Antecedentes Históricos

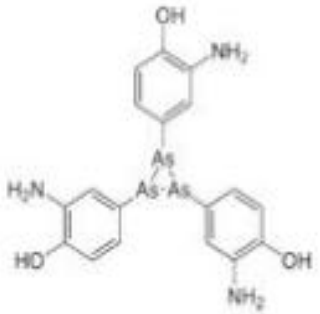


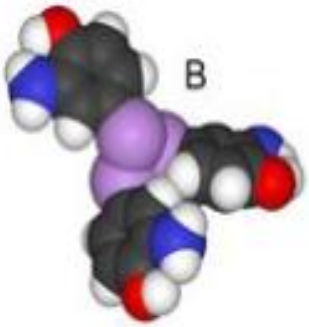
Treponema pallidum

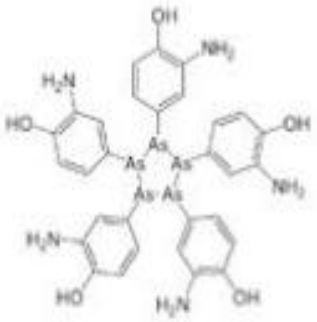
Estructura química del
Salvarsan o arsfenamina
"La bala mágica"

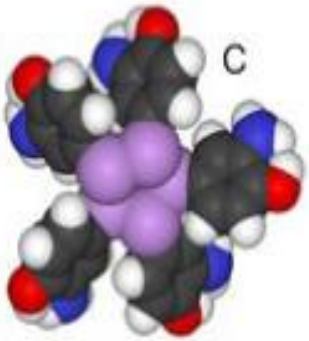












Paul Ehrlich

Concepto basico de la quimiter ápia: **Toxicidad selectiva**

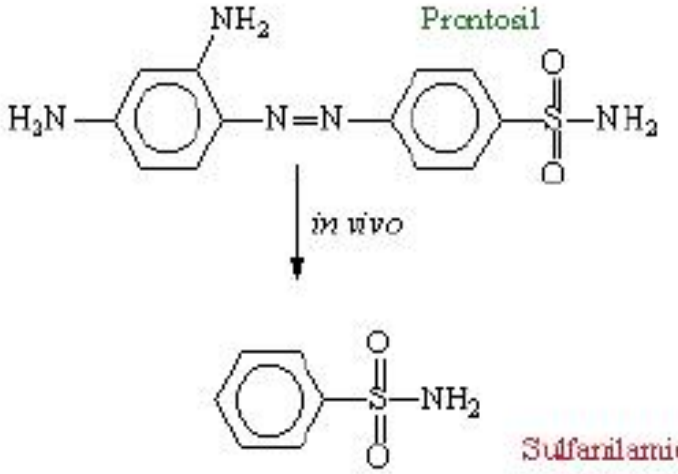
Creó el primer compuesto químico sintético (Salvarsan en 1901, 606 derivados) que podía curar una infección, la sífilis

Premio Nobel Medicina: 1908

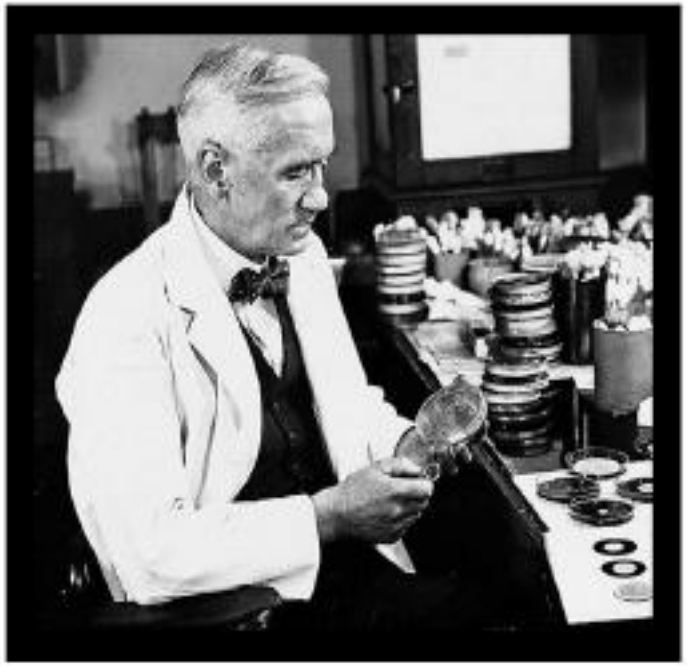
Antecedentes Históricos

Sulfamidas

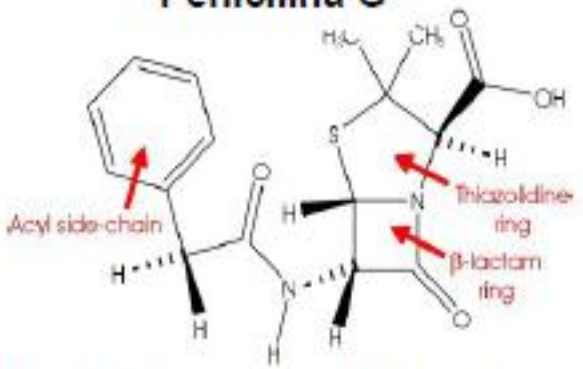
Descubiertas por Gerhard Domagk (1932) mientras buscaba colorantes para teñir *S. aureus*. Un colorante rojo (Prontosil Rubrum) protegía a ratones y conejos contra dosis letales de estáfilos y estreptococos hemolíticos.



Antecedentes Históricos



Penicilina G



A la sustancia se le dio el nombre de Penicilina, porque el hongo contaminante fue identificado como *Penicillium notatum*.
Efectiva contra las peores enfermedades infecciosas del momento, como la tuberculosis, sífilis, cólera o neumonía.

Alexander Fleming

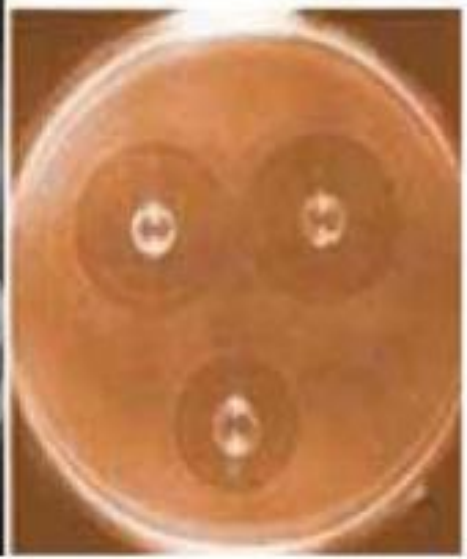
Descubrió el primer antibiótico, cuando por accidente se contaminó un cultivo de *Staphylococcus aureus* con un hongo y observó un halo transparente de inhibición de crecimiento de este microorganismo alrededor del hongo (1928).
Premio Nobel de Medicina 1945

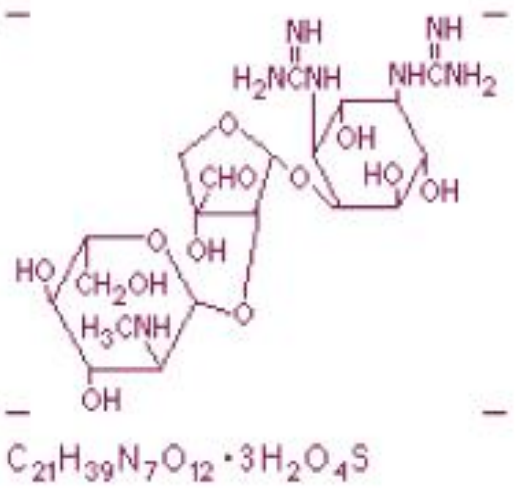
Antecedentes Históricos

Streptomicina

Fue aislada en 1944 por el Albert Schatz en el laboratorio de Selman Waksman a partir de *Streptomyces griseus* debido a las evidencias de que cepas de *Mycobacterias* se inactivaban al mezclarlos con muestras del suelo. Introduce el término "**antibiótico**".

Selman Waksman recibe el premio Nobel de medicina 1952



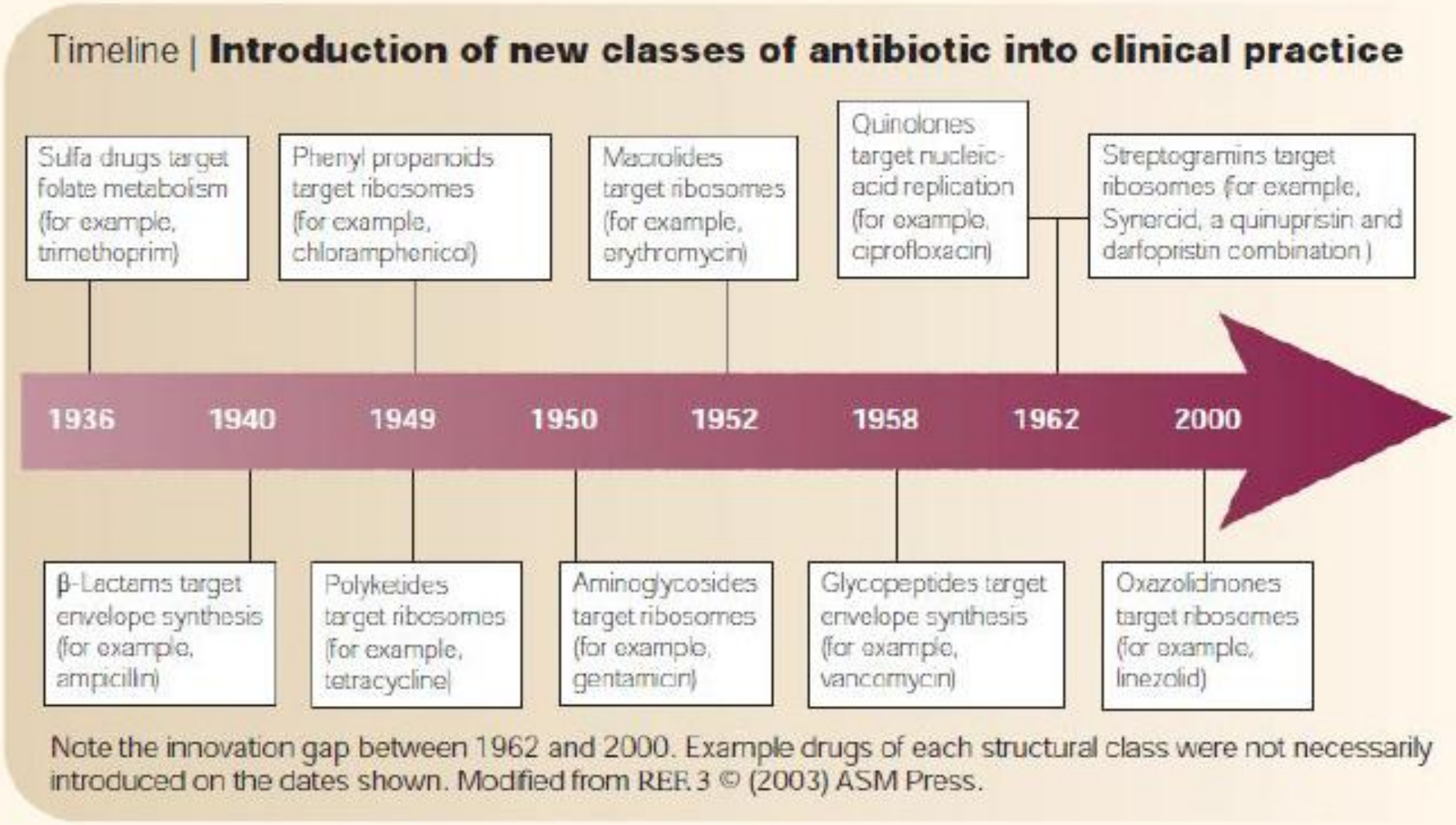


$C_{21}H_{39}N_7O_{12} \cdot 3H_2O_4S$

Aminoglicósidos

Son azúcares complejos unidos por enlaces glicosídicos. Atraviesan la membrana citoplasmática por un mecanismo oxígeno-dependiente. Los grupos $-NH_2$ y -OH interactúan con proteínas del ribosoma.

Antecedentes Históricos

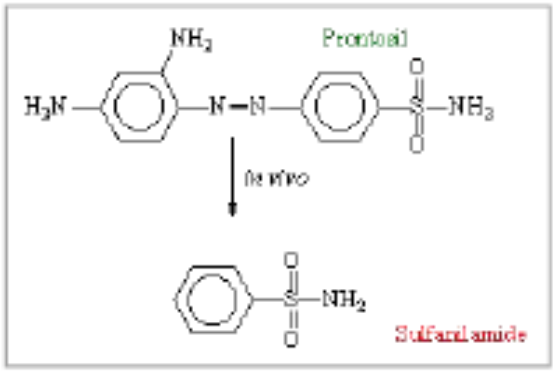


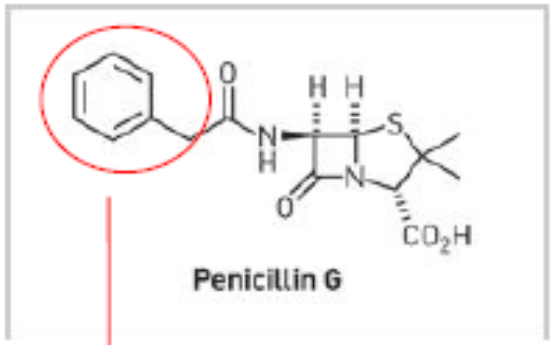
daptomycin (2000) descubierto 1980
pleuromutilins (2007) se usó en vetrinaria por mas de 30 años
fidaxomicin (2011) primer reporte en 1970

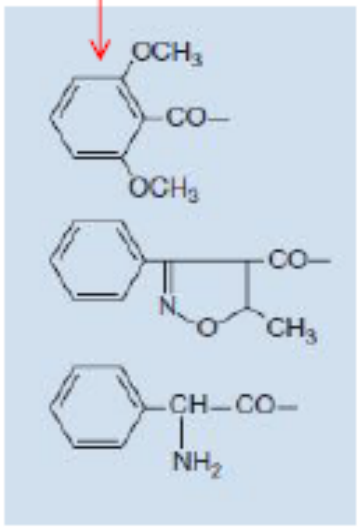
Clasificación de los antibióticos

1- Según su origen
2- Según su estructura química
3- Según su actividad sobre microorganismos
4- Según su espectro de acción
5- Según su mecanismo de acción

Clasificación según su origen







Quimioterapéutico o Sintético

Sustancia producida de manera sintética que posee la propiedad de inhibir el crecimiento o destruir microorganismos

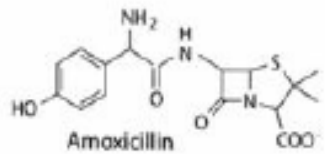
Productos Naturales

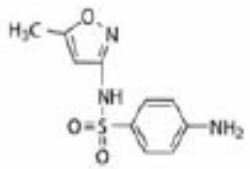
Sustancia producida por el metabolismo de organismos vivos, principalmente hongos y bacterias, que posee la propiedad de inhibir el crecimiento o destruir microorganismos

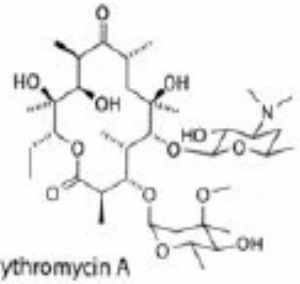
Semisintéticos

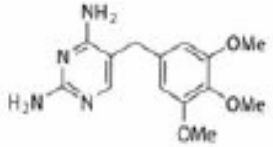
Productos naturales con modificaciones químicas en su estructura, que poseen mejoras en sus propiedades fisicoquímicas y/o farmacológicas

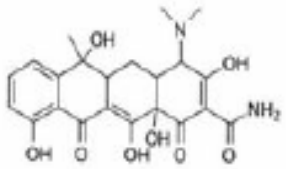
Clasificación según su estructura

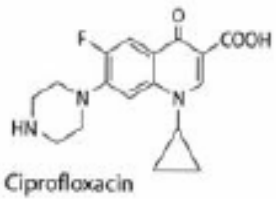

Amoxicillin

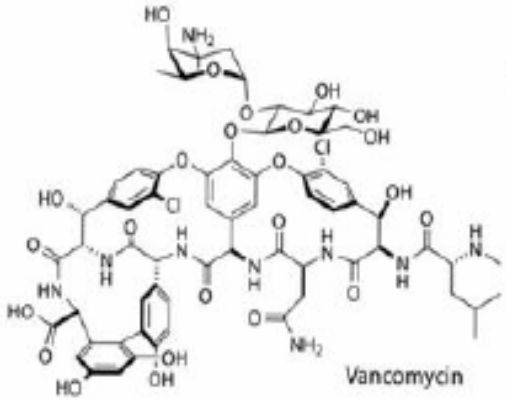

Sulfamethoxazole

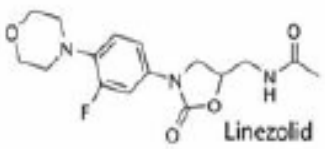

Erythromycin A

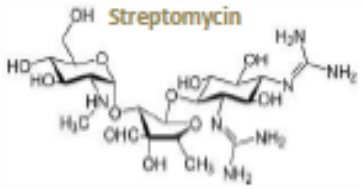

Trimethoprim


Tetracycline


Ciprofloxacin


Vancomycin


Linezolid


Streptomycin

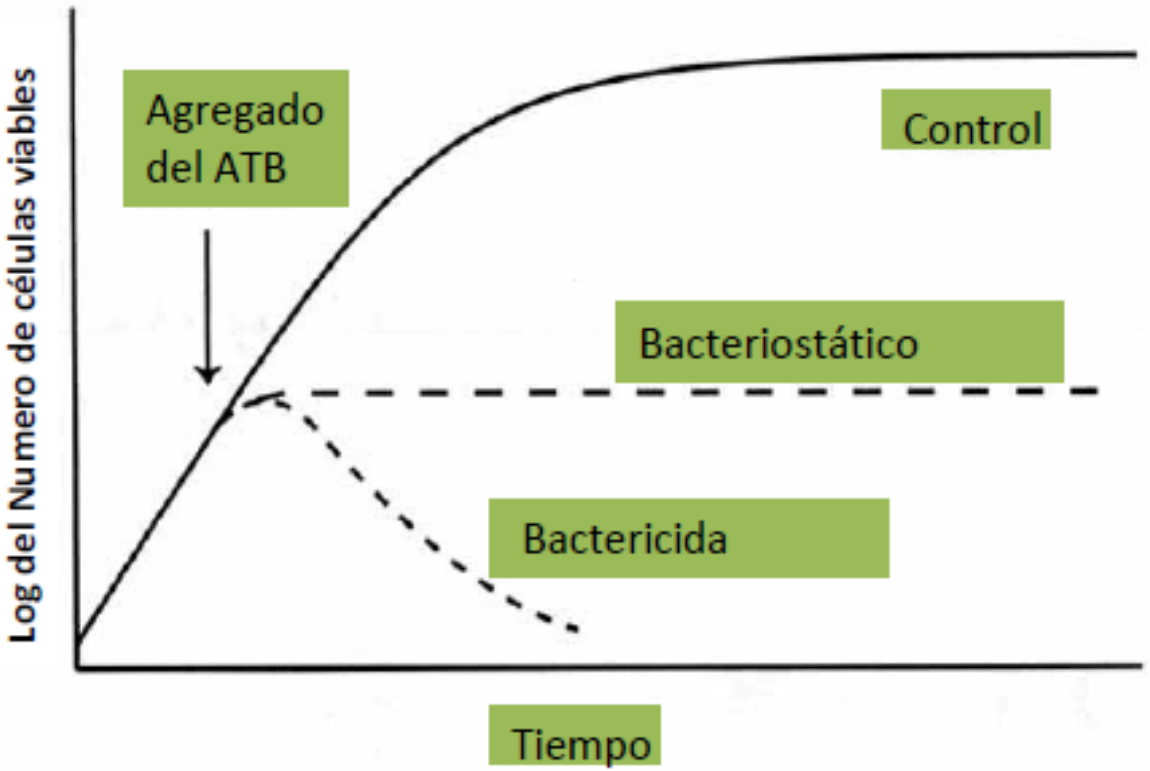
- β-lactámicos
  - penicilinas
  - cefalosporinas
- Tetraciclinas
- Aminoglicósidos
- Quinolonas
- Polipéptidos (síntesis ribosomal o no ribosomal)
- Macrólidos
- Cloramfenicol

Esta diversidad estructural les permite interactuar con diferentes sitios blancos en las bacteria

Efecto de los antimicrobianos sobre el crecimiento bacteriano

Bactericidas: producen la muerte de los agentes infecciosos

Bacteriost á ticos: inhiben el crecimiento bacteriano aunque el microorganismo permanece viable



Clasificación de según su espectro de acción

Un antibiótico frente a distintas bacterias

- **Espectro reducido**:

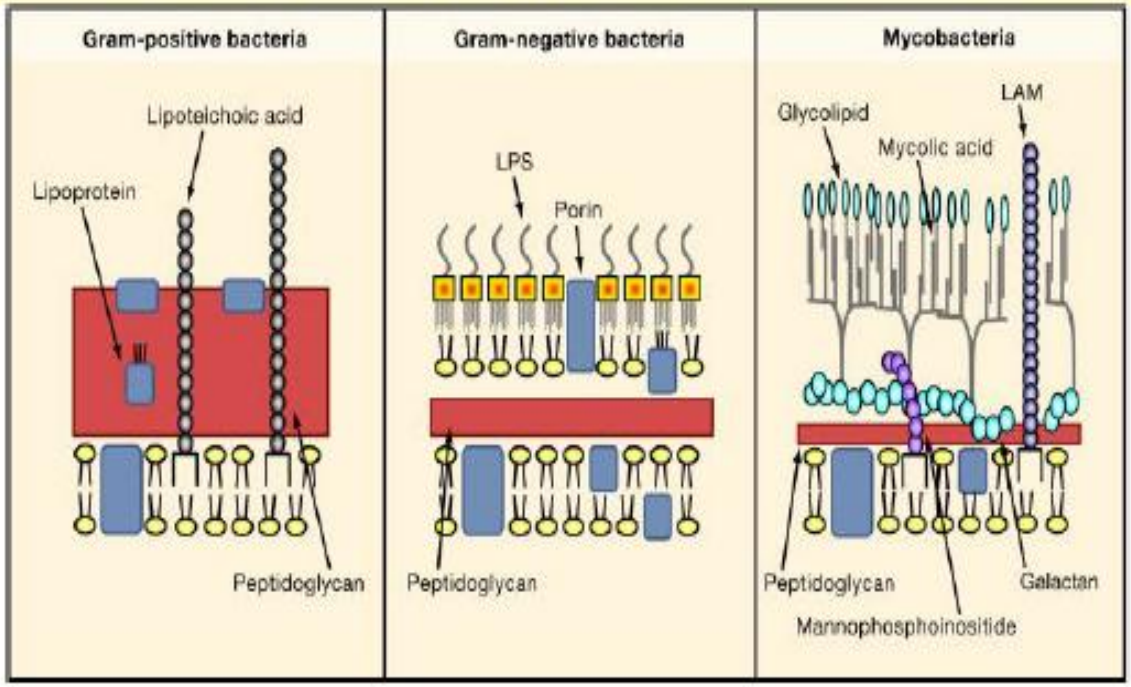
  Son activos selectivamente frente a un grupo determinado de bacterias

  Ej: **Macrólidos:** cocos Gram (+)
  Gentamicina: bacilos Gram (-)

- **Espectro amplio**:

  Presentan actividad frente a la mayoría de los grupos bacterianos de importancia clínica

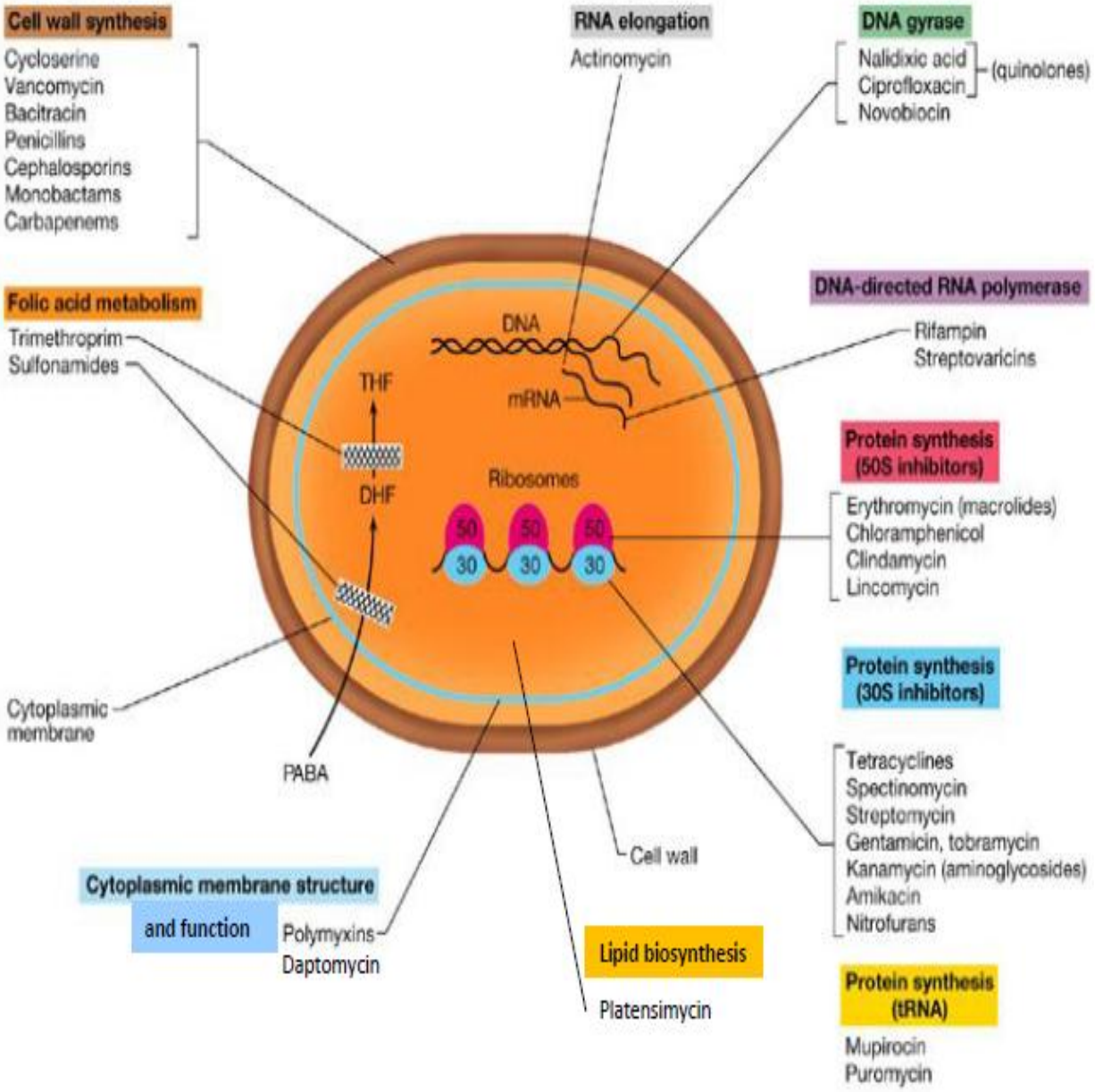
  Ej: **Penicilina:** cocos Gram (+), cocos Gram (-), bacilos Gram (+)
  Ampicilina: cocos Gram (+) y Gram (-), algunos bacilos Gram (-)



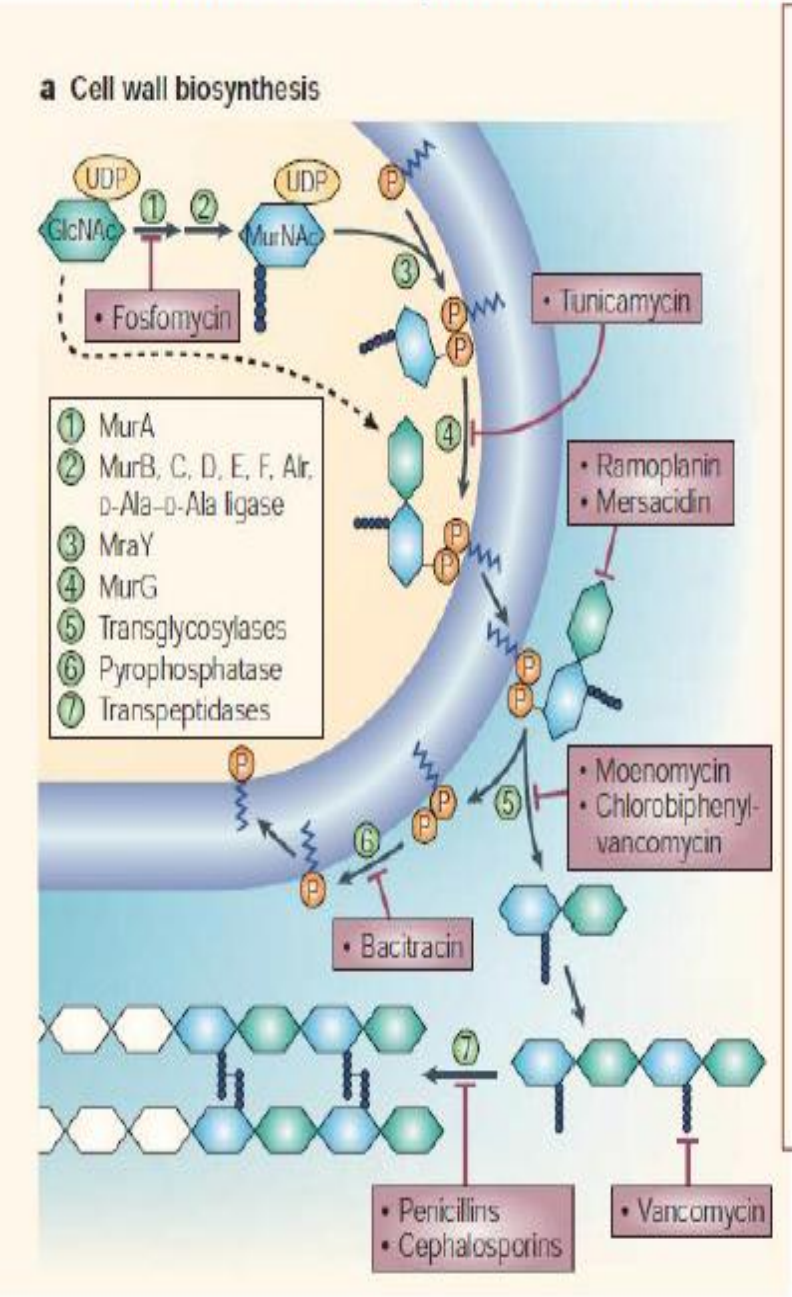
Cuál es la base de la toxicidad selectiva de los antibióticos?

- Procesos celulares presentes solo en microorganismos → Síntesis de pared o folato
- Procesos similares pero con diferencia estructurales suficientes → Ribosomas

Sitios blanco de acción de los principales antibacterianos



Antibióticos que inhiben la síntesis de pared celular



- Inhiben enzimas biosintéticas
  Fosfomicina, Cicloserina *
- Se combinan con moléculas transportadoras
  Bacitracina
- Secuestro de sustratos de la pared
  Vancomicina
- Inhiben las reacciones de entrecruzamiento del peptidoglicano
  Penicilinas, Cefalosporinas *

* Análogos de sustratos

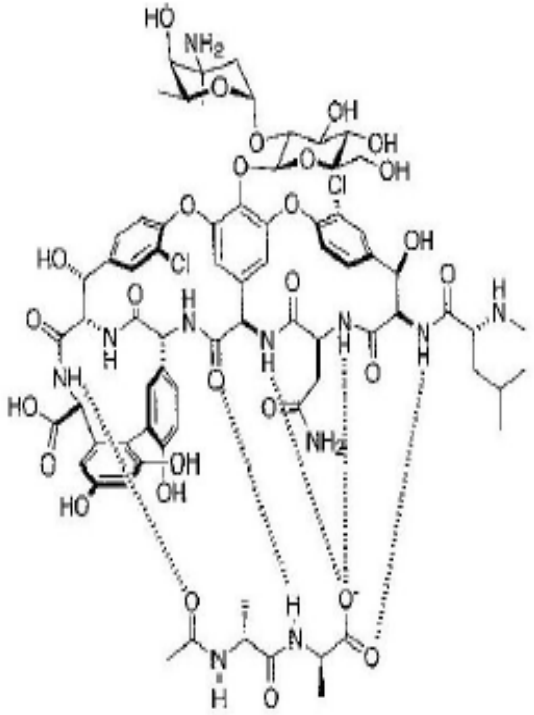
Antibióticos que inhiben la síntesis de pared celular

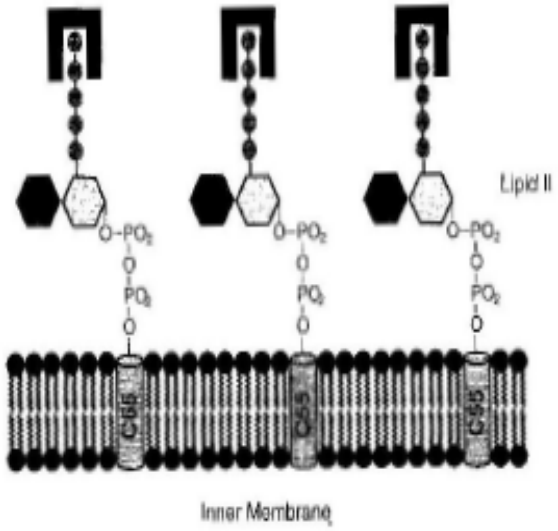
Secuestro del sustrato de la pared

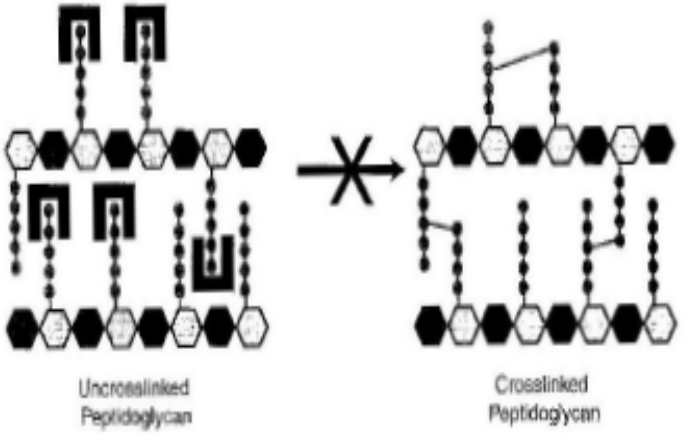
***Vancomicina*: glicopéptido**

Forma un complejo con los residuos de D-alanina del muramilpentapéptido, estabilizado por cinco puentes de hidrógeno.

Impide la transferencia de los precursores desde el transportador lipídico y la transpeptidación.



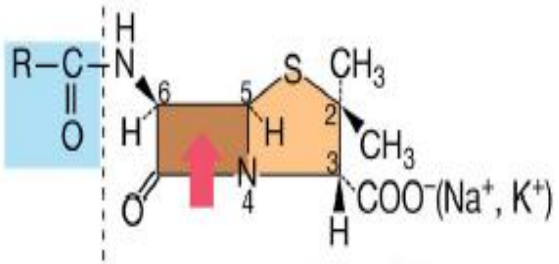


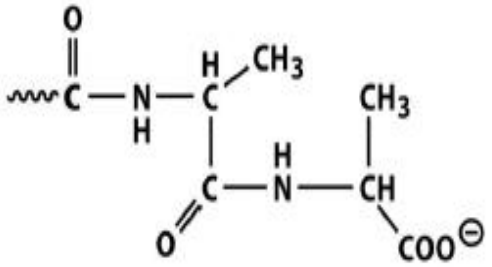


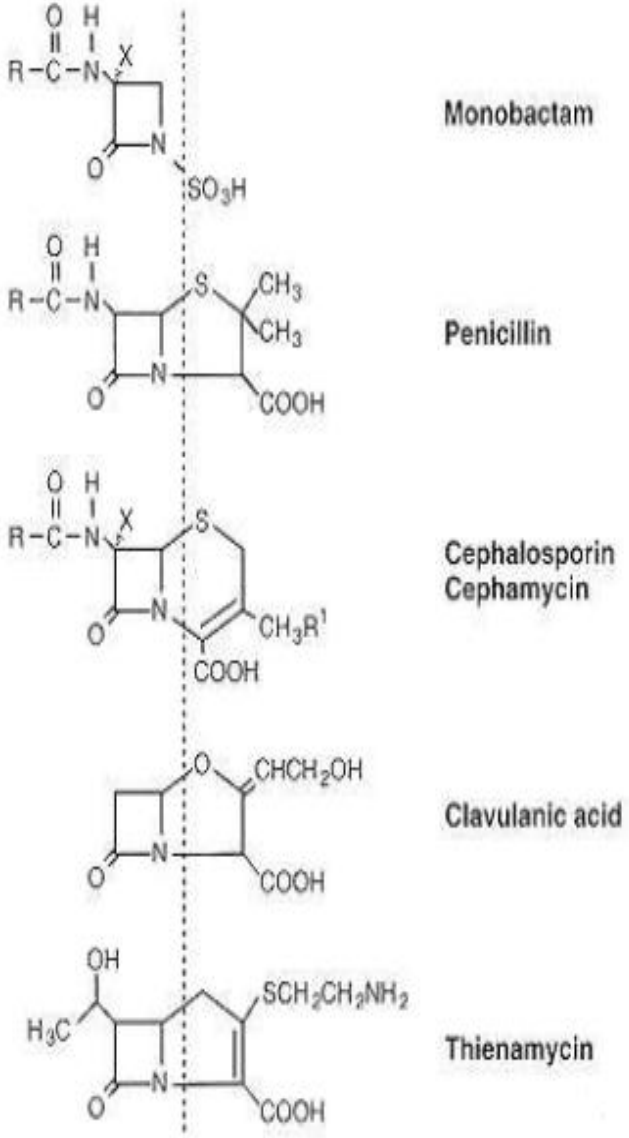
Antibióticos que inhiben la síntesis de pared celular

Inhibición de las reacciones de entrecruzamiento (transpeptidación) del peptidoglicano

β-lactámicos







Proteínas de unión a β-lactámicos (PBP)

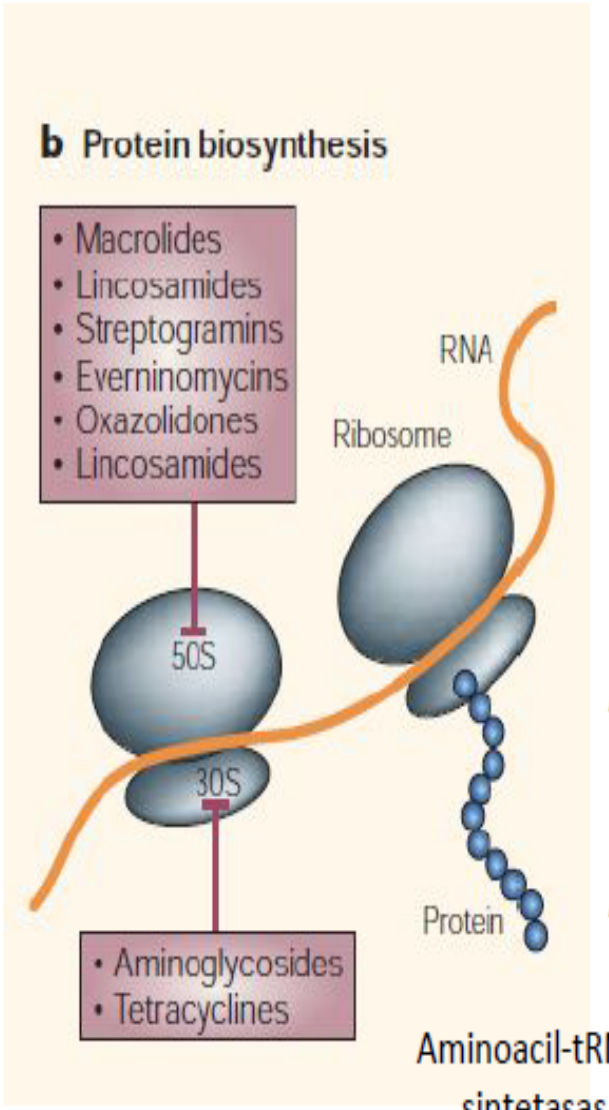
- ✓ Enzimas sensibles a penicilina o cefalosporinas
- ✓ Distinto grado de afinidad frente a los distintos β-lactámicos
- ✓ Existen distintas PBPs con diferentes actividades.
- ✓ Intervienen en elongación y en la forma de la bacteria
- ✓ No todas las especies bacterianas presentan idéntico perfil de PBPs.
- ✓ Algunas tienen actividad carboxipeptidasa (CP) que liberan la alanina terminal
- ✓ Algunas son bifuncionales (TP y CP)

SDS-PAGE
^{14}C-peniciloil-proteinas

PBPs con actividad transglucosidasa y transpeptidasa:	Función natural	Acción de la penicilina
PBP 1a y PBP 1b	Elongación del cilindro celular	lisis rápida
PBP 2	Condiciona la forma de la célula	la célula se redondea y muere
PBP 3	Formación del septo transversal	Filamentación y muerte
PBPs con actividad carboxipeptidasa (endopeptidasa)	**Función natural**	**Acción penicilina**
PBP 4, PBP 5, PBP 6	Eliminan la D-ala terminal delpentapéptido (maduración PG)	no letal

Inhibición de la síntesis de proteínas

Antibióticos que inhiben la síntesis de proteínas



Actúan sobre la subunidad 30S del ribosoma

Aminoglicósidos: Estreptomicina, Neomicina, Kanamicina, Gentamicina, Tobramicina, Amikacina

Tetraciclinas: Doxiciclina, Tetraciclina

Actúan sobre la unidad 50S del ribosoma

Cloranfenicol

Macrólidos: Eritromicina, Azitromicina, Claritromicina

Lincosamidas: Clindamicina

Actúan sobre el ensamblado del ribosoma

2-Oxazolidona: Linezolida

Actúan sobre las aminoacil-tRNA sintetasas

Mupirocina

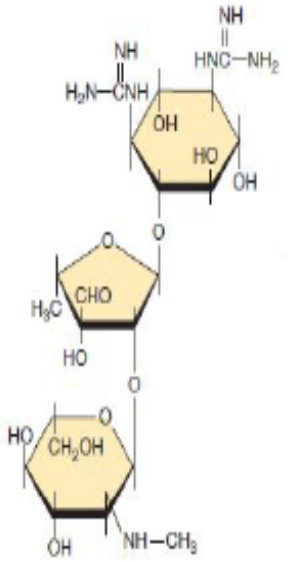
Antibióticos que inhiben la síntesis de proteínas

Actúan sobre la subunidad 30S del ribosoma

Aminoglicósidos

Son azúcares complejos unidos por enlaces glicosídicos. Atraviesan la membrana citoplasmática por un mecanismo oxígeno-dependiente. Los grupos $-NH_2$ y -OH interactúan con proteínas del ribosoma.

Dr. Selman A. Waksman

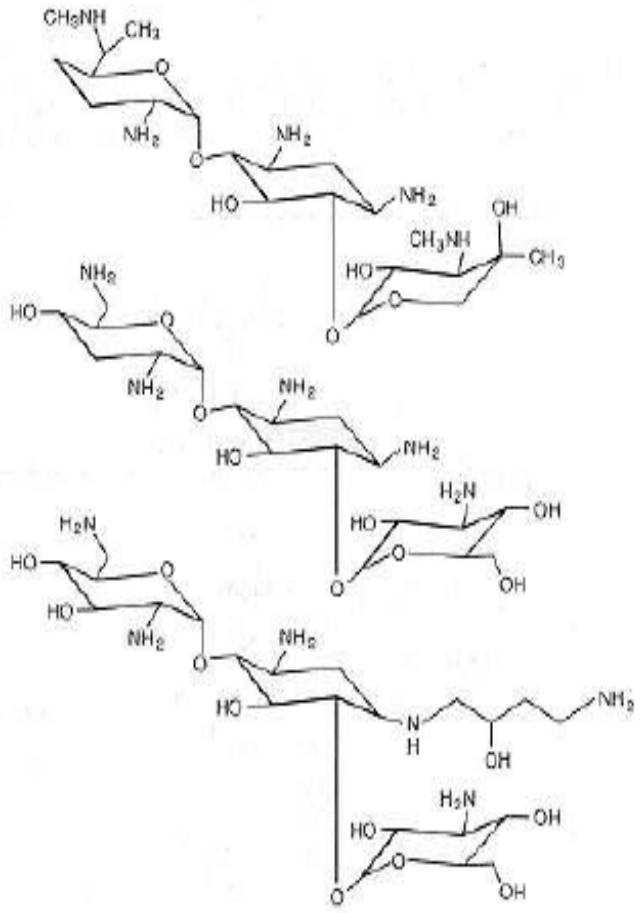


La **estreptomicina** fue aislada en 1944 por el Dr. Selman Waksman a partir de *Streptomyces griseus*

Gentamicin

Tobramycin

Amikacin



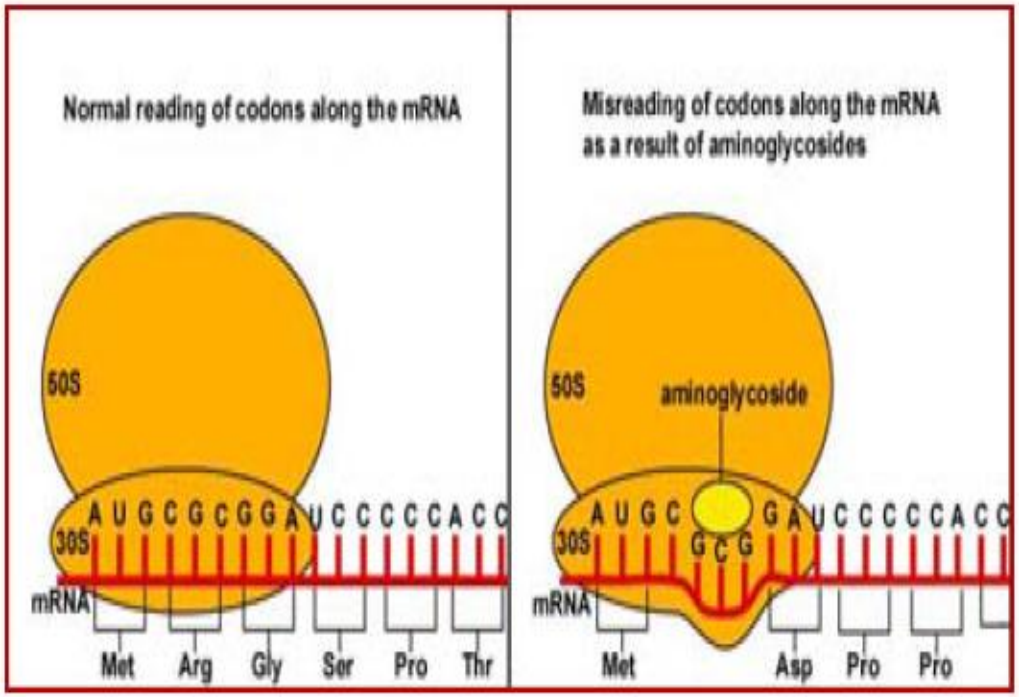
Antibióticos que inhiben la síntesis de proteínas

Que actúan sobre la subunidad 30S del ribosoma

Aminoglicósidos: Mecanismo de Acción

Se unen a la subunidad 30S del ribosoma:

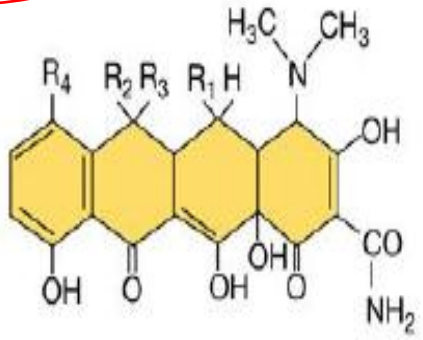
- Bloquea la formación del complejo de iniciación
- Produce lectura errónea del mensaje: proteína defectuosa
- El resultado final es la muerte de la bacteria, son bactericidas

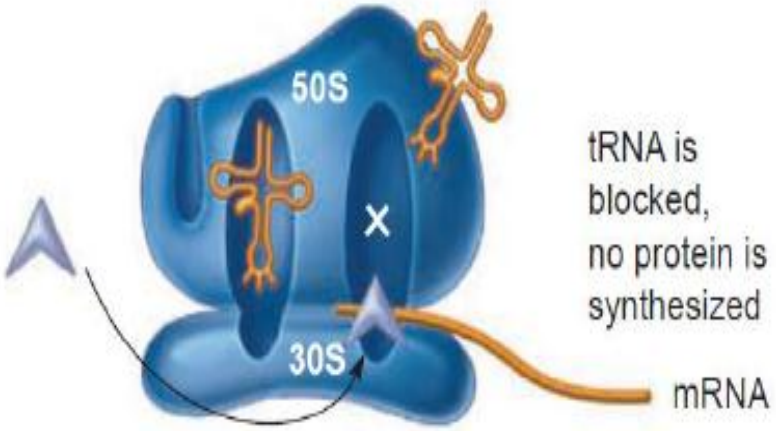


Antibióticos que inhiben la síntesis de proteínas

Que actúan sobre la subunidad 30S del ribosoma

Tetraciclinas: Estructura química y Mecanismo de acción





Tetracycline analog	R_1	R_2	R_3	R_4
Tetracycline	H	OH	CH_3	H
7-Chlortetracycline (aureomycin)	H	OH	CH_3	Cl
5-Oxytetracycline (terramycin)	OH	OH	CH_3	H

Bloquean la inserción del aminoacil-tRNA

La unión es transitoria, por lo que su efecto es reversible: son bacteriostáticos.

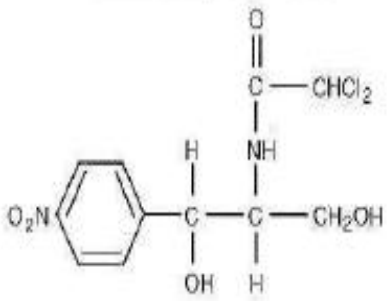
Antibióticos que inhiben la síntesis de proteínas

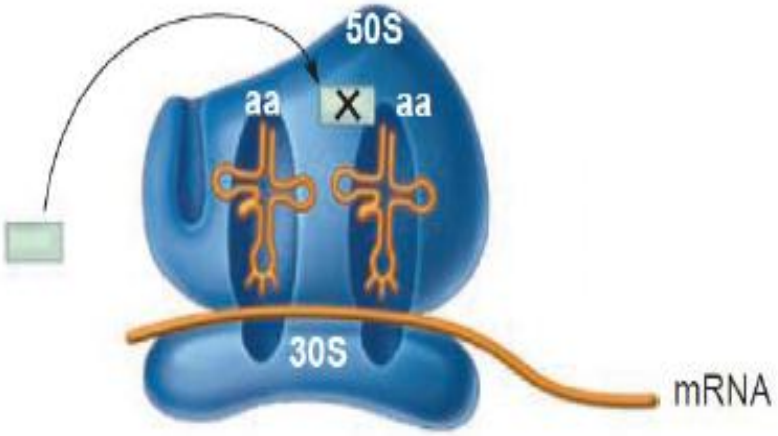
Que actúan sobre la subunidad 50S del ribosoma

Cloranfenicol: *Estructura química y Mecanismo de acción*

Originalmente producido por *Streptomyces venezuelae,* actualmente se sintetiza químicamente.

Chloramphenicol





Se une a la enzima peptidil transferasa

en la subunidad 50S

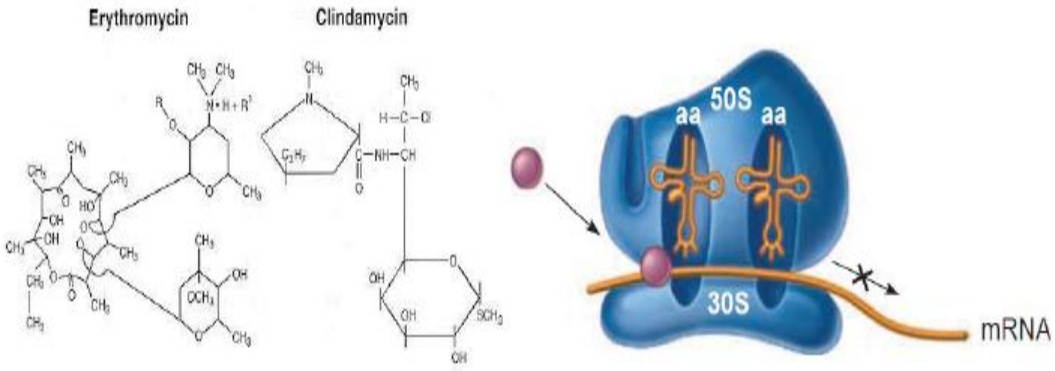
Inhibe la formación del enlace peptídico. Detiene la síntesis de proteínas.

Es un agente bacteriostático.

Antibióticos que inhiben la síntesis de proteínas

Que actúan sobre la subunidad 50S del ribosoma

Macrólidos y Lincosamidas: Estructura y Mecanismo de acción



Inhiben la peptidil transferasa y la translocación

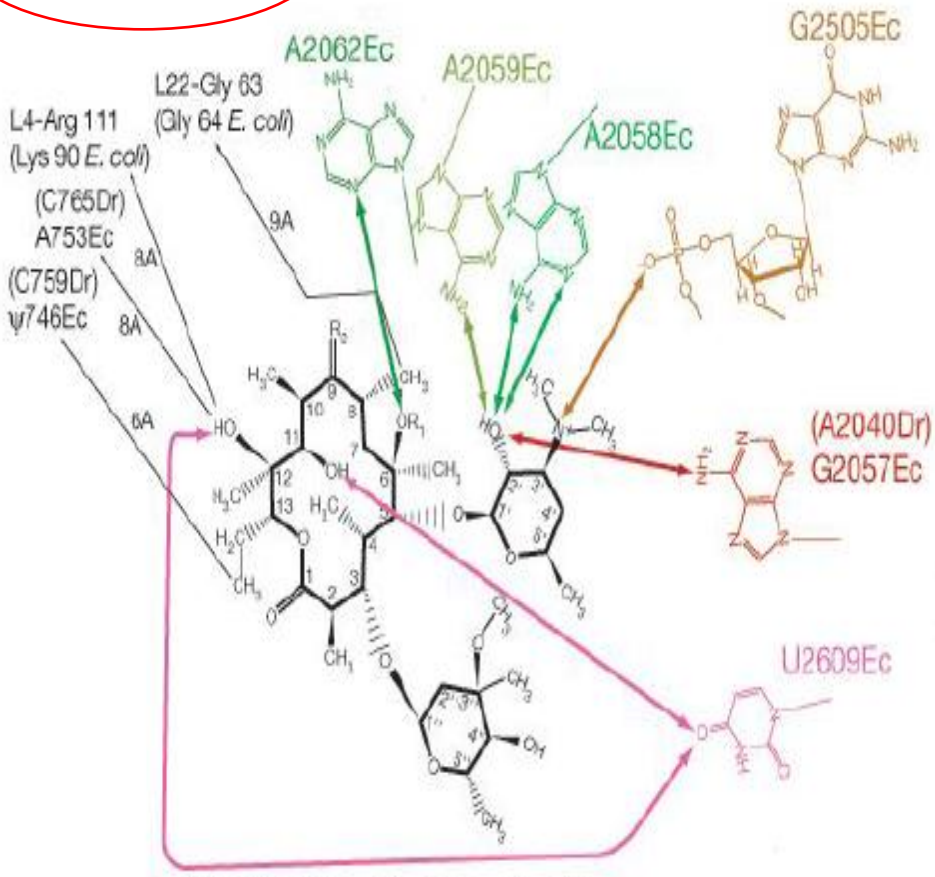
Se detiene la síntesis de proteínas liberando prematuramente el peptidil tRNA.

Son bacteriostáticos.

Antibióticos que inhiben la síntesis de proteínas

Que actúan sobre la subunidad 50S del ribosoma

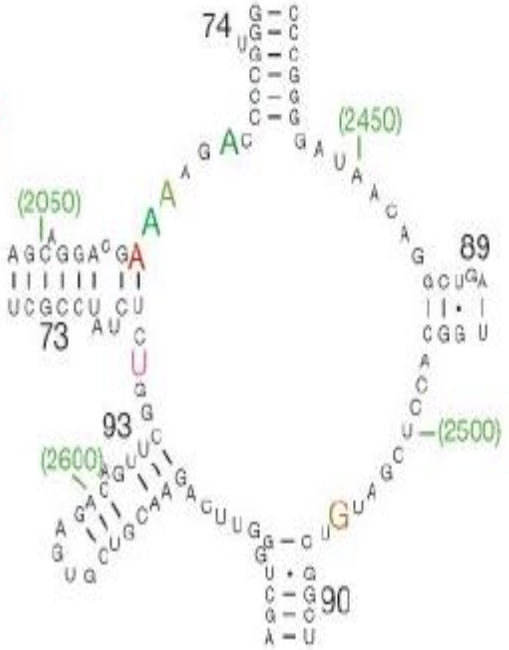
Macrólidos: Unión al ribosoma



Erythromycin $R_1 = H$; $R_2 = O$

Clarithromycin $R_1 = CH_3$; $R_2 = O$

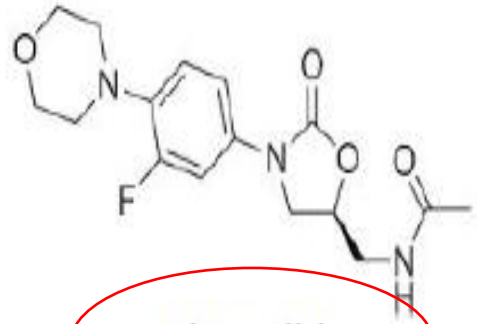
Roxithromycin $R_1 = H$; $R_2 = N\text{-}O\text{-}CH2\text{-}O\text{-}CH2\text{-}CH2\text{-}O\text{-}CH3$



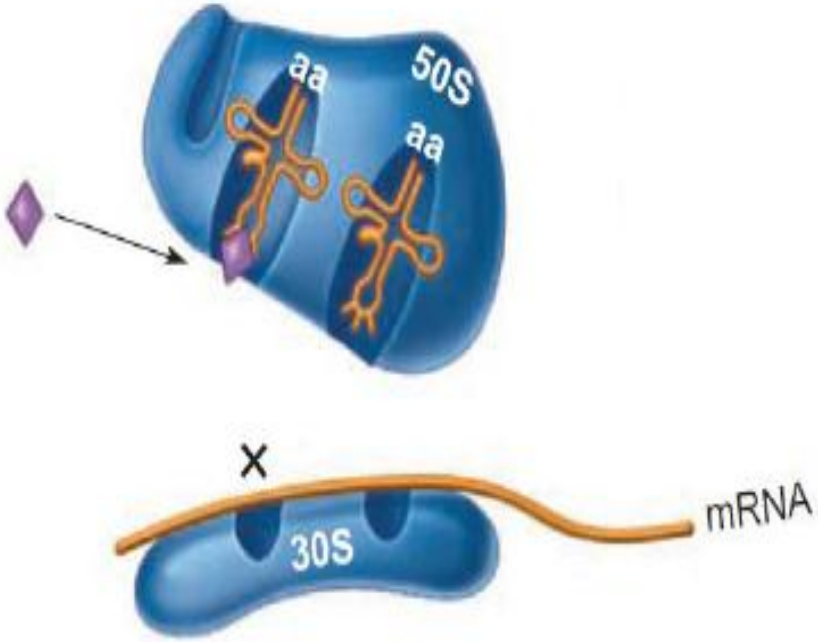
Antibióticos que inhiben la síntesis de proteínas

Inhibición del ensamblado de los Ribosomas

2-Oxazolidona: Estructura y Mecanismo de acción



Linezolida

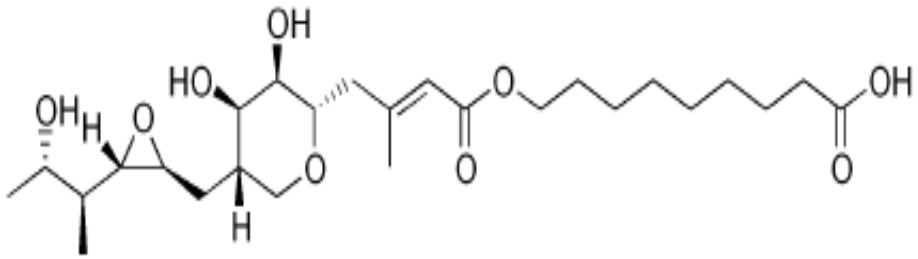


Previenen la iniciación y bloquean el ensablado del ribosoma

Antibióticos que inhiben la síntesis de proteínas

Inhibición de aminoacil-tRNA sintetasas

Mupirocina: Estructura y Mecanismo de acción



Se une a la isoleucil-tRNA sintetasa del microorganismo, de manera que impide la incorporación de la isoleucina a las proteínas.

Bacteriostático a bajas concentraciones y bactericida a altas concentraciones.

Inhibición de la síntesis de ácidos nucleicos

Antibióticos que inhiben la síntesis de ácidos nucleicos

- Inhibición de la DNA girasa

  Quinolonas

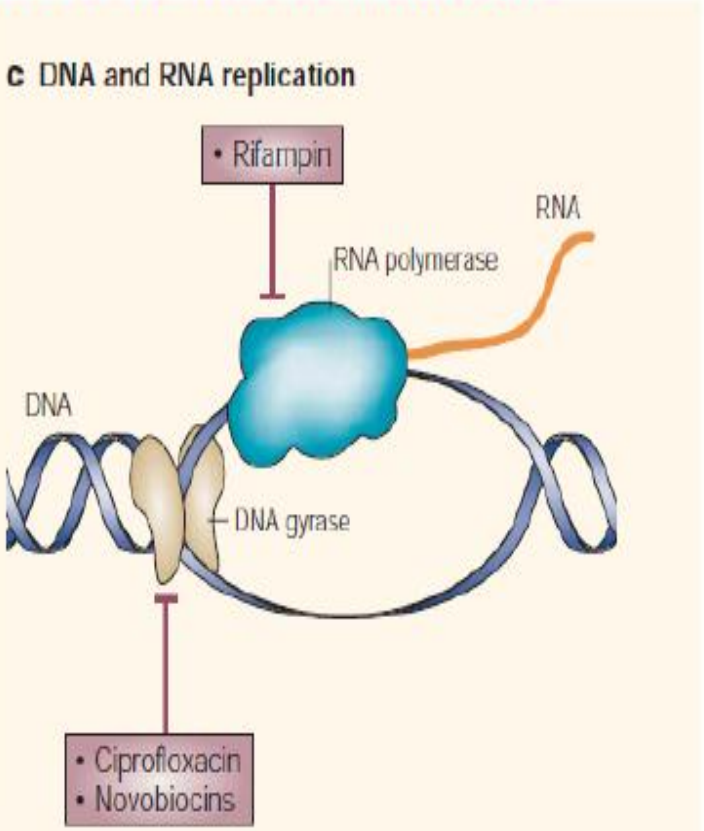
- Inhibición de la RNA polimerasa

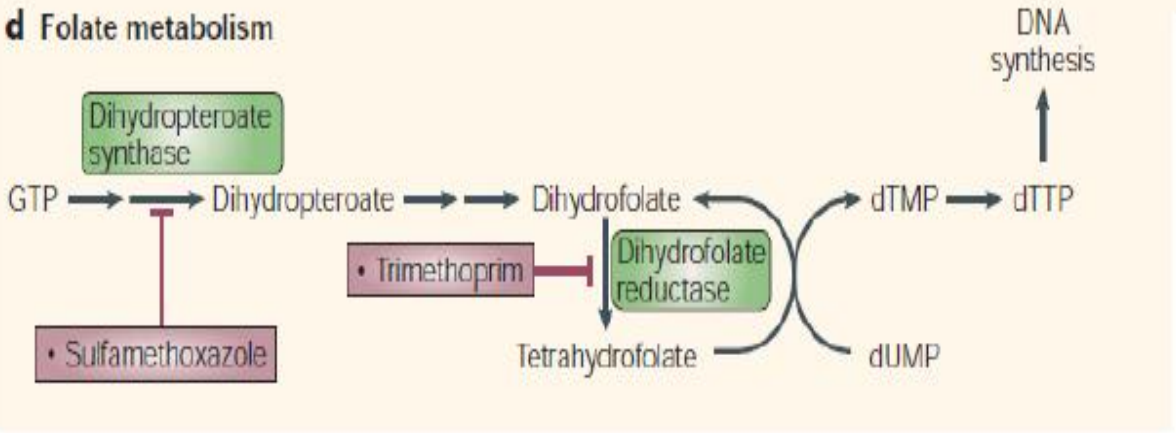
  Rifampicina

- Inhibición de la síntesis de precursores

  Sulfamidas

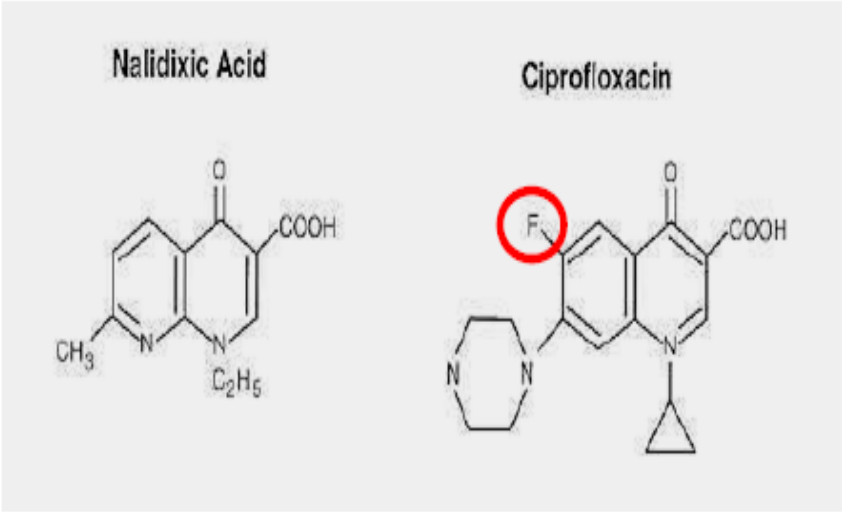
  Trimetoprima





Antibióticos que inhiben la síntesis de ácidos nucleicos

Quinolonas



Interfieren la síntesis de ADN, bloquean la reacción de superenrollamiento dependiente de ATP catalizada por la girasa.

En altas concentraciones pueden inhibir la Topoisomerasa II (enzima que presenta homología con la girasa).

En bacterias G(+) actúan sobre Topoisomerasa IV

No afectan la estructura de cromosomas humanos.

Tienen un efecto bactericida

Antibióticos que inhiben la síntesis de ácidos nucleicos

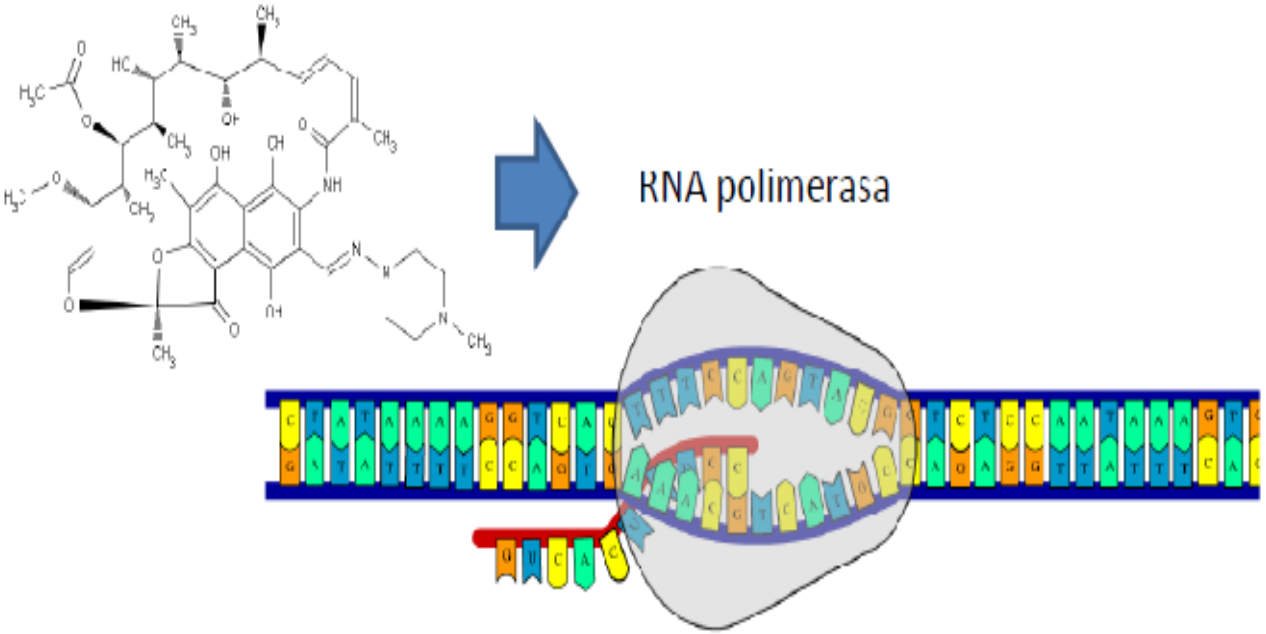
Rifampicina:

Es un antibiótico semisintético derivado de *Amycolatopsis rifamycinica* (previamente conocido como *Streptomyces mediterranei*).

Se une de modo no covalente a la subunidad ß de la ARN polimerasa RNA polimerasa bloqueando la síntesis del mRNA.

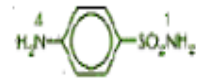
Es útil en el tratamiento de la Tuberculosis, en combinación con drogas antituberculosas, como Isoniazida (inhibe la síntesis de lípidos de *Mycobacterium tuberculosis*) y Etambutol.

También se usa en combinación con esta drogas para el tratamiento de la Lepra.

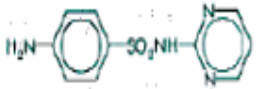


Antibióticos que inhiben la síntesis de ácidos nucleicos

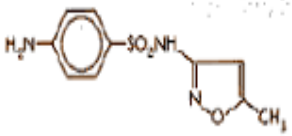
Sulfamidas



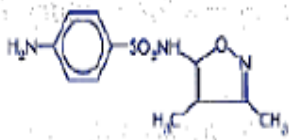
SULFANILAMIDE



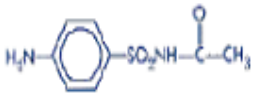
SULFADIAZINE



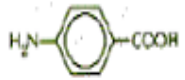
SULFAMETHOXAZOLE



SULFISOXAZOLE



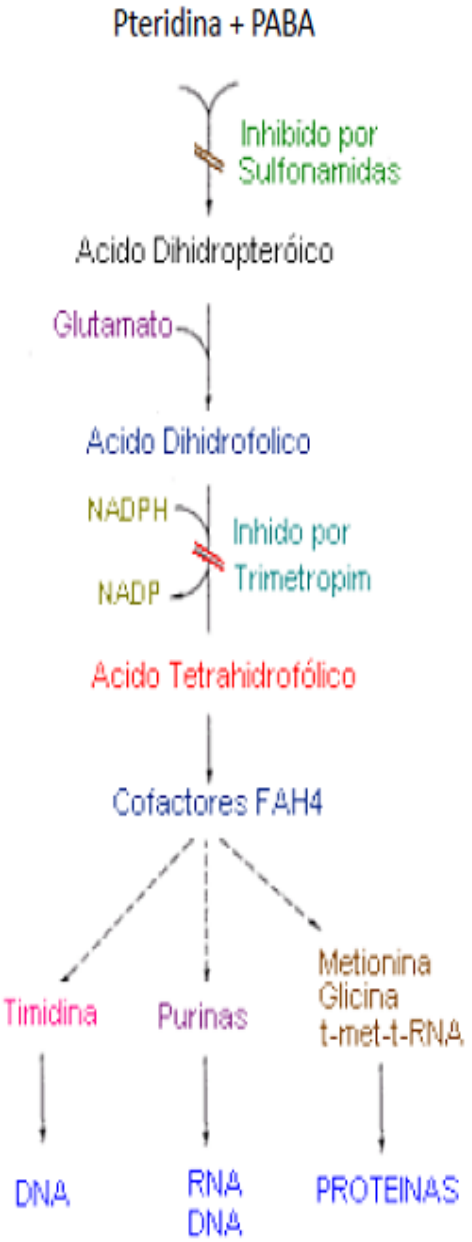
SULFACETAMIDE



PARA-AMINOBENZOIC ACID

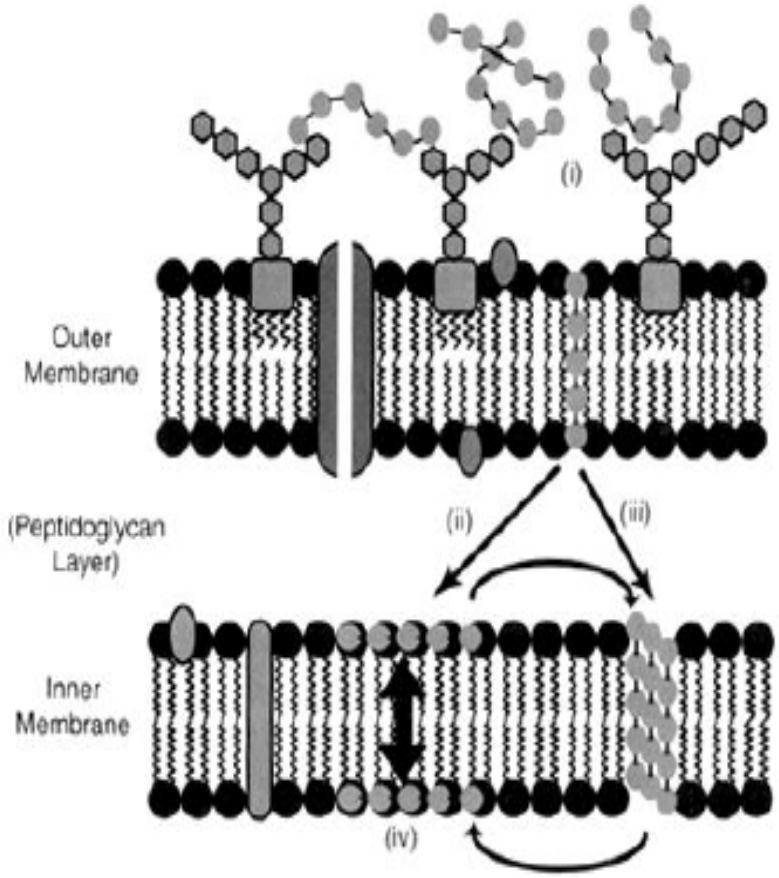
Análogos del PABA, antagonistas competitivos en la síntesis del ácido fólico bacteriano.

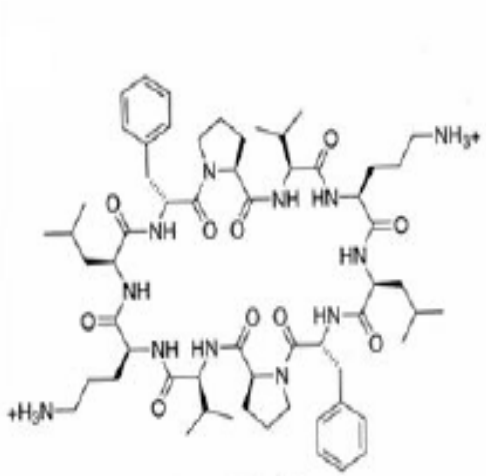
También inhiben la dihidropteroato sintetasa, necesaria para la incorporación del PABA al ácido dihidropteroico (precursor del ácido fólico).

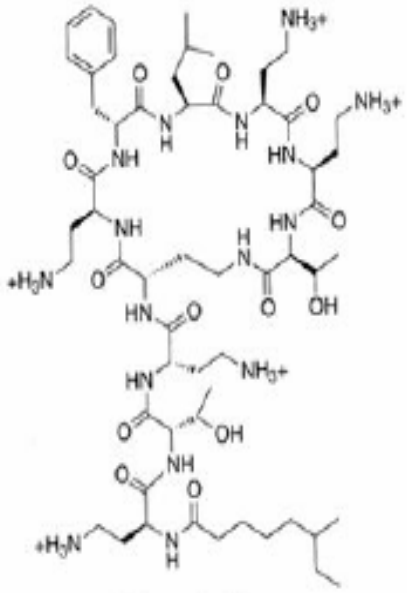


Antibióticos que interfieren con la membrana celular

Polimixina B: Péptidos catiónicos que se insertan en la membrana. ATB se une al LPS o a las cargas negativas de la membrana, desorganizando la MC y generando permeabilidad de la misma y despolarización de la membrana. Relativamente tóxicos, poca utilidad terapéutica.

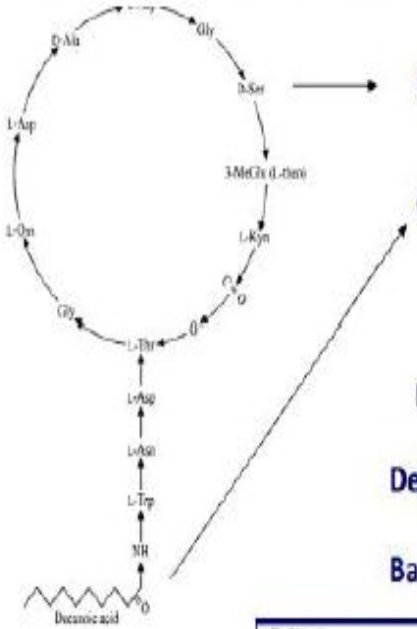






Antibióticos que interfieren con la membrana celular

Daptomicina: Antibiótico lipopéptido cíclico, producido por *Streptomyces roseosporus*. Es bactericida por unión a la membrana celular de Gram (+). En presencia de Ca^{+2} produce una rápida despolarización de la membrana y muerte bacteriana, sin lisis celular.



Cadena cíclica de 13 péptidos

Cadena lateral altamente lipofílica (Acido decanoico)

↓

Se inserta en la membrana plasmática e induce la salida de K+

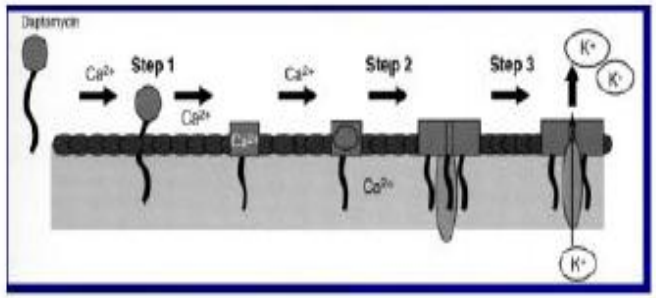
↓

Depolarización de la membrana

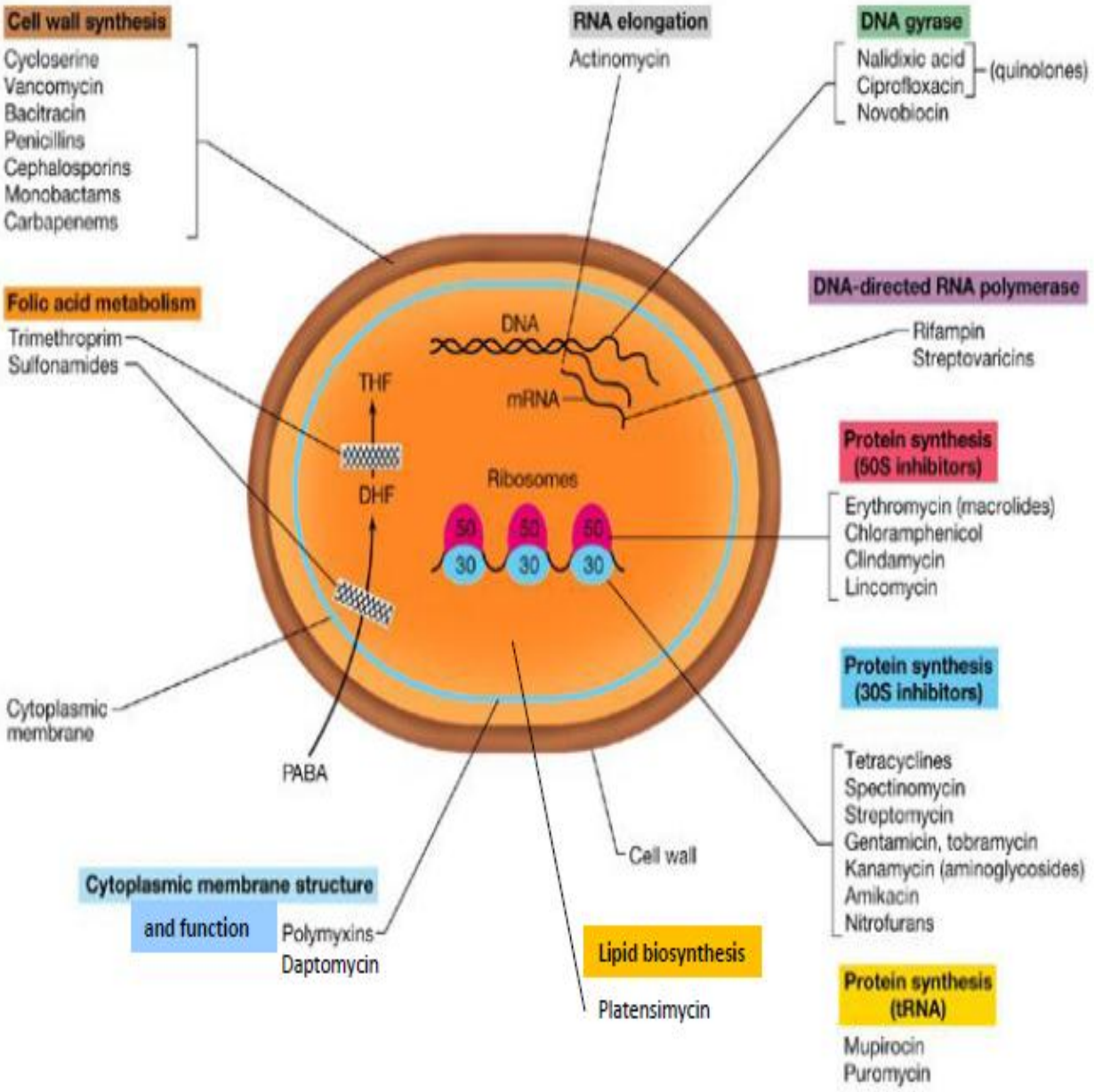
↓

Bacteria no puede sintetiza ATP

Requiere formar un complejo con Ca++



Sitios blanco de acción de los antimicrobianos

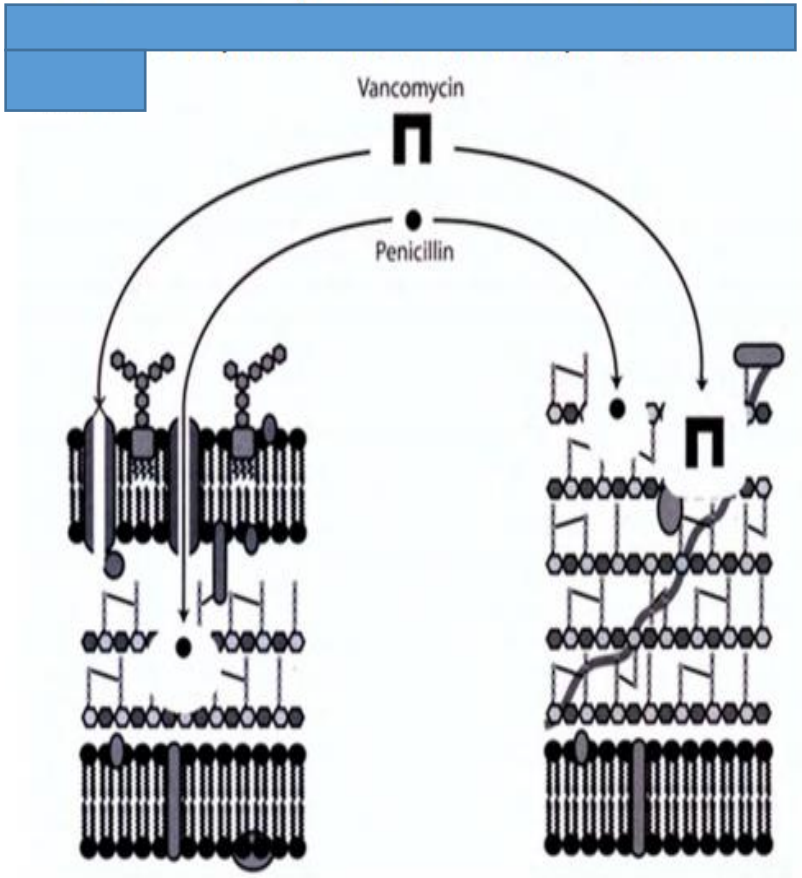


Resistencia microbiana a los antibióticos

Perdida de sensibilidad de un microorganismo a un ATB

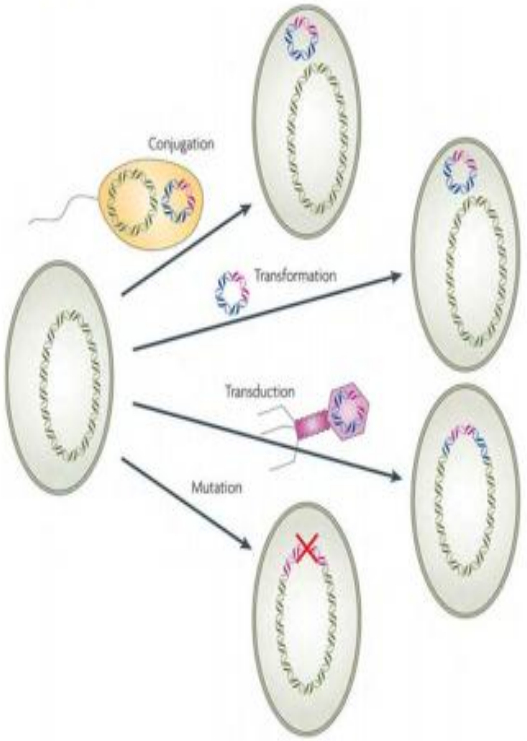
Intrínseca

- La bacteria no tiene la molécula/reacción enzimática que es el blanco del antibiótico
- Diferencias en la permeabilidad



Adquirida

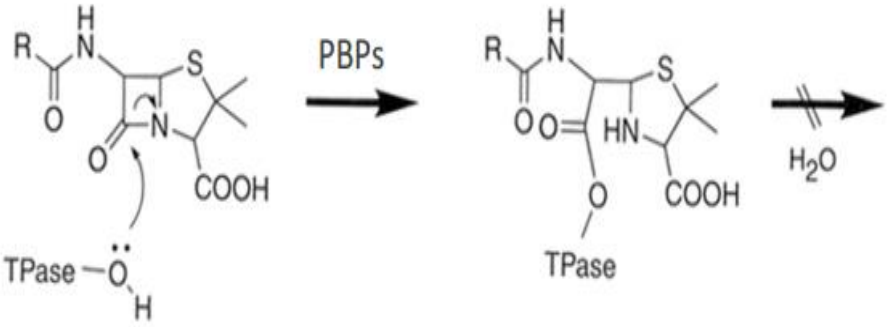
- Adquisición de genes por transferencia horizontal:
  - Conjugación
  - Transformación
  - Transducción
  - Mutaciones



Destrucción o modificación de la droga

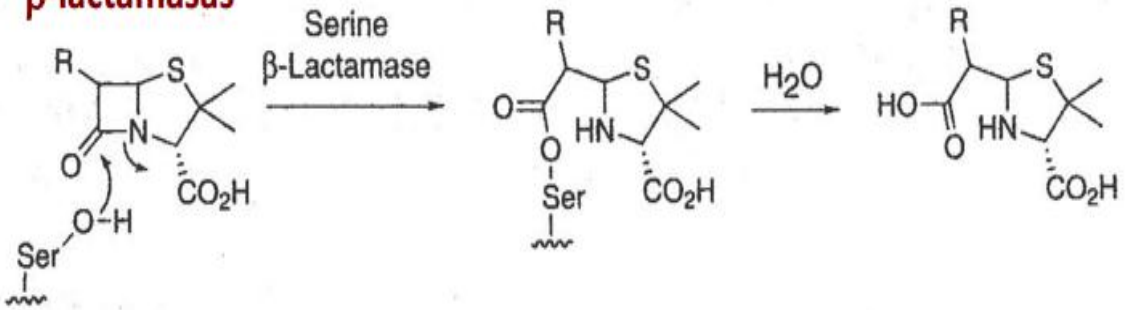
Destrucción de la droga

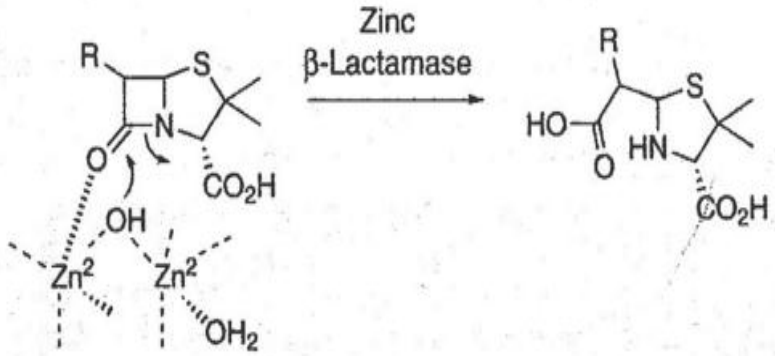
Transpeptidasas



Inhibición de la reacción de transpeptidación y del entrecruzamiento del peptidoglicano
Activan el mecanismo autolítico endógeno bacteriano

β-lactamasas





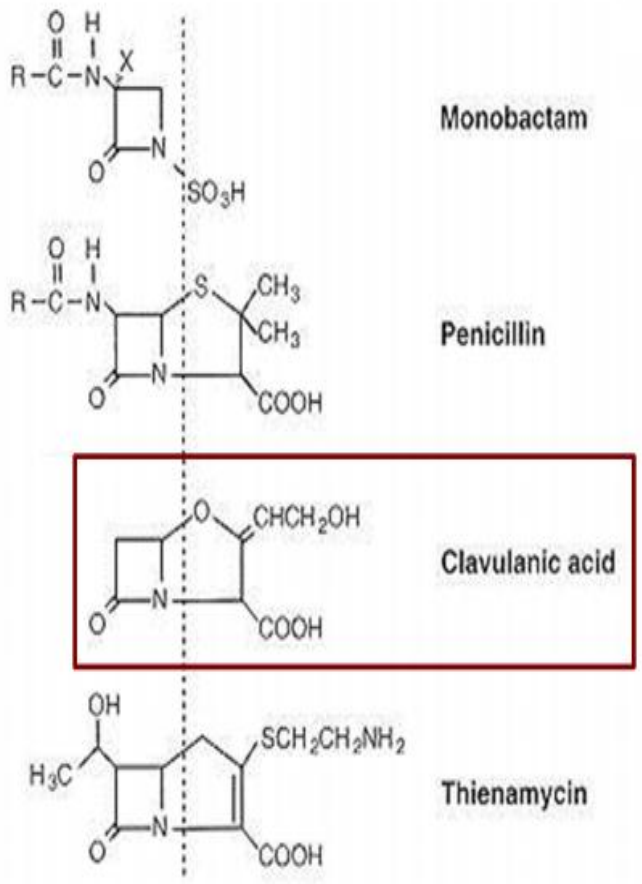
Existe enzimas de diferentes tipo:

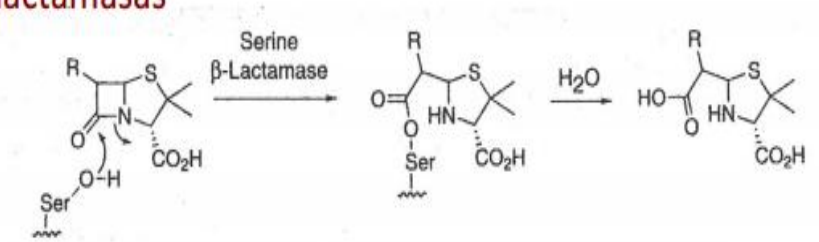
- Serin-β-lactamasas (Tipo A, C y D)
- Metalo-β-lactamasas (Zn^{+2})
- Periplasmicas o unidas a membrana interna (Gram -) o extracelulares (Gram +)
- Constitutivas o inducibles

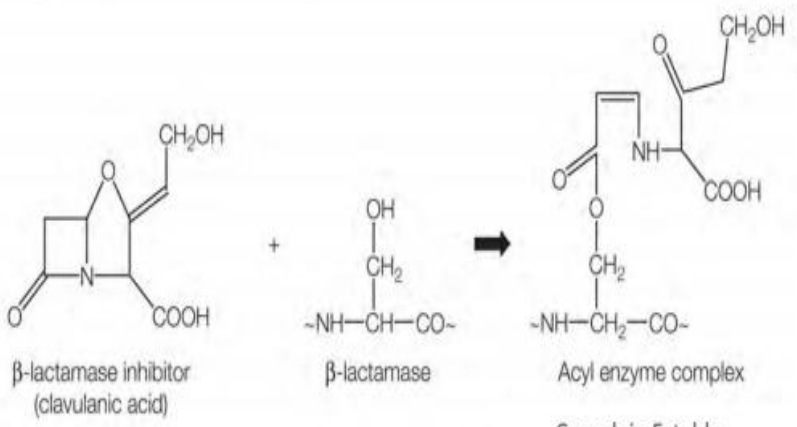
Estrategias para neutralizar las β-lactamasas

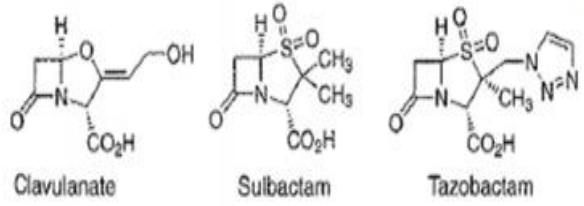
Desarrollo de β-lactámicos semisintéticos que sean hidrolizados lentamente (ej: carbapenem, monobactam)

Inhibidores (competitivos) de β-lactamasas









Clavulanate-Amoxicillin → Augmentin
Clavulanate-Ticarcillin → Timentin
Sulbactam-Ampicillin → Unasyn
Tazobactam-Piperacillin → Zocin

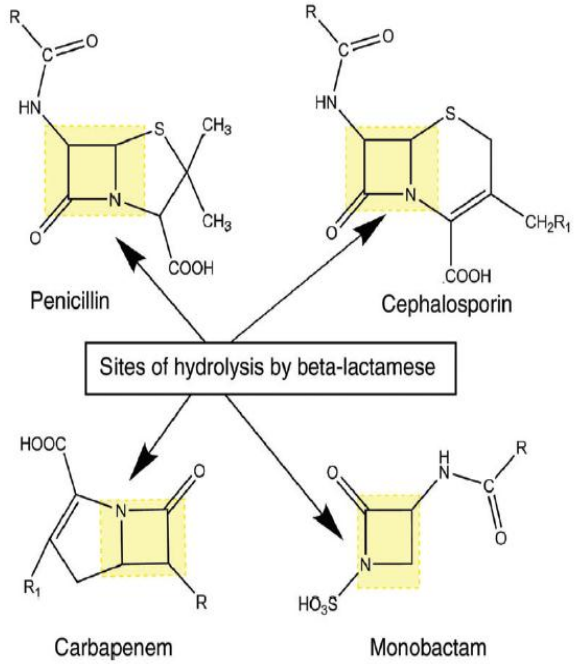


Figure 3. The sites of hydrolysis by beta-lactamase of four important beta-lactam groups

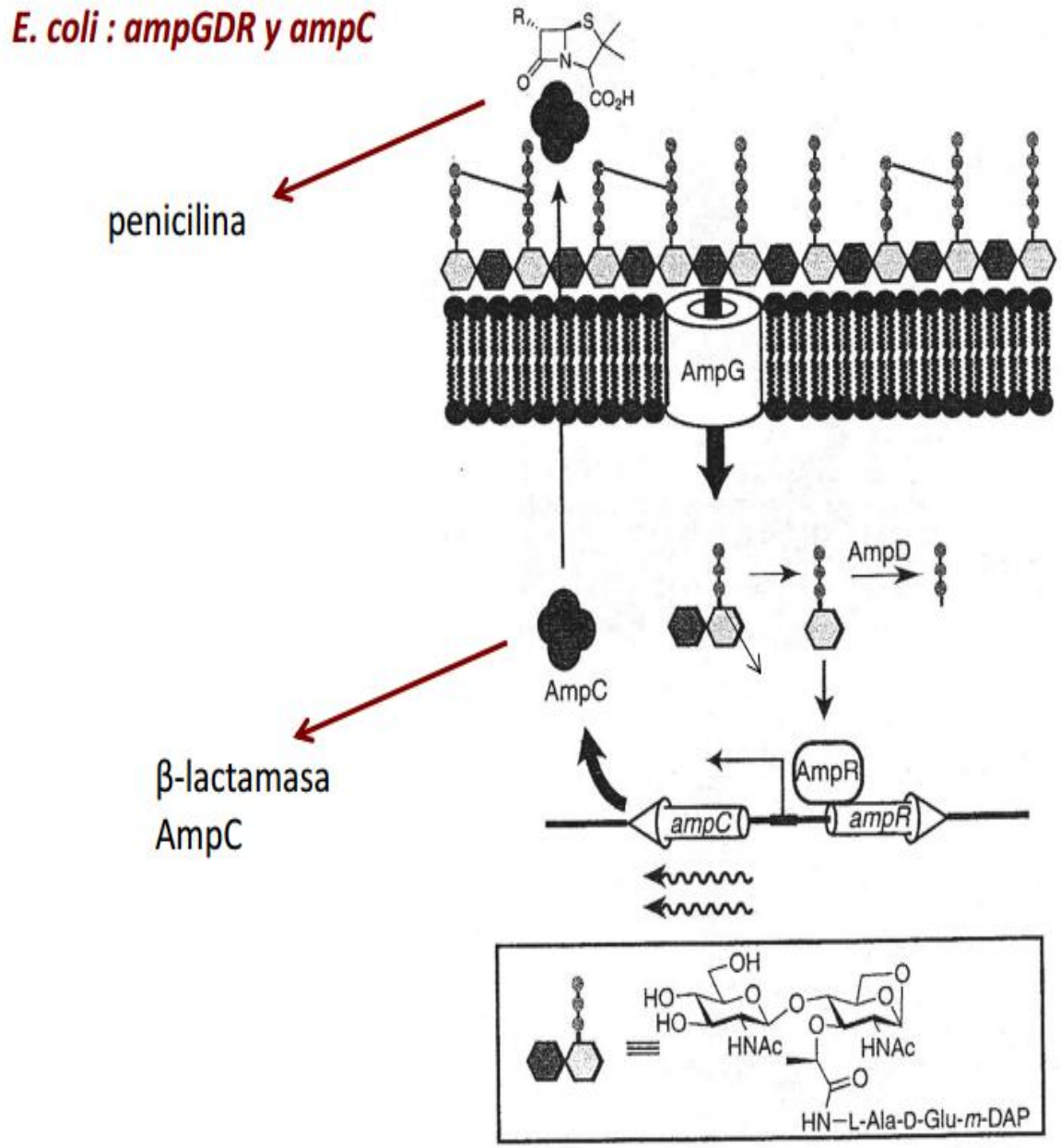
Group	Molecular class*	Characteristic	Types that are mediated by MGEs in *Pseudomonas aeruginosa*
1	C	Serine at active site Inducible enzymes Overproduction due to mutation Hydrolysis of cephalosporins Resistance to inactivation by clavulanate and tazobactam	CMY
2	A and D	Serine at active site Broad spectrum penicillinase Inactivation by clavulanate and tazobactam	TEM, SHV, CTX-M, PER, VEB, GES, PSE, KPC, OXA
3	B	Metallo-beta-lactamase Require Zn for activation Lack of inhibition by tazobactam and clavulanate Effective against all beta-lactams except monobactam Inhibition by EDTA	IMP, VIM, GIM, and SMP

*Ambler classification on the basis of amino acid sequences[99].
CMY: active on cephamycins, CTX-M: active on cefotaxime, first isolated at Munich, GES: Guiana-extended spectrum, GIM: German imipenemase, IMP: active on imipenem, KPC: *Kleibsiella pneumoniae* carbapenemase, MGE: mobile genetic elements, OXA: active on oxacillin, PER: *Pseudomonas* extended resistant, PSE: *Pseudomonas*-specific enzyme, SHV: sulfhydryl reagent variable, SMP: Sao Paulo metallo-β-lactamase, TEM: named after the patient (Temoneira), VEB: Vietnam extended-spectrum β-lactamase, VIM: Verona integron-encoded metallo-β-lactamase.

Table 3. Beta-lactamase classification[93]

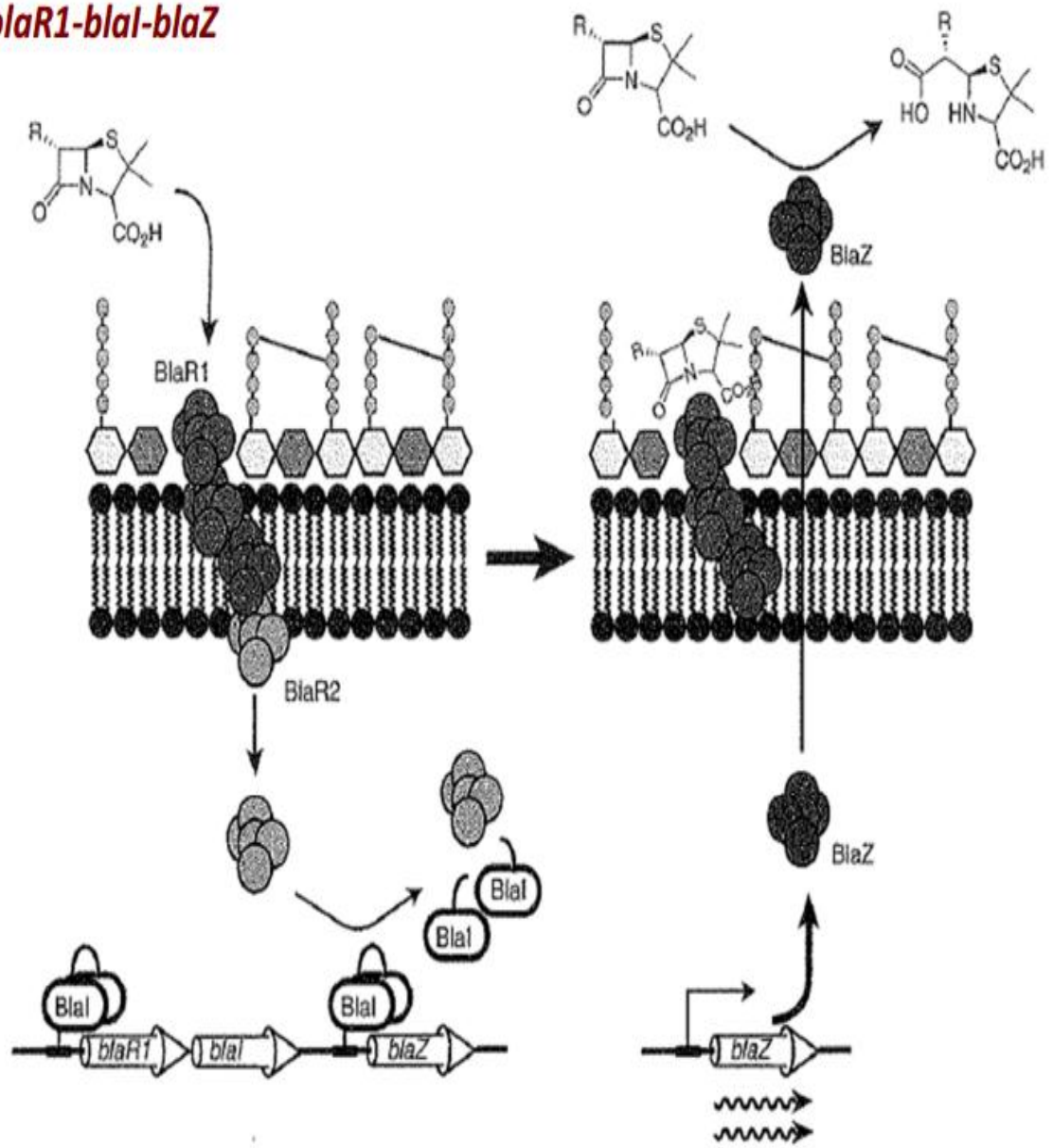
Regulación de la expresión de los genes de las β-lactamasas

E. coli : ampGDR y ampC

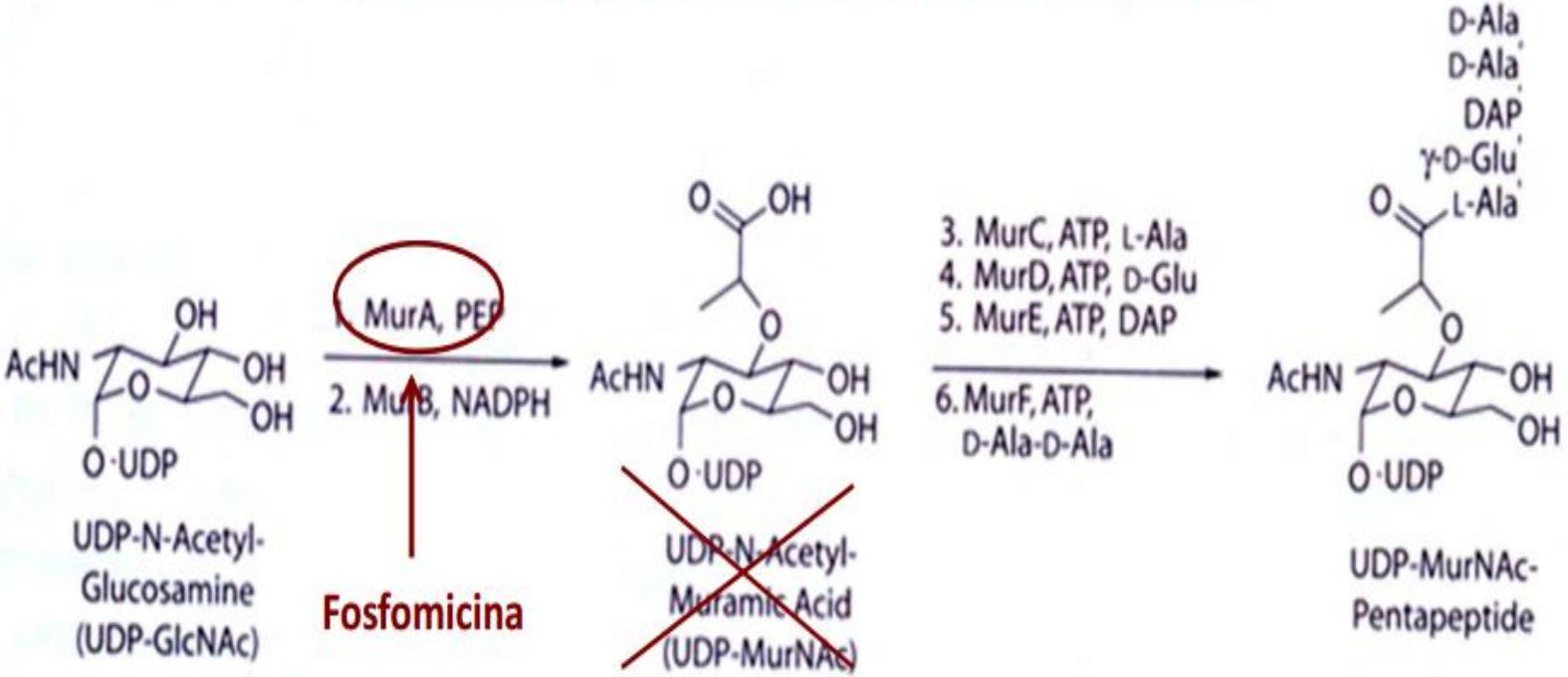


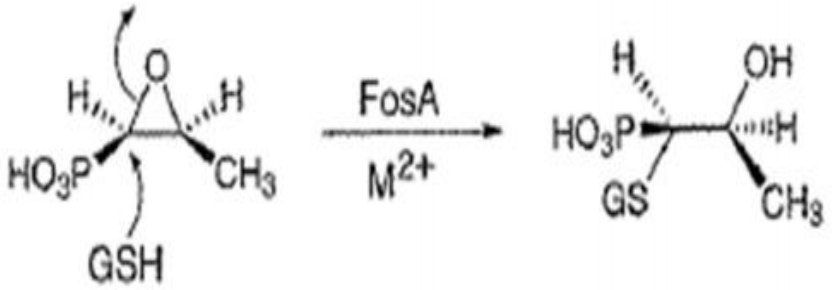
Regulación de la expresión de los genes de las β-lactamasas

S. aureus : blaR1-blaI-blaZ



Inactivación enzimática de la fosfomicina

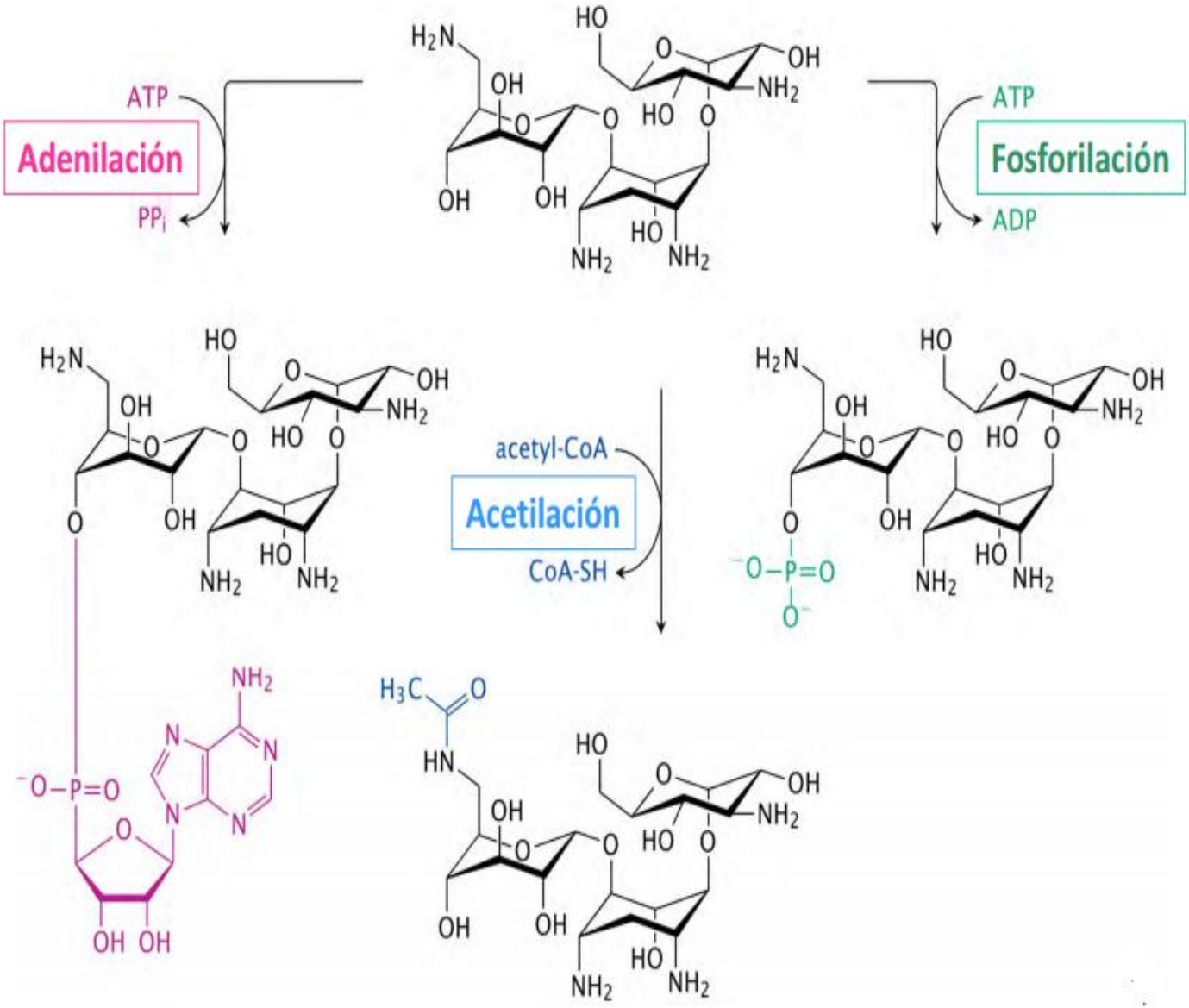




FosA: fosfomicin-glutatión S-transferasa

Modificación de la droga

Modificaciones químicas de aminoglicosidos: **Kanamicina**



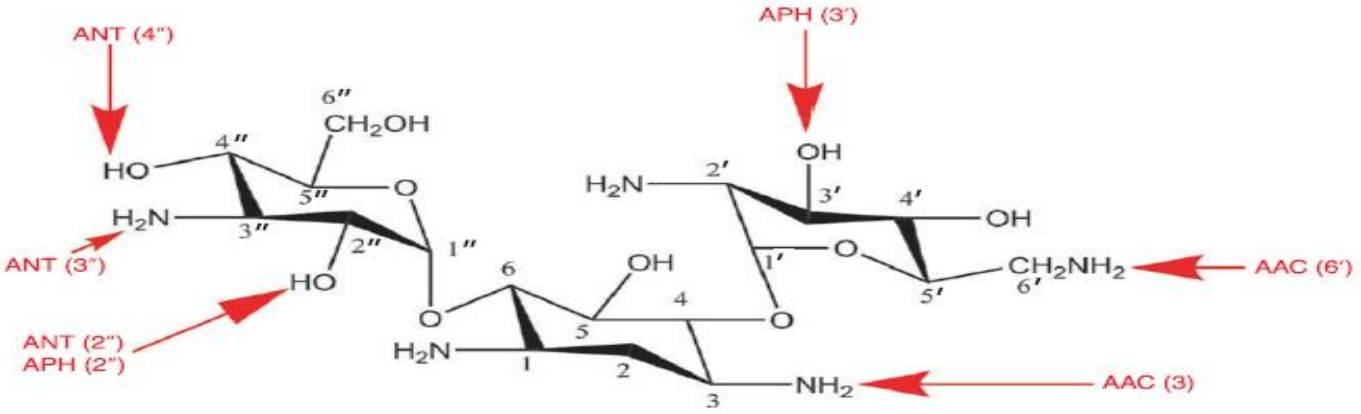


Figure 4. General structure of aminoglycoside and sites of action by aminoglycoside modifying enzymes. AAC: aminoglycoside acetyltransferase, ANT: aminoglycoside nucleotidyltransferase, APH: aminoglycoside phosphoryltransferase.

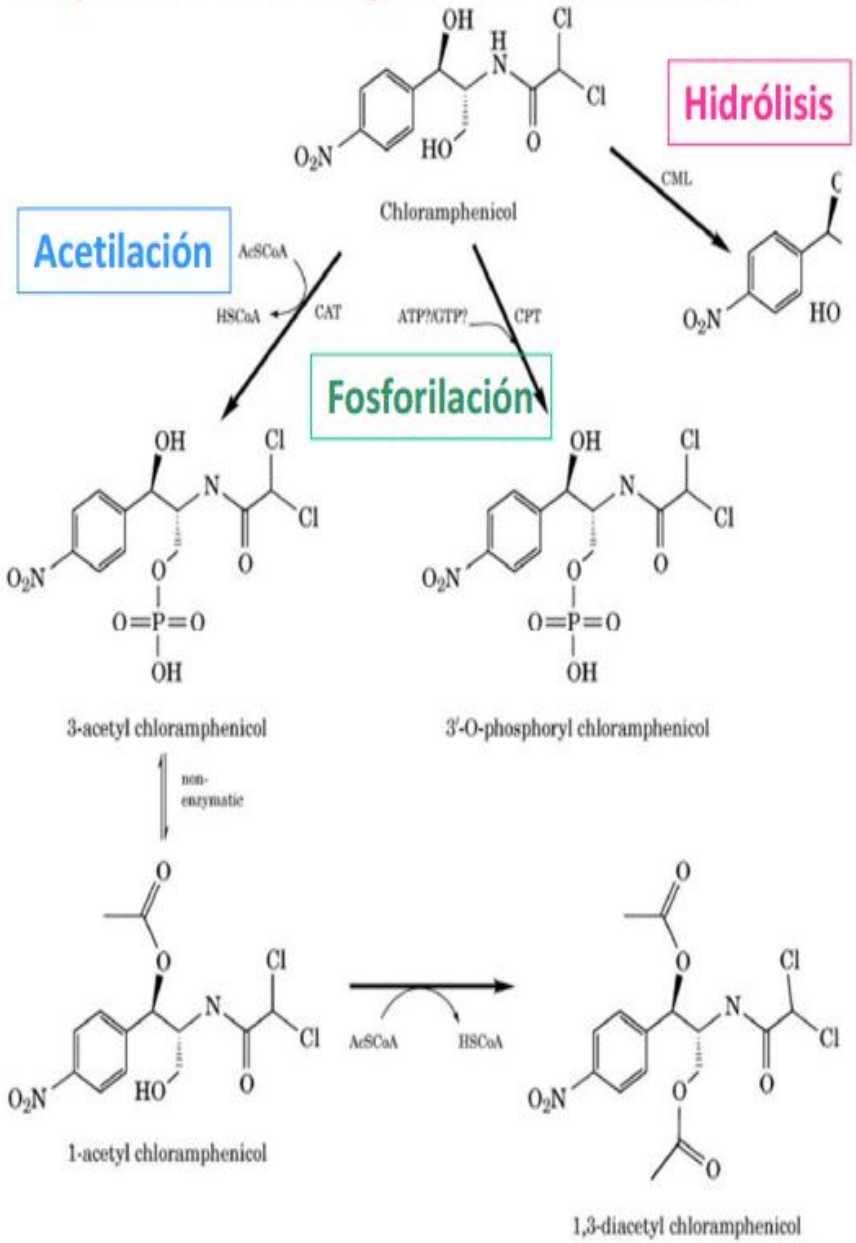
AMEs	Type (variants)	Resistant to
Aminoglycoside acetyltransferase (AAC)	AAC(6′)-I	Tobramycin, netilmicin, kanamycin, amikacin
	AAC(6′)-II*	Tobramycin, netilmicin, kanamycin, gentamicin
	AAC(3)-I [Ia,Ib,Ic]	Gentamicin
	AAC(3)-II	Gentamicin, tobramycin, netilmicin
	AAC(3)-III	Gentamicin, tobramycin
	AAC(3)-IV	Gentamicin, tobramycin, netilmicin
Aminoglycoside nucleotidyltransferase (ANT)	ANT(2″)-I†	Gentamicin, tobramycin
	ANT(3″)-I	Tobramycin, netilmicin, amikacin
	ANT(4″)-II [IIa, IIb]	Amikacin, tobramycin, isepamicin
Aminoglycoside phosphoryltransferase (APH)	APH(2″)-I	Gentamicin, tobramycin, amikacin
	APH(3′)-I	Kanamycin, neomycin
	APH(3′)-II	Kanamycin, neomycin, gentamicin
	APH(3′)-IV	Kanamycin, neomycin, amikacin

*Most common AAC of *P. aeruginosa*.
†Most common ANT in *P. aeruginosa*.

Table 4. Enzymes frequently reported in ***Pseudomonas aeruginosa*** responsible for aminoglycosides resistance

Modificación de la droga

Modificaciones químicas de aminoglicósidos: **Cloranfenicol**

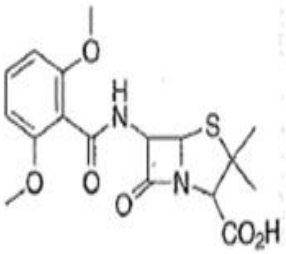


Modificación del blanco

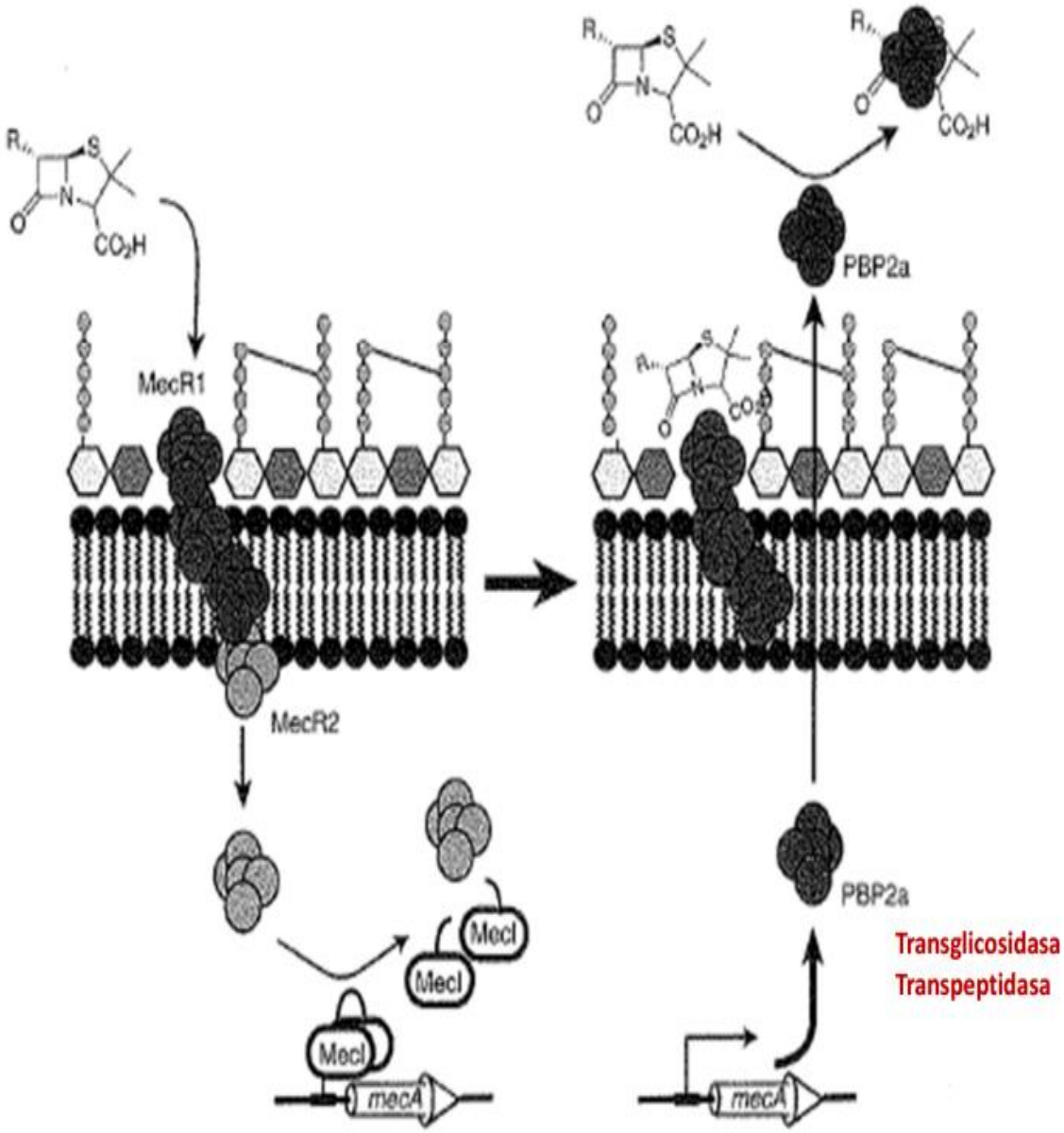
Reemplazo del sitio blanco

Resistencia a β-lactámicos: Síntesis de PBP2a insensible a β-lactámicos

***S. aureus* MRSA**

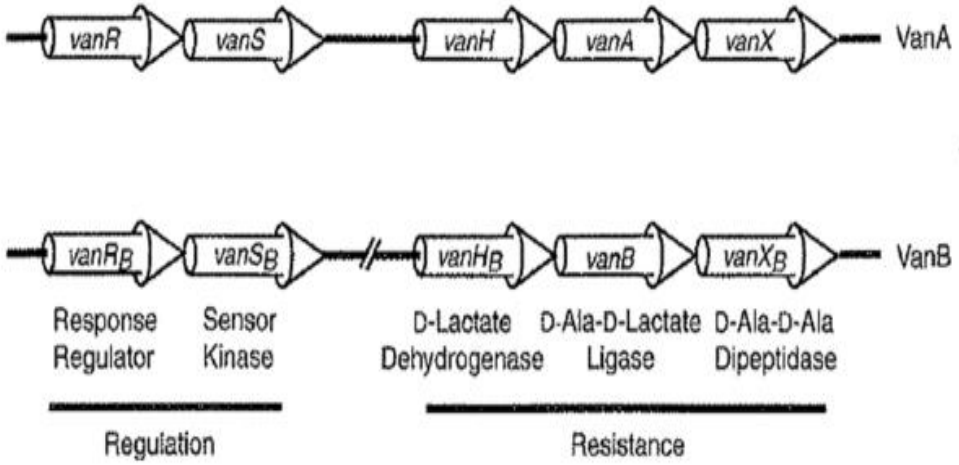


Methicillin



Modificación del sitio blanco

Resistencia a Vancomicina: *vanR, vanS, vanH, vanA,, vanX, vanY* y *vanZ*



VanS: Proteina sensora

VanR: Activador transcripcional

VanH: Piruvato deshidrogenasa

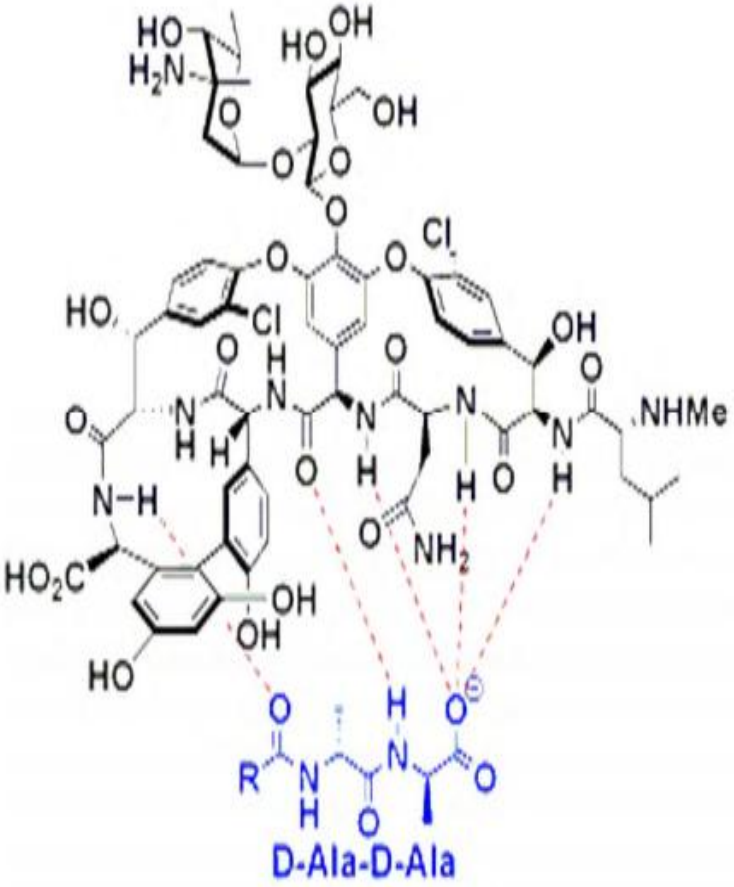
VanA: Ligasa A-ala-X

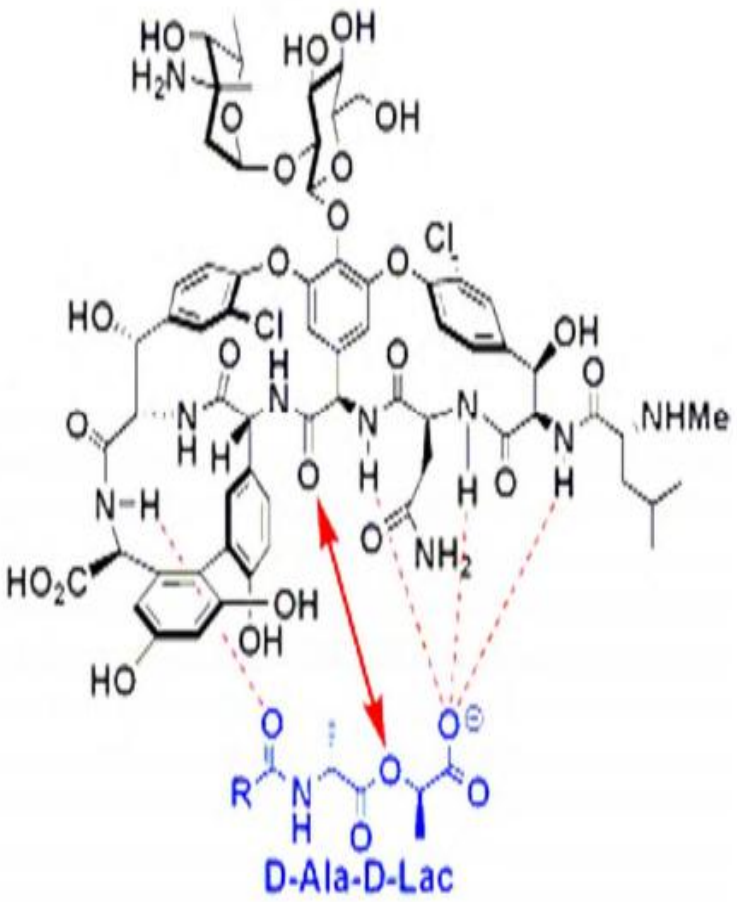
VanX: D-ala-D-ala dipeptidasa

VanY: D-D Carboxipeptidasa

Modificación del sitio blanco

Resistencia a Vancomicina por modificación de D-Ala por D-Lac





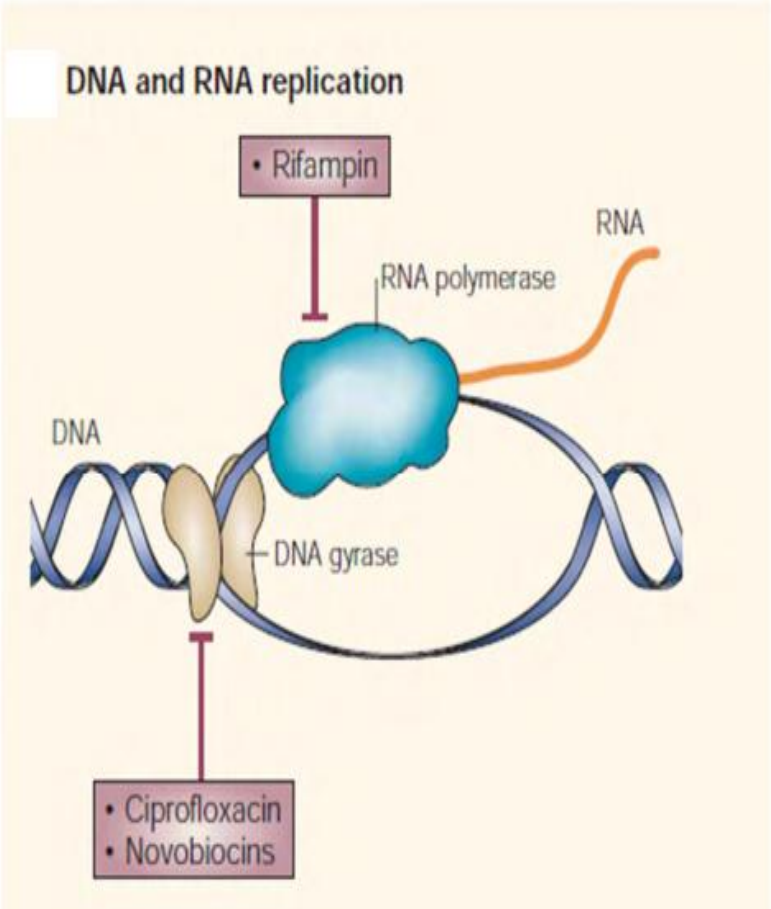
Modificación del sitio blanco

Resistencia a Rifampicina:

Mutaciones puntuales de subunidad β RNA Pol

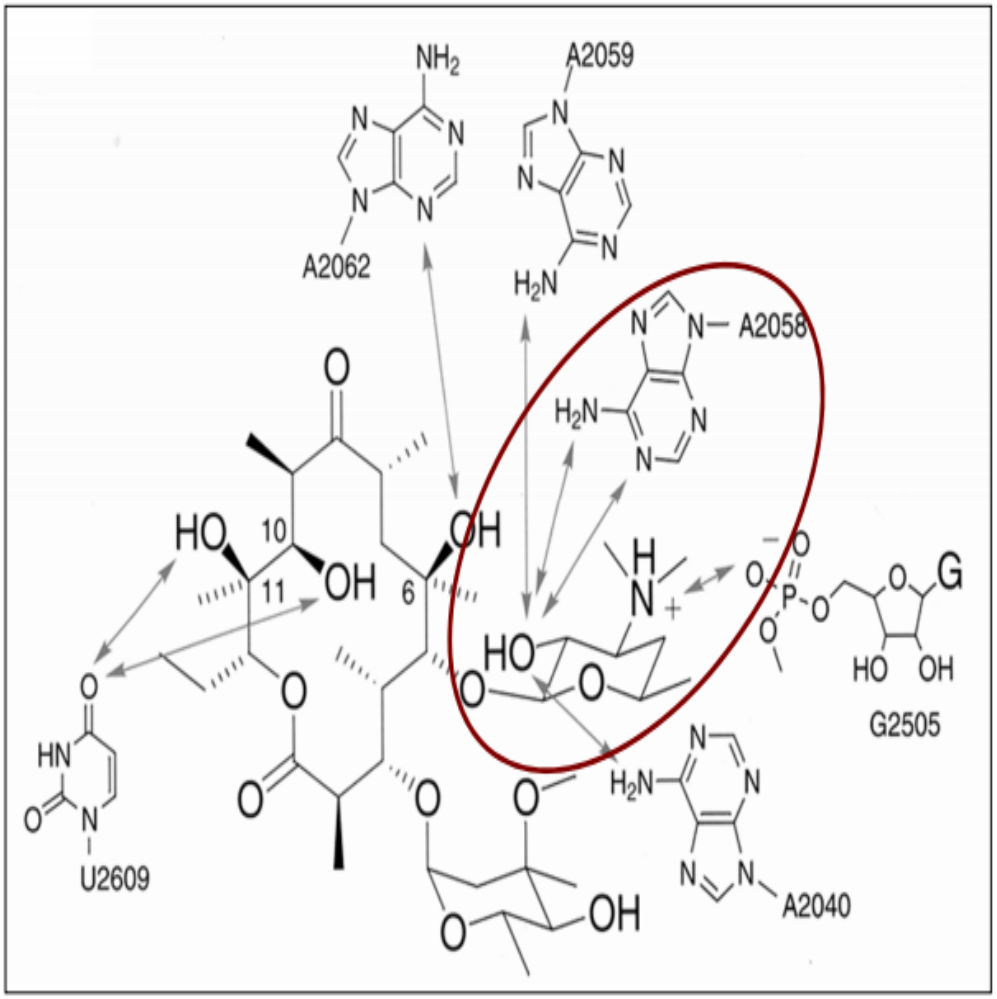
Resistencia a Quinolonas:

Mutaciones puntuales de subunidad A de DNA Girasa



Modificación del sitio blanco

Unión de **Macrólidos** al ribosoma



Dimetilación de residuo Adenina rRNA 23S

Mutaciones puntuales de rRNA o proteínas ribosomales

Future MICROBIOLOGY

REVIEW

For reprint orders, please contact: reprints@futuremedicine.com

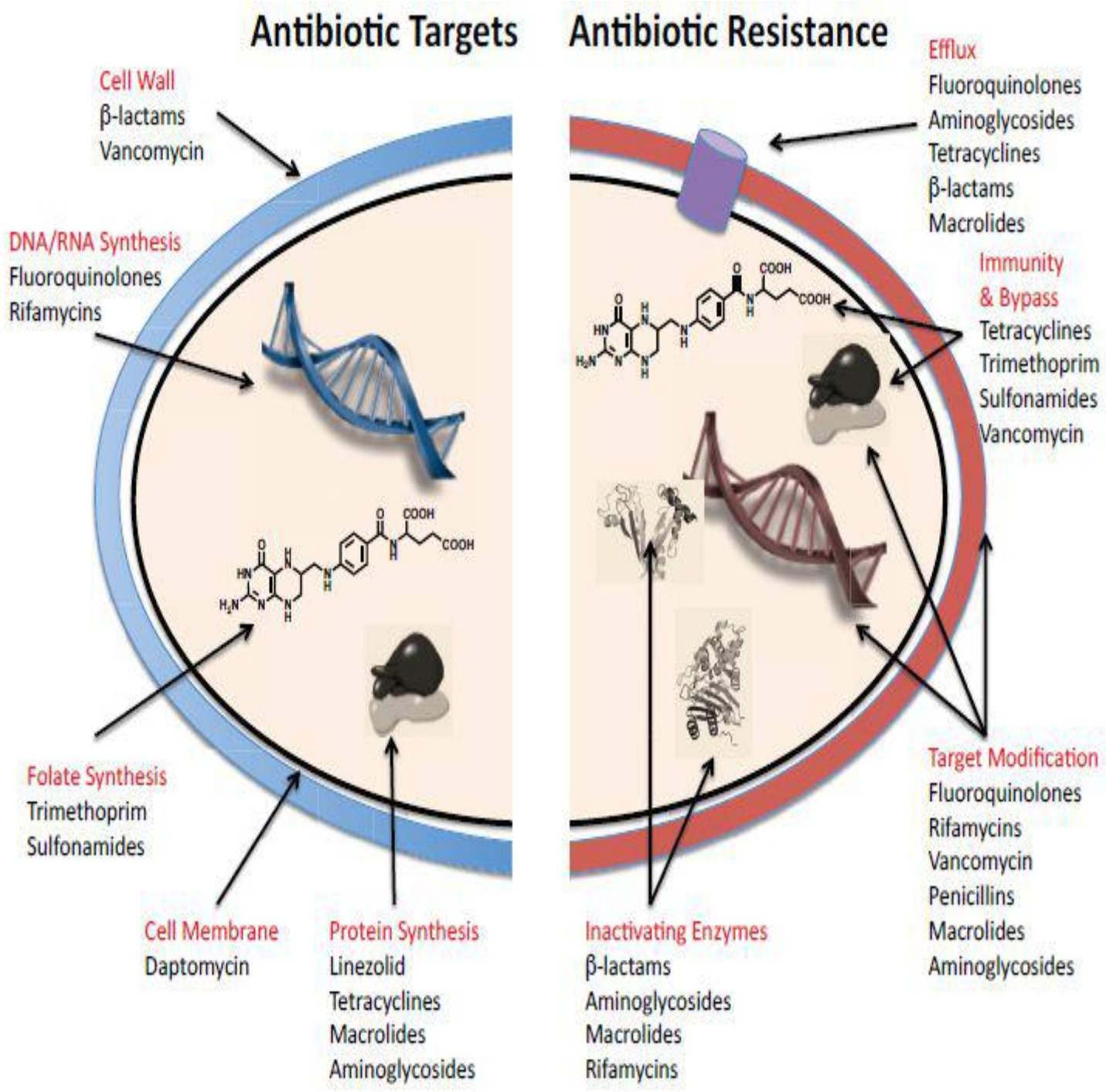
Multidrug efflux pumps in Gram-negative bacteria and their role in antibiotic resistance

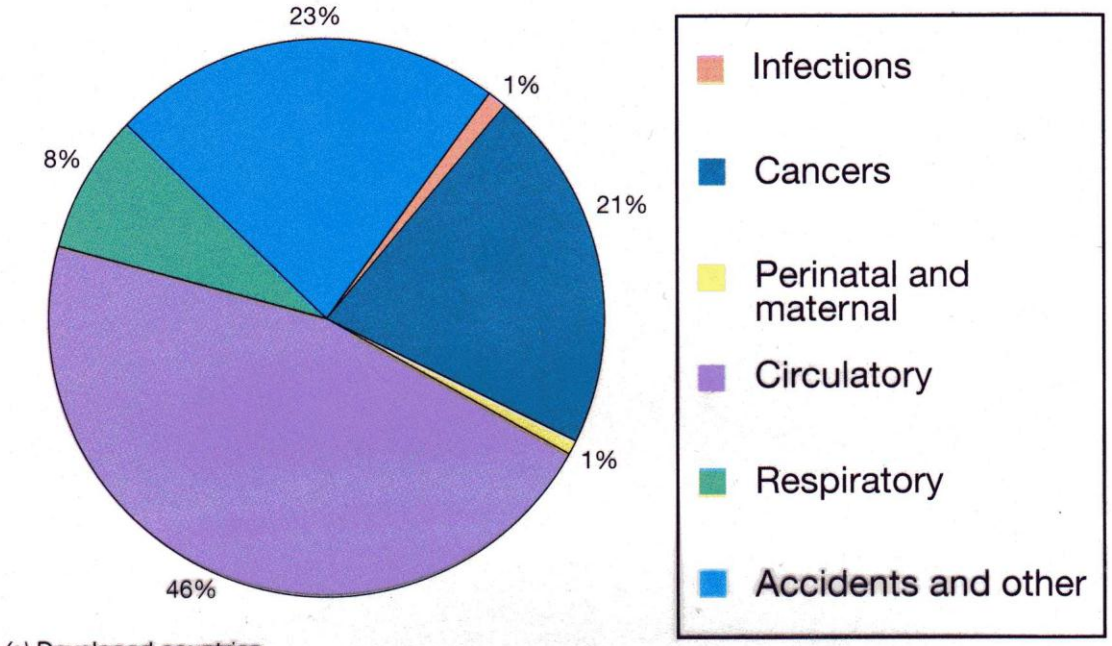
Jessica MA Blair[1], Grace E Richmond[1] & Laura JV Piddock*[,1]

ABSTRACT Gram-negative bacteria express a plethora of efflux pumps that are capable of transporting structurally varied molecules, including antibiotics, out of the bacterial cell. This efflux lowers the intracellular antibiotic concentration, allowing bacteria to survive at higher antibiotic concentrations. Overexpression of some efflux pumps can cause clinically relevant levels of antibiotic resistance in Gram-negative pathogens. This review discusses the role of efflux in resistance of clinical isolates of Gram-negative bacteria, the regulatory mechanisms that control efflux pump expression, the recent advances in our understanding of efflux pump structure and how inhibition of efflux is a promising future strategy for tackling multidrug resistance in Gram-negative pathogens.

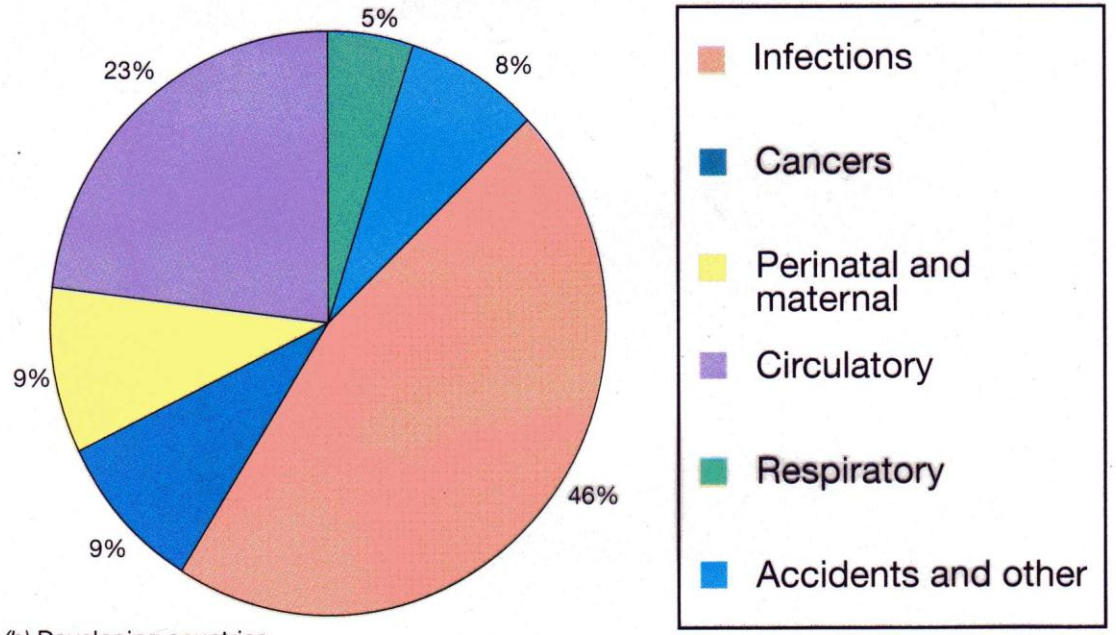
Table 1. Examples of clinically relevant efflux pumps in Gram-negative bacteria.

Organism	Efflux pump	Substrates	Ref.
Escherichia coli	AcrAB-TolC	Aromatic hydrocarbons, benzalkonium, β-lactams, novobiocin, erythromycin, fusaric acid, fluoroquinolones, tetracycline, chloramphenicol, ethidium bromide, acriflavine, crystal violet, SDS, Triton X-100, bile salts, triclosan, fatty acids, methotrexate, linezolid	[3–5]
Salmonella enterica	AcrAB-TolC	Bile salts, SDS, deoxycholate, acriflavine, fatty acids, novobiocin, erythromycin, chloramphenicol, Triton X-100, crystal violet, rifampicin, tetracycline, cholate, norfloxacin, nalidixic acid, β-lactams, fluoroquinolones	[6–8]
Pseudomonas aeruginosa	MexAB-OprM	Quinolones, macrolides, tetracyclines, lincomycin, chloramphenicol, novobiocin, β-lactams except imipenem	[9–11]
	MexCD-OprJ	Quinolones, macrolides, tetracyclines, lincomycin, chloramphenicol, novobiocin, penicillins except carbenicillin and sulbenicillin, cephems except ceftazidime, flomoxef, meropenem, S-4661	[9–11]
	MexXY-OprM	Quinolones, macrolides, tetracyclines, lincomycin, chloramphenicol, aminoglycosides, penicillins except carbenicillin and sulbenicillin, cephems except cefsulodin and ceftazidime, meropenem, S-4661	[9–11]
Acinetobacter baumannii	AdeABC	Aminoglycosides, cefotaxime, fluoroquinolones, tetracyclines, chloramphenicol, erythromycin and trimethoprim	[12]
Campylobacter jejuni	CmeABC	Fluoroquinolones, erythromycin, β-lactams, rifampicin, tetracycline, ethidium bromide, SDS, deoxycholate, chloramphenicol, gentamicin, acridines	[13,14]
Neisseria gonorrhoeae	MtrCDE	Capric acid, palmitic acid, cholic acid, crystal violet, Triton X-100, erythromycin	[15]

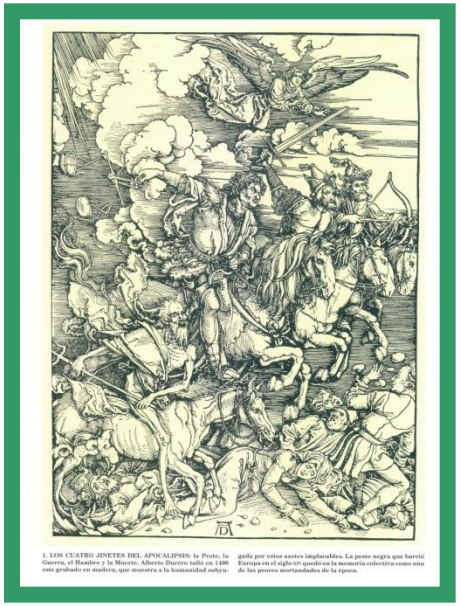

Antibiotic Targets
Antibiotic Resistance
Cell Wall
β-lactams
Vancomycin
DNA/RNA Synthesis
Fluoroquinolones
Rifamycins
Folate Synthesis
Trimethoprim
Sulfonamides
Cell Membrane
Daptomycin
Protein Synthesis
Linezolid
Tetracyclines
Macrolides
Aminoglycosides
Efflux
Fluoroquinolones
Aminoglycosides
Tetracyclines
β-lactams
Macrolides
Immunity
& Bypass
Tetracyclines
Trimethoprim
Sulfonamides
Vancomycin
Target Modification
Fluoroquinolones
Rifamycins
Vancomycin
Penicillins
Macrolides
Aminoglycosides
Inactivating Enzymes
β-lactams
Aminoglycosides
Macrolides
Rifamycins

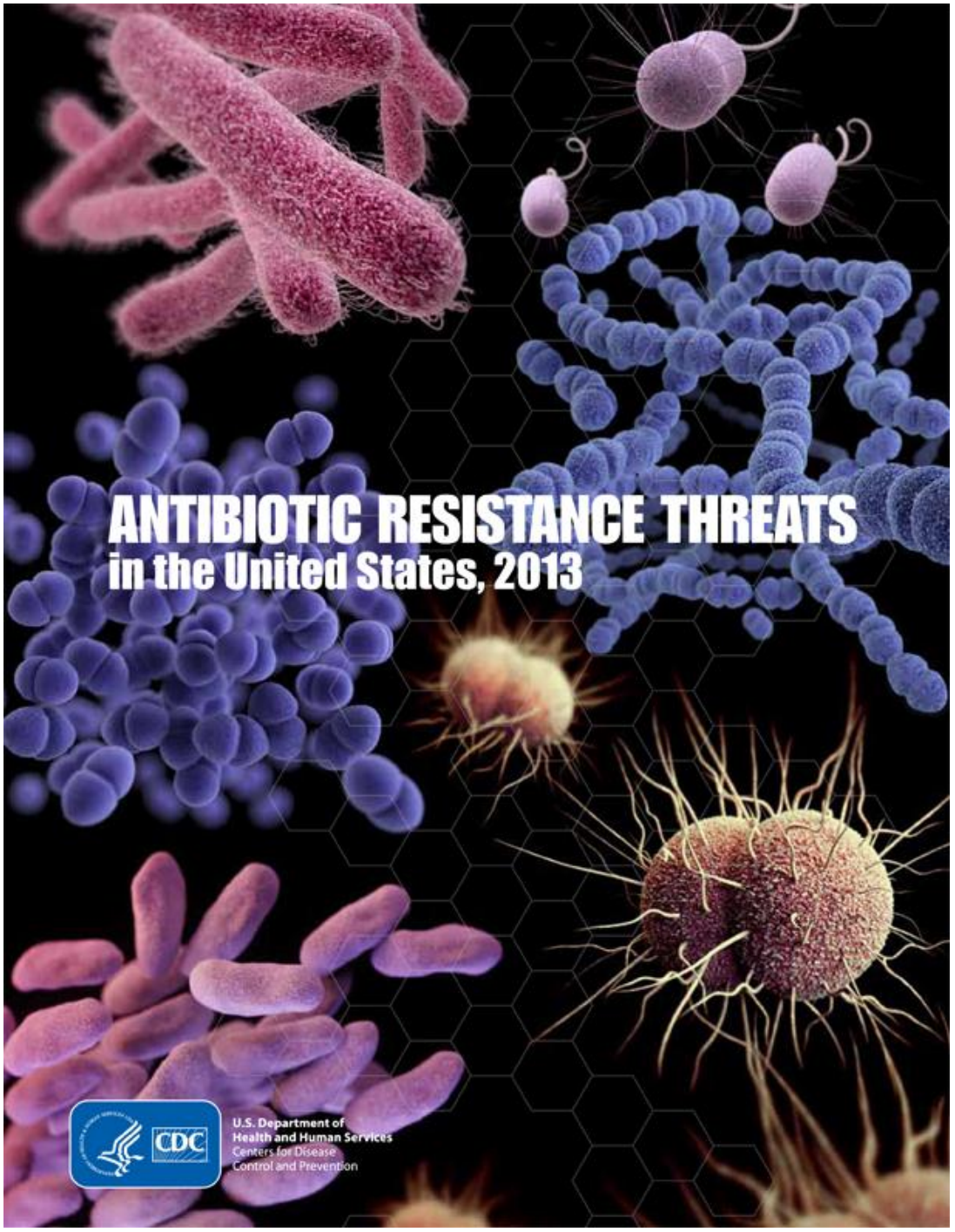


(a) Developed countries



(b) Developing countries




ANTIBIOTIC RESISTANCE THREATS
in the United States, 2013
CDC
U.S. Department of
Health and Human Services
Centers for Disease
Control and Prevention

NATIONAL SUMMARY DATA

Estimated minimum number of illnesses and deaths caused by antibiotic resistance*:

At least **2,049,442** illnesses,

23,000 deaths

**bacteria and fungus included in this report*

+

Estimated minimum number of illnesses and death due to *Clostridium difficile* (*C. difficile*), a unique bacterial infection that, although not significantly resistant to the drugs used to treat it, is directly related to antibiotic use and resistance:

At least **250,000** illnesses,

14,000 deaths

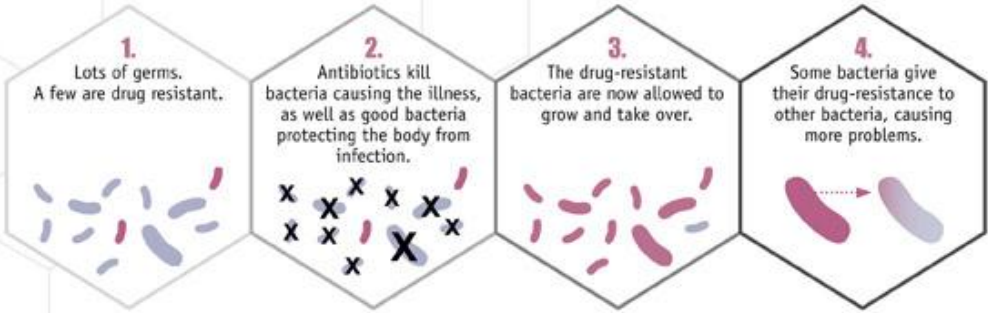
WHERE DO INFECTIONS HAPPEN?

Antibiotic-resistant infections can happen anywhere. Data show that most happen in the general community; however, most deaths related to antibiotic resistance happen in healthcare settings, such as hospitals and nursing homes.

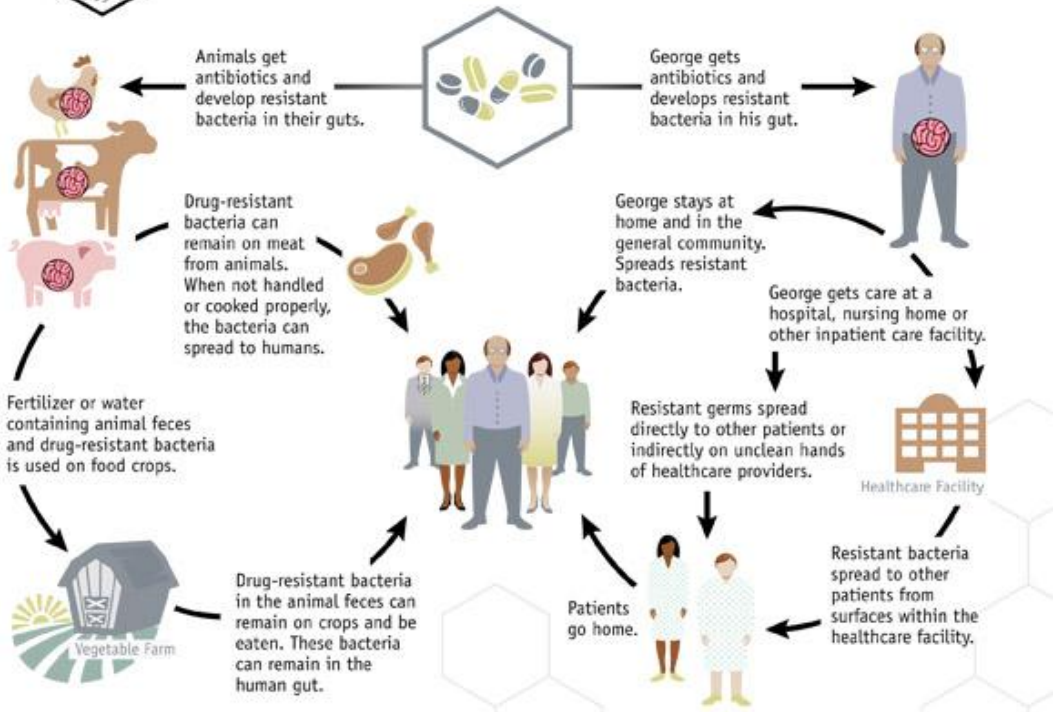
U.S. Department of Health and Human Services
Centers for Disease Control and Prevention

CS239559

How Antibiotic Resistance Happens



Examples of How Antibiotic Resistance Spreads



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

CS239559

Deng *et al. Ann Clin Microbiol Antimicrob (2015) 14:45*
DOI 10.1186/s12941-015-0100-6

ANNALS OF CLINICAL MICROBIOLOGY AND ANTIMICROBIALS

REVIEW Open Access

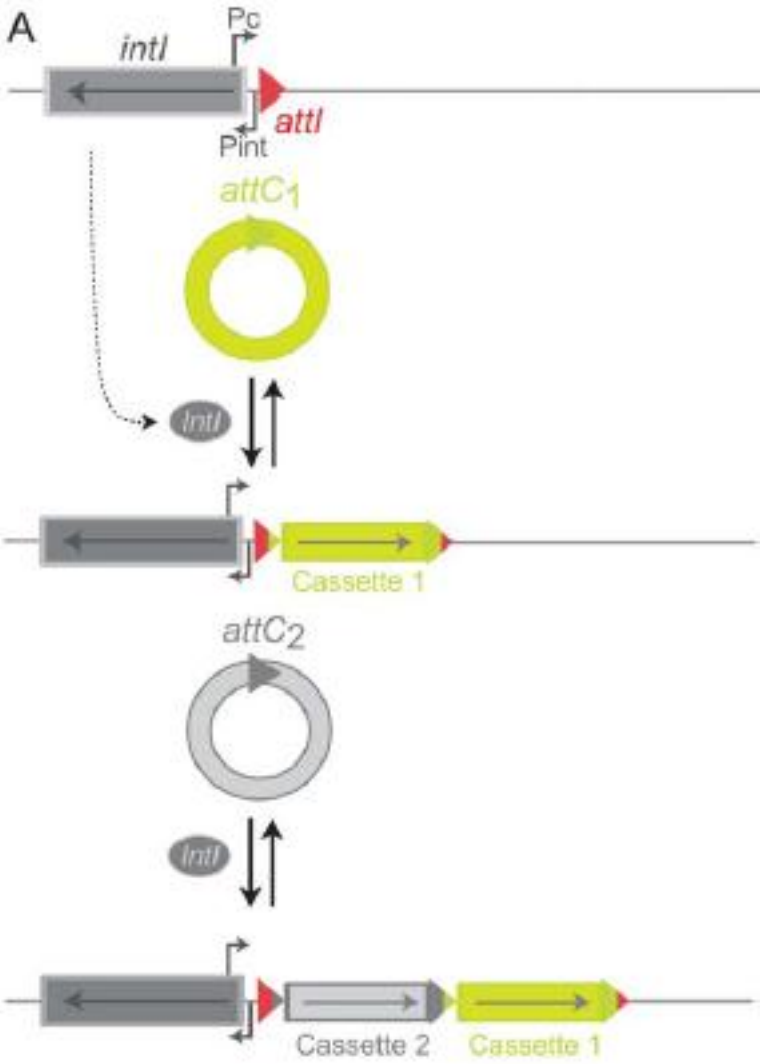
Resistance integrons: class 1, 2 and 3 integrons

Yang Deng[1], Xuerui Bao[1], Lili Ji[1], Lei Chen[2], Junyan Liu[1], Jian Miao[1], Dingqiang Chen[3], Huawei Bian[4], Yanmei Li[5*] and Guangchao Yu[6*]

Abstract

As recently indiscriminate abuse of existing antibiotics in both clinical and veterinary treatment leads to proliferation of antibiotic resistance in microbes and poses a dilemma for the future treatment of such bacterial infection, antimicrobial resistance has been considered to be one of the currently leading concerns in global public health, and reported to widely spread and extended to a large variety of microorganisms. In China, as one of the currently worst areas for antibiotics abuse, the annual prescription of antibiotics, including both clinical and veterinary treatment, has approaching 140 gram per person and been roughly estimated to be 10 times higher than that in the United Kingdom, which is considered to be a potential area for the emergence of "Super Bugs". Based on the integrons surveillance in Guangzhou, China in the past decade, this review thus aimed at summarizing the role of integrons in the perspective of both clinical setting and environment, with the focus on the occurrence and prevalence of class 1, 2 and 3 integrons.

Keywords: Antimicrobial resistance, Mobile genetic elements, Horizontal transfer, Resistance integrons



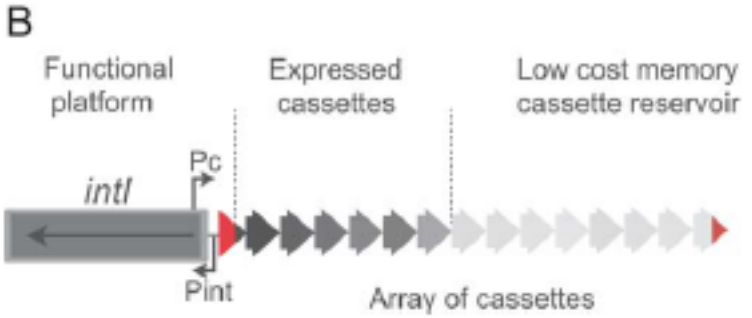
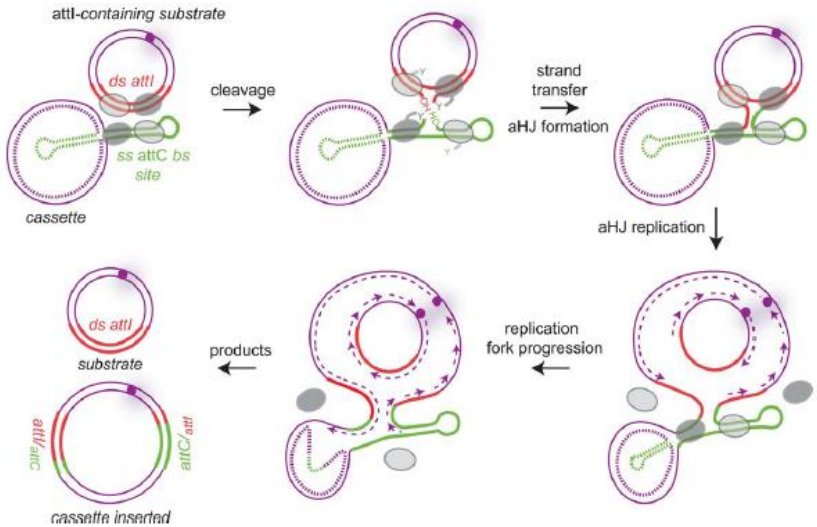


FIGURE 1 Organization of integrons. (A) Insertion and excision of cassettes: the functional platform, composed of the integrase-encoding *intI1* gene, the cassette (P_C) and integrase promoters (Pint), and the primary *attI* recombination site (red triangle), is shown. Cassette insertion (*attC*×*attI*) and excision (*attC*×*attC*) catalyzed by the IntI integrase are represented. Hybrid *attI* and *attC* sites are indicated. Arrows inside the cassettes indicate the direction of the open reading frame. (B) Expression of cassettes: cassettes of the array are represented by small arrows. Their expression level is reflected by the color intensity of each arrow. Only the first cassettes of the array are expressed, and the subsequent ones can be seen as a low-cost cassette reservoir. doi:10.1128/microbiolspec.MDNA3-0019-2014.f1



ated resistance to their host microorganisms. As consequence, acquisition of resistance genes has been regarded as major contributor for the wide distribution and spread of antimicrobial resistance, via either vertical transfer and horizontal transfer, with the latter mechanism involving mobile genetic elements such as plasmids and transposons [3]. As mostly carried by plasmids or contained within a transposon, integrons as well as its mechanism and role played in the distribution of microorganisms have been well established and documented [6, 7], which

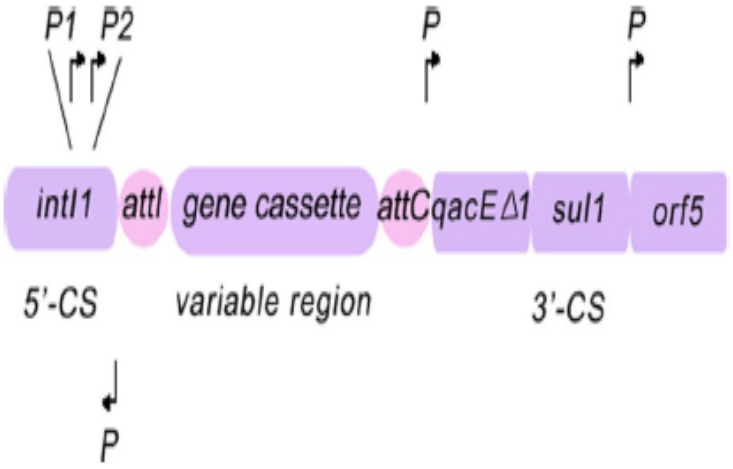


Fig. 1 Schematic representation of a class 1 integron. *P1* promoter for transcription of gene cassettes, *P2* second promoter that is usually inactive, *int* integrase gene, *attI1* integration site, *qacE* partially deleted gene that encodes quaternary ammonium compound resistance, *sull* sulphonamide resistance, *orf5* unknown function, *P* promoters of the *qacE*△ and *sull* genes, *attC* sequence on the gene cassette recognized by the integrase

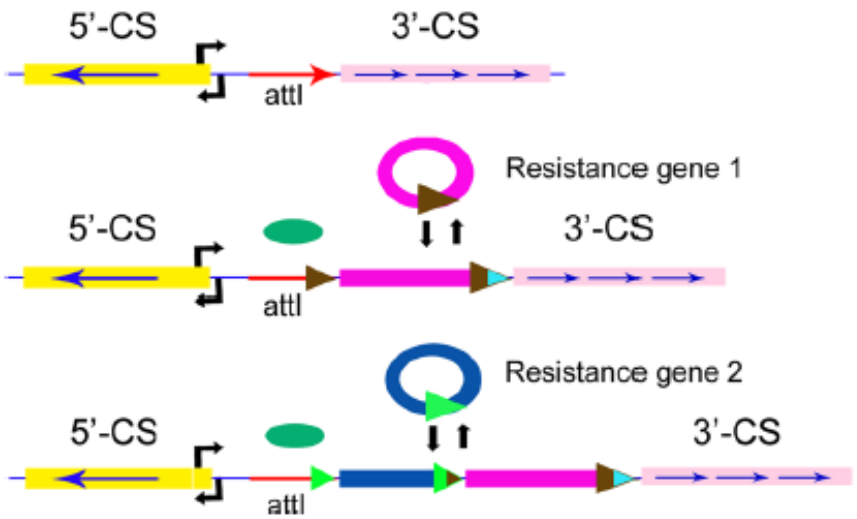


Fig. 3 Integration and excision of gene cassettes by site-specific recombination. IntI encoded by the *intI* gene in the integron catalyses recombination between the *attI1* site of the integron and/or the attC site(s) of gene cassette(s) resulting in insertion or excision of a cassette. One or more noncassette resistance genes may be inserted at the position of the 3′-CS. *Horizontal arrows* indicate the opposite orientations of *intI* and cassette-borne genes

Table 1 Occurrence and prevalence of class 1 integron in Gram-negative microorganisms

Date	Bacterial	Occurrence of class 1 Integron and the array of gene cassettes	Sampling	References
2006	*Shigella*	*EstX-aadA1* (3.85 %, 1/26)	Hiroshima prefecture, Japan; 2000–2004	[34]
2002	*Salmonella*	36.2 % (34/94); *aadA2-bla (PSE-1)* (61.76 % 21/34); *aadA1-aadA2-bla (PSE-1)* (38.23 %,13/34)	Animals, Japan	[33]
2000	*V. cholerae*	44/176; *aadB-aadA2-blaP1-dfrA1-dfrA15*	Thailand	[39]
2002	*Burkholderia*	29.4 % (5/17); *oxa-aac* (6′-1a)	Ireland	[38]
2004	*Campylobacter*	62/378	Ireland	[37]
2008	*Enterobacteriaceae*	50/226	Addenbrooke's Hospital	[62]
2005	*Escherichia coli*	4/32 (12.5 %); *sat-1-aadA*	Meat and meat products, Norwey	[42]
2008	*E. coli*	59.5 % (355/597)	South Thailand	[65]
2011	*E. coli*		Preliminary study in Guangzhou, China	[3]
2009	*P. aeruginosa*	45.8 % (54/118)	Preliminary study in Guangzhou, China	[19]
2008	*Serratia*	1/30; *aacC1-ORFX-ORFY-aadA1*	Canada	[17]
2004	*Stenotrophomonas maltophilia*	22 % (20/93)	Kaohsiung Medical University	[36]
2013	*P. aeruginosa*	43 % 37/182	Guilan, Iran	[44]
2011	*K. pneumoniae*	18/26	Blood stream infections	[2]
2013	*S. enteritidis*	11.9 % (59)	Taiwan	[41]
2013	*S. panama*	40.0 % (20)	Taiwan	[41]
2010	*P. aeruginosa*	High prevalence	Iran	[40]
2009	*Aeromonas*	16/41 (39.02 %); *dfrA15-cmlA4-aadA2*	Hidalgo, Mexico	[32]

Table 3 Summary of different structures of class 2 integrons reported in previous studies

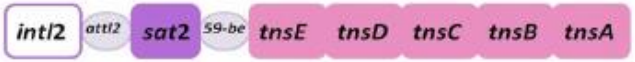
Name	Genes	Accession no.	Cassette arrays	Reference
Tn*7*	*dfrA1-sat2-aadA1*	NC_002525		[1]
Tn*1825*	*sat1-aadA1*	X56815		[48]
Tn*4132*	*dfrA1b-sat2-aadA1*	Z50804		[15]
Tn*7*::IS*1-ereA*	*dfrA1-sat1-ereA-aadA1*	AY183453		[50]
AB161461	*sat-sat1-aadA1*	AB161461		[27]
AB161462	*estX*	AB161462		[55]
Tn*7*::In*2-1*	*sat2*	DQ082896		[12]
Tn*7*::In*2-8*	*sat2-aadB-catB2-dfrA1-sat2-aadA1*	DQ176450		[51]

Table 4 Occurrence and prevalence of class 2, 3, and 4 integrons in Gram-positive and Gram-negative bacteria

Bacterial	Occurrence of Integrons and the array of gene cassettes	Sampling	Reference
Class 2 integrons			
Escherichia coli	7.4 % (31/417); *dfrA1-sat2-aadA1* (77.4 %, 24/31), *estX-sat2-aadA1* (19.4 %, 6/31) and *estX-sat2-*$\triangle$*aadA1* (3.2 %, 1/31)	BfT-GermVet monitoring study, Germany, 2004–2006	[67]
Enterobacteriaceae	34.9 % (52/149); II2 (Tn*7*), III2 (*estX-sat2-aadA1-orfX*, most widely distributed) and IV2 (*aadA1*, first reported)	*E. coli* amd *K. pneumoniae* strains from swine and chickens, Portugal	[62]
E.coli	3.0 % (3/100)	Spain	[65]
E. coli	3.6 % (4/111); *dfrA1-sat1-aadA1*	Preliminary study, Guangzhou, China	[68]
E. coli	One out of 322	Irrigation water and associated sediments, El Paso, Presidio and Weslaco	[69]
Coliforms	2.7 % (5/183)	Rivers in northern region of Turkey	[63]
Pseudomona aeruginosa	19.5 % (23/118); *dfrA1-sat1-aadA1*, first report of class 2 integron in this species of bacteria	Preliminary study, Guangzhou, China	[19]
Shigella flexneri	100 % (58/58); *dfrA1-sat1-aadA1*	Stool samples of sporadic diarrheic patients, China, 2005–2006	[70]
S. sonneii	93 % (2/43)	Adult patients with diarrhoea, Senegal	[71]
S. enterica	85 contemporary multi-drug resistant D-Tartrate-Positive isolates; *dfrA1-sat1-aadA1*	*S. enterica* Serovar Paratyphi B isolates Germany, 1995–2001	[72]
S. enteritidis	4.3 %; *estX-sat2-aadA1*	Poultry samples, Japan	[33]
E. faecalis	Two strains harboring Class 1 and 2 integrons; *dfrA1-sat1-aadA1*, first evidence of class 2 integron in G^+ bacteria	Preliminary study, Guangzhou, China	[52]
Class 3 integrons			
E.coli		Australia	[73]
E.coli	*ges1/oxa10:aac(6′)*	Switzerland	[74]
Serratia marcescens	*imp1/aacA4*	Japan	[75]
Klebsiella pneumoniae	*ges1/oxa10:aacA4*	The urine of an intensive care unit patient in Portugal	[76]
Class 4 integrons			
Vibrio cholerae		Collection de l'Institut Pasteur (CIP)	[77, 78]
V. metschnikovii			[77]

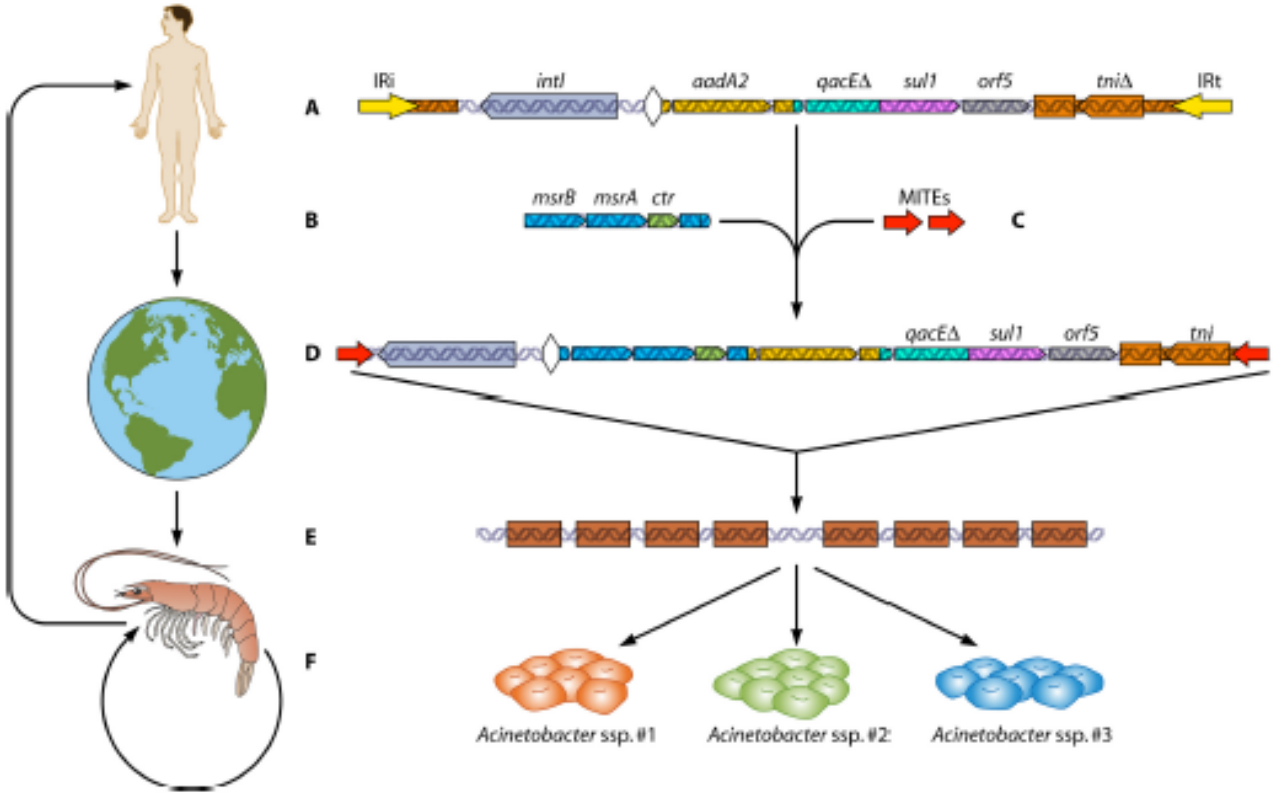


FIG 6 Role of resistance gene pollution in generating novel, complex DNA elements. (A) A typical class 1 integron from human-pathogenic or commensal bacteria. This type of DNA element commonly pollutes aquatic environments. It consists of inverted DNA repeats IRi and IRt, the class 1 integron integrase gene *intI1*, and a gene cassette, *aadA2*, which confers streptomycin resistance. The 3′ conserved segment consists of fused genes for disinfectant and sulfonamide resistance (*qacEΔ/sul1*), ORF5, and the remnants of genes encoding transposition functions (*tniΔ*). (B and C) In an aquatic environment, such an integron was modified by acquiring a novel gene cassette encoding two methionine sulfoxide reductases (*msrB* and *msrA*) (B) and replacing the inverted repeats IRi and IRt with miniature inverted-repeat transposable elements (MITEs) (C). (D) This event generated a compound MITE/integron element. (E) Mobility conferred by the MITEs allowed insertion of the compound integron into a genomic island. (F) This genomic island moved into at least three different species of the genus *Acinetobacter*, carrying the integron with it. Consequently, resistance determinants released from human waste streams may interact with gene cassettes and mobile DNA elements in aquatic ecosystems to generate new combinations of potential virulence genes in environmental bacteria. The presence of these bacteria in food items provides a readily accessible route for contamination of the food chain and the emergence of novel, virulent pathogens.

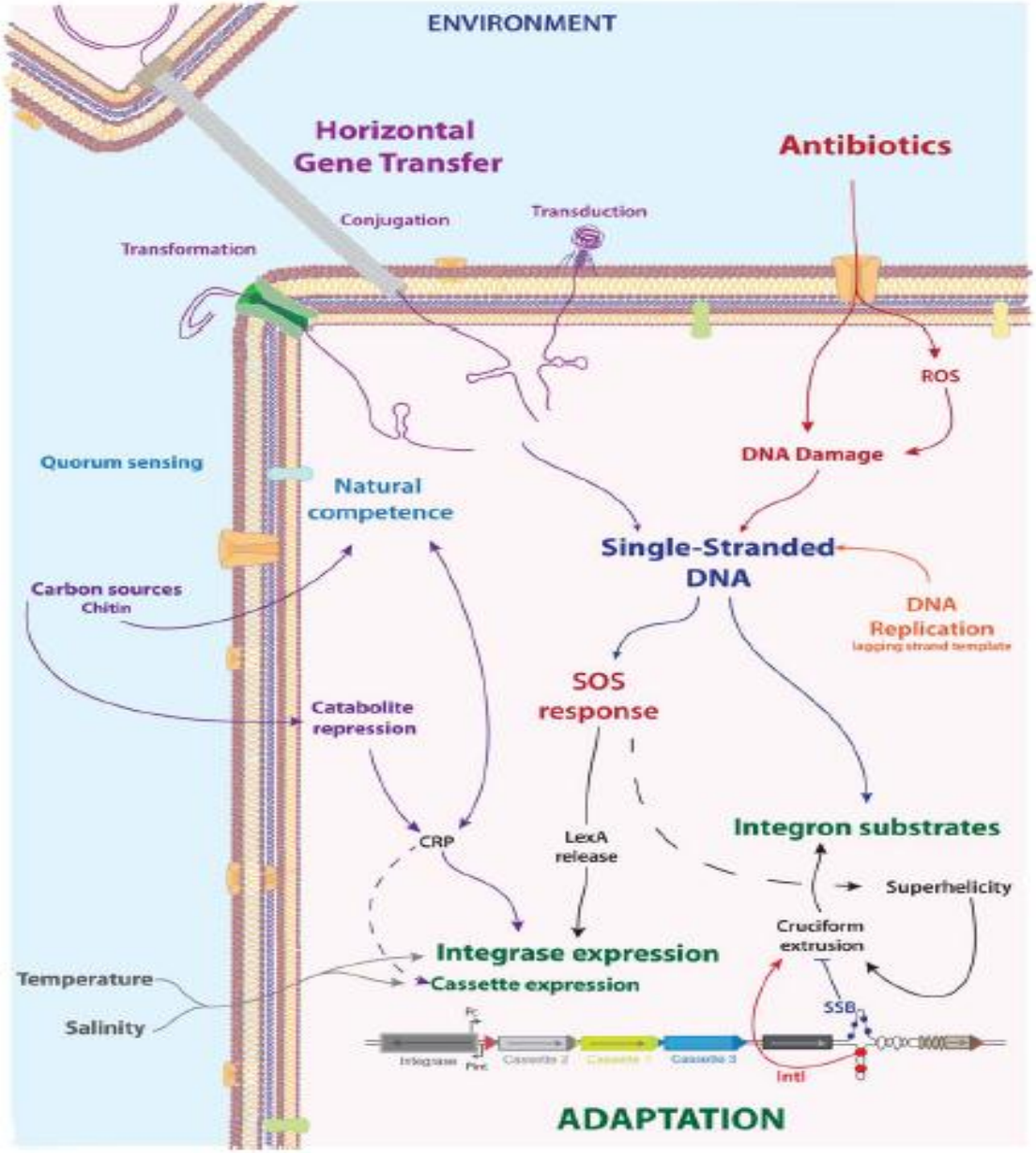


FIGURE 6 Intimate connection between the integron and cell physiology. A snapshot representation of the links between integrons' activity and bacterial physiology is shown. The main triggering signal for integrase expression is the bacterial SOS response. A detailed description of these connections is depicted in the section entitled: *A system intimately connected to cell physiology.* doi:10.1128/microbiolspec.MDNA3-0019-2014.f6

Developing Resistance

Timeline of Key Antibiotic Resistance Events

Dates are based upon early reports of resistance in the literature. In the case of pan drug-resistant (PDR)-*Acinetobacter* and *Pseudomonas*, the date is based upon reports of healthcare transmission or outbreaks. Note: penicillin was in limited use prior to widespread population usage in 1943.

Resistencia frente a ATB

Antibiótico	Uso Clínico	Resistencia observada
Sulfonamidas	1930	1940
Penicilina	1943	1946
Estreptomicina	1943	1959
Cloramfenicol	1947	1959
Vancomicina	1956	1988
Cefalosporinas	1960	Fines de 1960
Meticilina	1960	1961

Necesidad constante de encontrar o desarrollar nuevos antibióticos

Examples of Recently Approved Drugs

Drug Name	Year Approved	Key Targeted Pathogens	Drug's Use and Resistance Trends
Quinupristin/ Dalfoprisitin	1999	*Staphylococcus* *Streptococcus*	This is a combination of two drugs that can be used to treat gram-positive infections. Because side effects are common, this drug is usually not a first choice for therapy. Resistance in target pathogens has been described, but the percentage in the United States is still low.
Moxifloxacin	1999	Enterobacteriaceae *Staphylococcus* *Streptococcus*	Moxifloxacin, like other fluoroquinolones, demonstrates broad spectrum activity, and it can be used to treat a range of infections. Unfortunately, there is cross-resistance among the fluoroquinolones, and resistance is increasing in all targeted pathogens, especially Enterobacteriaceae.
Linezolid	2000	*Staphylococcus* *Enterococcus*	Linezolid can be used to treat serious gram-positive infections. Resistance has occurred but it is still uncommon.
Ertapenem	2001	Enterobacteriaceae *Staphylococcus* *Streptococcus*	Ertapenem is a carbapenem that can be used to treat a wide range of infections. Dissemination of carbapenem-resistant Enterobacteriaceae (CRE) is impacting the drug's overall effectiveness.
Gemifloxacin	2003	Enterobacteriaceae *Streptococcus*	Gemifloxacin is a fluoroquinolone that can be used to treat mild to moderate community-associated respiratory disease. Like moxifloxacin, there is cross-resistance with other fluoroquinolone drugs so resistance is increasing.
Daptomycin	2003	*Staphylococcus* *Streptococcus* *Enterococcus*	Daptomycin is often used for treatment of serious gram-positive infections. Resistance is emerging in all of the targeted pathogens, but the resistance rates are currently low.
Tigecycline	2005	Enterobacteriaceae *Staphylococcus* *Streptococcus* *Enterococcus*	Tigecycline is often one of the only active agents for carbapenem-resistant gram-negative infections, and resistance is emerging. However, even in the absence of resistance, the effectiveness of this agent for treatment of the most serious infections is a concern.
Doripenem	2007	Enterobacteriaceae *Pseudomonas aeruginosa* *Acinetobacter spp.* *Streptococcus spp.*	Doripenem is a carbapenem drug most commonly used to treat serious gram-negative infections. Dissemination of carbapenem-resistant gram-negative pathogens like CRE is reducing the overall effectiveness of this drug.
Telavancin	2008	*Staphylococcus* *Streptococcus* *Enterococcus*	Telavancin is approved for treatment of gram-positive skin and soft tissue infections. Use is limited because it is administered intravenously and is therefore difficult to use in an outpatient setting. In addition, it should not be used in a woman of childbearing age without a pregnancy test.

Drug Name	Year Approved	Key Targeted Pathogens	Drug's Use and Resistance Trends
Ceftaroline	2010	Enterobacteriaceae *Staphylococcus* *Streptococcus*	Ceftaroline is a cephalosporin drug, but unlike other cephalosporins, this one can be used to treat MRSA infections. Resistance has been identified but is rare. Ceftaroline does not demonstrate any enhanced activity compared to other cephalosporins for Enterobacteriaceae. ESBL-producing isolates and CRE isolates are resistant to this drug as well. ESBL (extended-spectrum β-lactamase) is an enzyme that allows bacteria to become resistant to a wide variety of penicillins and cephalosporins .Bacteria that contain this enzyme are known as ESBLs or ESBL-producing bacteria.

Patents on Quorum Quenching: Interfering with Bacterial Communication as a Strategy to Fight Infections

Manuel Romero[1], Laura Acuña[2] and Ana Otero[1]*

[1]*Departamento de Microbiología y Parasitología, Facultad de Biología, Edificio CIBUS, Campus Vida, Universidad de Santiago de Compostela, 15782 Santiago de Compostela, Spain;* [2]*Área de Valorización, Transferencia y Emprendimiento, Edificio Emprendia, Campus Vida, Universidad de Santiago de Compostela*

Received: September 13, 2011 Revised: October 12, 2011 Accepted: November 01, 2011

Abstract: Numerous bacterial functions, such as virulence and biofilm formation, are controlled by a cell density-dependent communication mechanism known as Quorum Sensing (QS), in which small diffusible molecules are released, allowing bacteria to coordinate their behavior once a minimal effective quorum has been reached. The interference with these signaling systems, also known as Quorum Quenching (QQ), represents a promising strategy to tackle bacterial infections. The growing interest in this approach is reflected by the increasing number of patents within the field (45 up to now), especially in the last few years, as shown by patent applications published since 2009. The fact that biofilm formation is also controlled by QS systems expands the application of QQ to clinically-relevant biofilms such as those responsible for periodontal disease. Moreover, since biofilms increase bacterial resistance to antimicrobials, QQ could represent a new way to fight some of the most recurrent human pathogens, such as nosocomial multiresistant strains, and this deserves further exploration, especially through more proofs of concept. In this article we review the best known QS and QQ systems to date and we describe recent patents on the interference with this type of bacterial communication.

Keywords: Acylase, agonist, AHL, AI-2, antagonist, bacterial communication, biofilm, lactonase, peptide, quorum quenching, quorum sensing, signal, virulence.

Different drugs for bad bugs: antivirulence strategies in the age of antibiotic resistance

Seth W. Dickey, Gordon Y. C. Cheung and Michael Otto

Abstract | The rapid evolution and dissemination of antibiotic resistance among bacterial pathogens are outpacing the development of new antibiotics, but antivirulence agents provide an alternative. These agents can circumvent antibiotic resistance by disarming pathogens of virulence factors that facilitate human disease while leaving bacterial growth pathways — the target of traditional antibiotics — intact. Either as stand-alone medications or together with antibiotics, these drugs are intended to treat bacterial infections in a largely pathogen-specific manner. Notably, development of antivirulence drugs requires an in-depth understanding of the roles that diverse virulence factors have in disease processes. In this Review, we outline the theory behind antivirulence strategies and provide examples of bacterial features that can be targeted by antivirulence approaches. Furthermore, we discuss the recent successes and failures of this paradigm, and new developments that are in the pipeline.

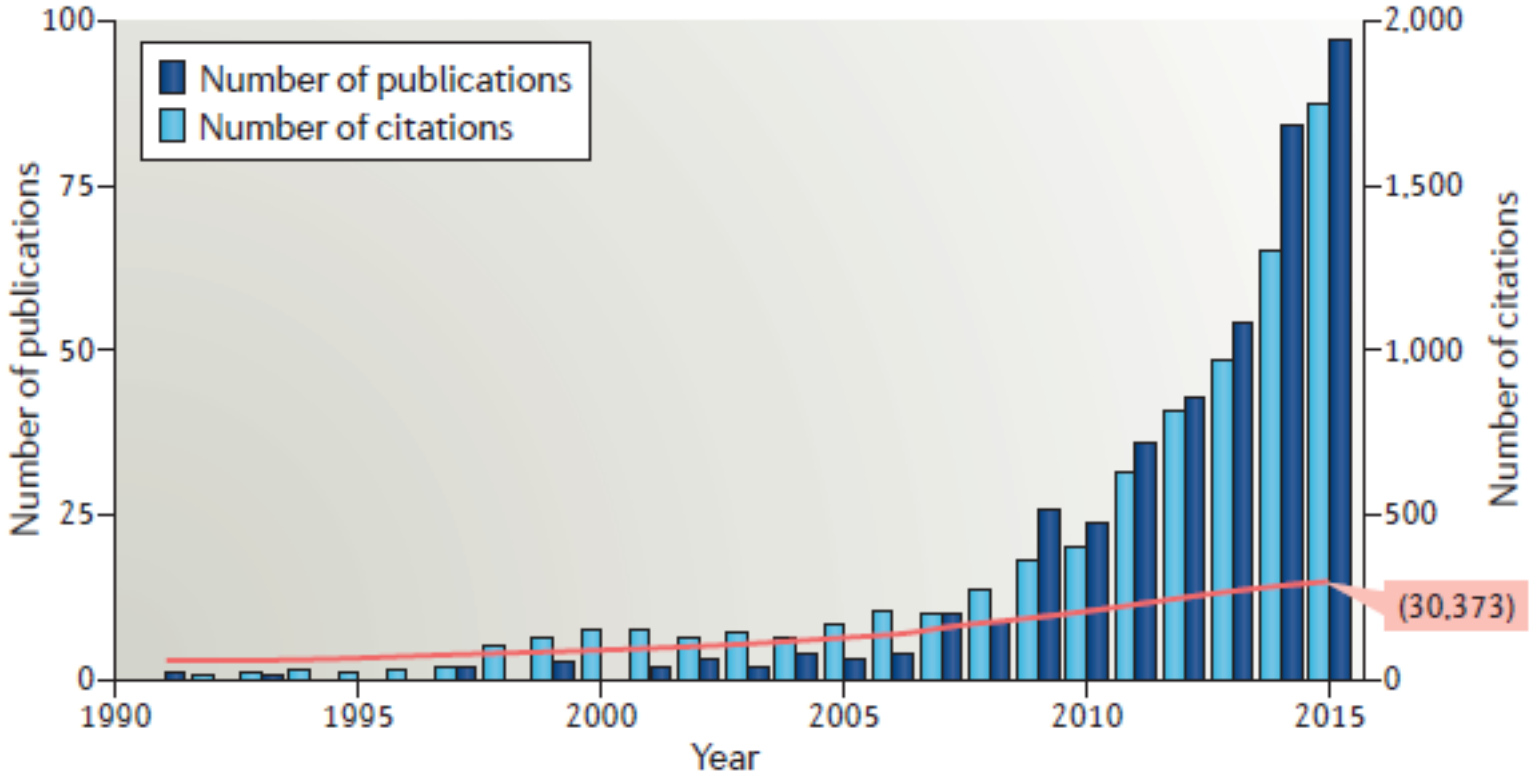


Figure 1 | **The rise of antivirulence approaches.** The number of antivirulence publications and citations is sharply increasing over time. Web of Science (Thomson Reuters) was queried with the following search terms: "antivirulence" OR "anti-virulence" OR "virulence inhibition" OR "virulence inhibitor" OR "virulence factor inhibition". The baseline of antibiotic publications was generated with the query: "antibacterial" OR "antibiotic" OR "antimicrobial". The total number of publications on Web of Science is given as a baseline (red), scaled to the number in 2015, which is indicated in parentheses.

Bacterial Quorum Sensing Inhibitors: Attractive Alternatives for Control of Infectious Pathogens Showing Multiple Drug Resistance

Ashima K. Bhardwaj*, Kittappa Vinothkumar and Neha Rajpara

Department of Human Health and Diseases, Indian Institute of Advanced Research, Koba Institutional Area, Gandhinagar 382 007, Gujarat, India

Received: December 13, 2012; Revised: January 31, 2013; Accepted: January 31, 2013

Abstract: Quorum sensing (QS) is a bacterial communication process that depends on the bacterial population density. It involves small diffusible signaling molecules which activate the expression of myriad genes that control diverse array of functions like bioluminescence, virulence, biofilm formation, sporulation, to name a few. Since QS is responsible for virulence in the clinically relevant bacteria, inhibition of QS appears to be a promising strategy to control these pathogenic bacteria. With indiscriminate use of antibiotics, there has been an alarming increase in the number of antibiotic resistant pathogens. Antibiotics are no longer the magic bullets they were once thought to be and therefore there is a need for development of new antibiotics and/or other novel strategies to combat the infections caused by multidrug resistant organisms. Quorum sensing inhibition or quorum quenching has been pursued as one of such novel strategies. While antibiotics kill or slow down the growth of bacteria, quorum sensing inhibitors (QSIs) or quorum quenchers (QQs) attenuate bacterial virulence. A large body of work on QS has been carried out in deadly pathogens like *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Vibrio fischeri, V. harveyi*, *Escherichia coli* and *V. cholera*e etc to unravel the mechanisms of QS as well as identify and study QSIs. This review describes various aspects of QS, QSI, different model systems to study these phenomena and recent patents on various QSIs. It suggests QSIs as attractive alternatives for controlling human, animal and plant pathogens and their utility in agriculture and other industries.

Keywords: Biofilms, multidrug resistance, patents, *Pseudomonas aeruginosa*, quorum sensing, quorum sensing inhibitors, *Staphylococcus aureus*, *Vibrio cholerae*.

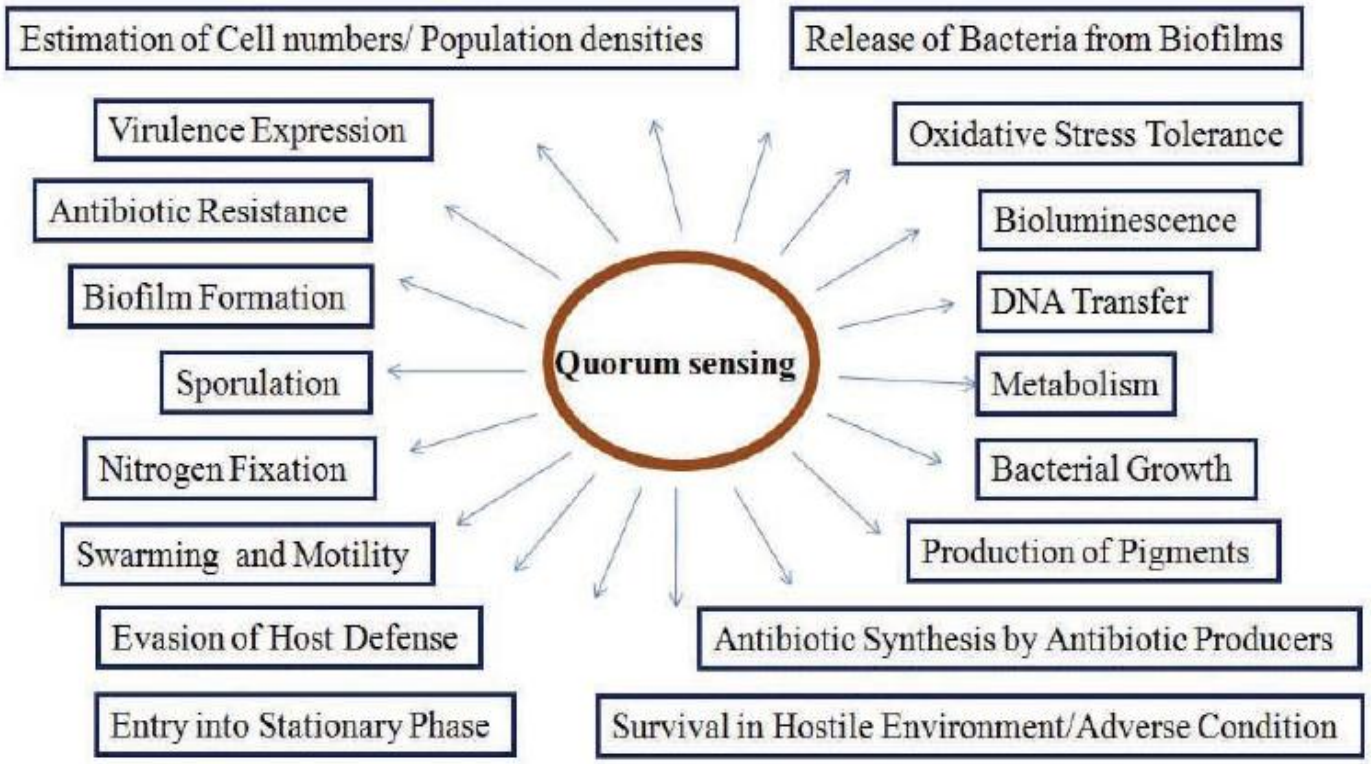
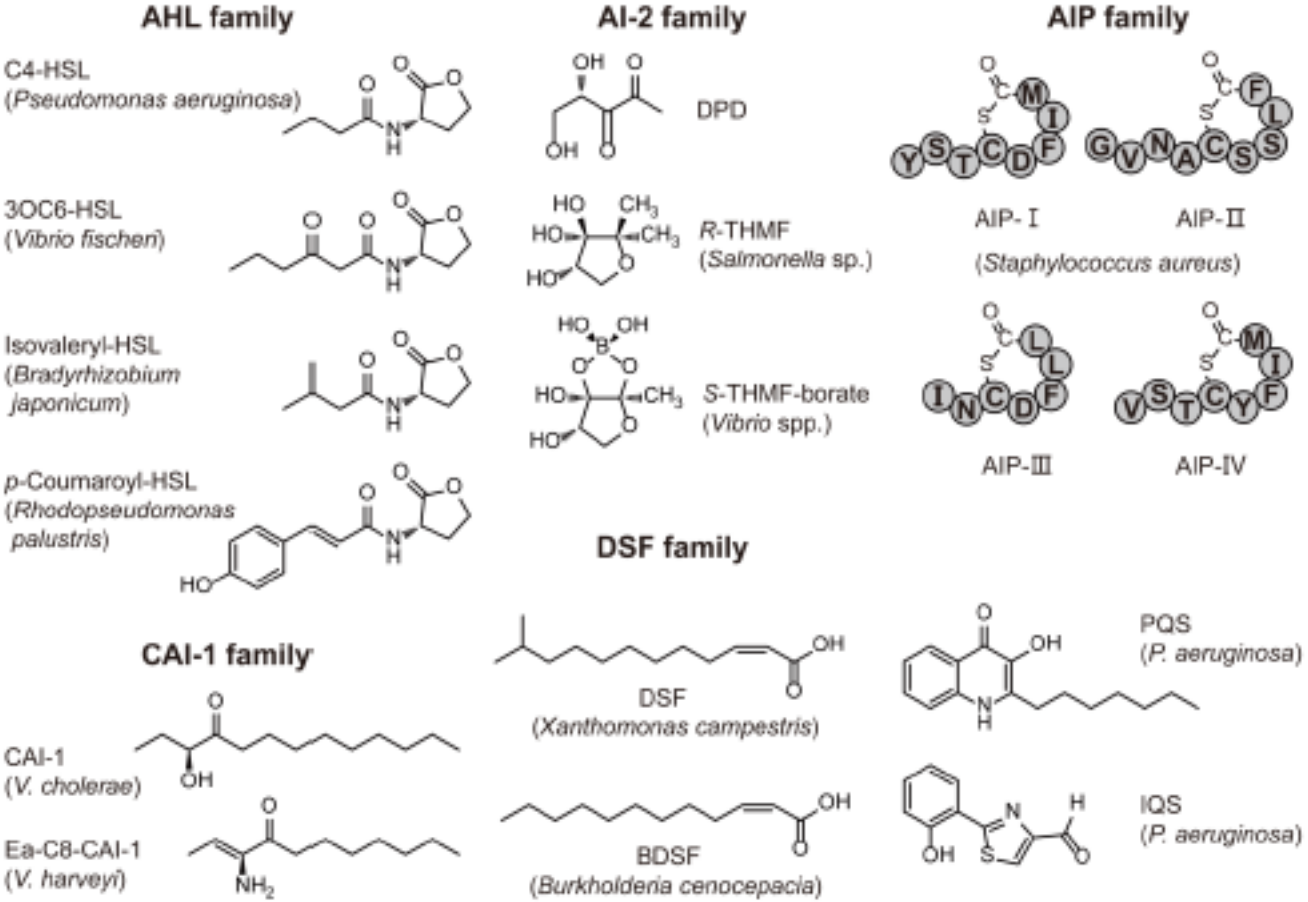


Fig. (1). Quorum sensing: A central component of multiple functions in bacterial communities.

Figure 1. Structures of representative quorum sensing (QS) signals.



AHL: *N*-Acyl-homoserine lactonse; AI-2: Autoinducter-2; AIP: Autoinducing peptides; CAI-1: *Cholerae* autoinducer-1; Ea-C8-CAI-1: (*Z*)-3-Aminoundec-2-en-4-one; DSF: Diffusible signal factor; BDSF: *Burkholderia cenocepacia* diffusible signal factor; PQS: *Pseudomonas* quinolone signal; IQS: Integrating QS signal; *R*-THMF: (*2R,4S*)-2-Methyl-2,3,3,4-tetrahy droxytetrahydrofuran ; *S*-THMF-borate: (*2S,4S*)-2-Methyl-2,3,3,4-tetrahydroxytetrahydrofuran-borate; DPD: 4,5-Dihydroxy-2,3-pentanedione.

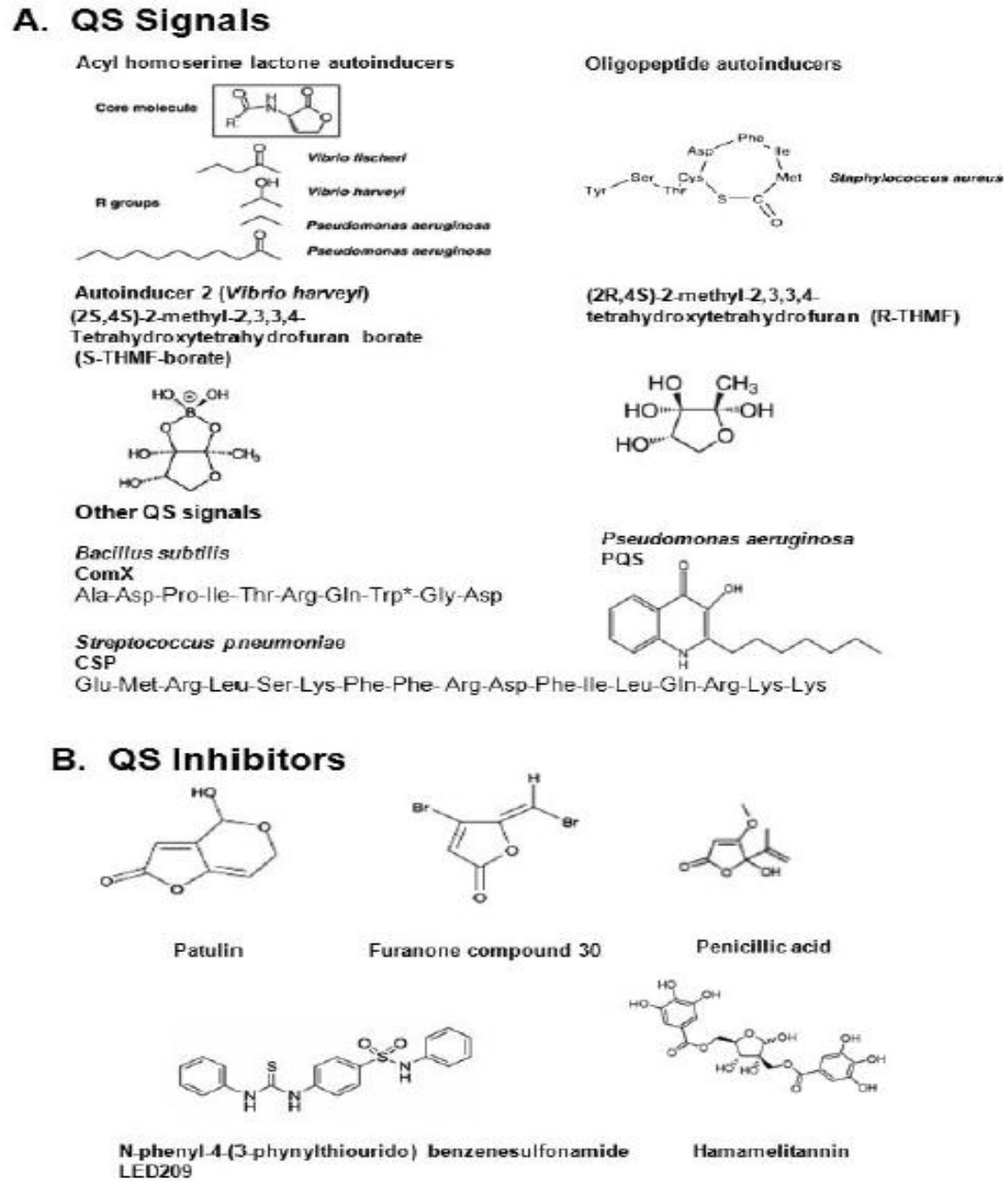


Fig. (2). Structures of some representative QS signals (A) and QSIs (B)

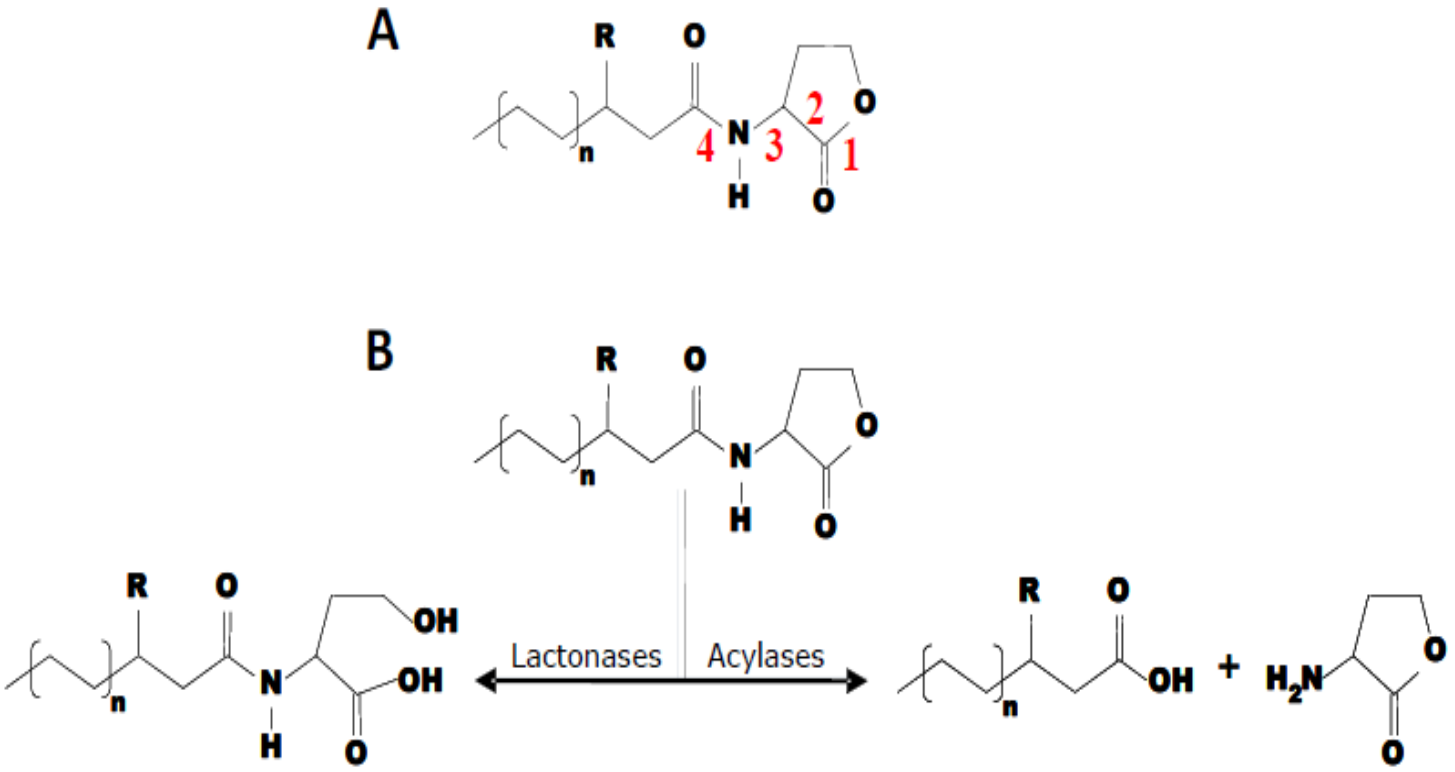


Fig. (1). A) Four possible enzyme cleavage sites of an AHL. B) AHL degradation mechanisms of lactonases and acylases. [Modified from 25].

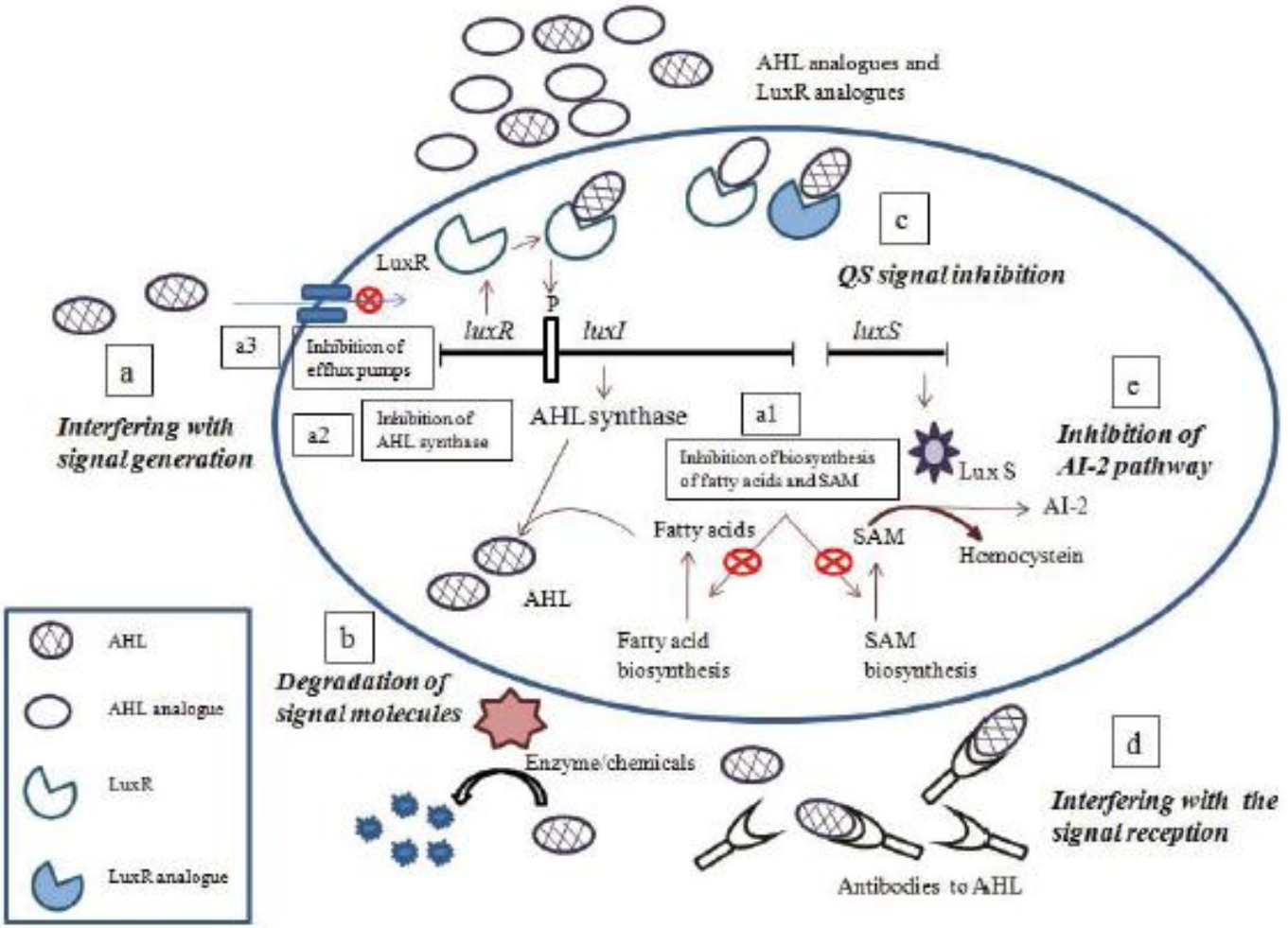


Fig. (3). Targets for QSI: **a**) interfering with signal generation by: **a1**) Inhibition of biosynthesis of fatty acids and SAM, **a2**) Inhibition of AHL synthase, **a3**) Inhibition of efflux pumps that allow the accumulation of signal molecules inside the cell; **b**) Degeneration of signal molecules either enzymatically or chemically; **c**) QS signal inhibition by AHL analogues or LuxR analogues; **d**) Interfering with the signal reception by antibodies raised against signal molecules and **e**) Inhibition of AI-2 pathway.

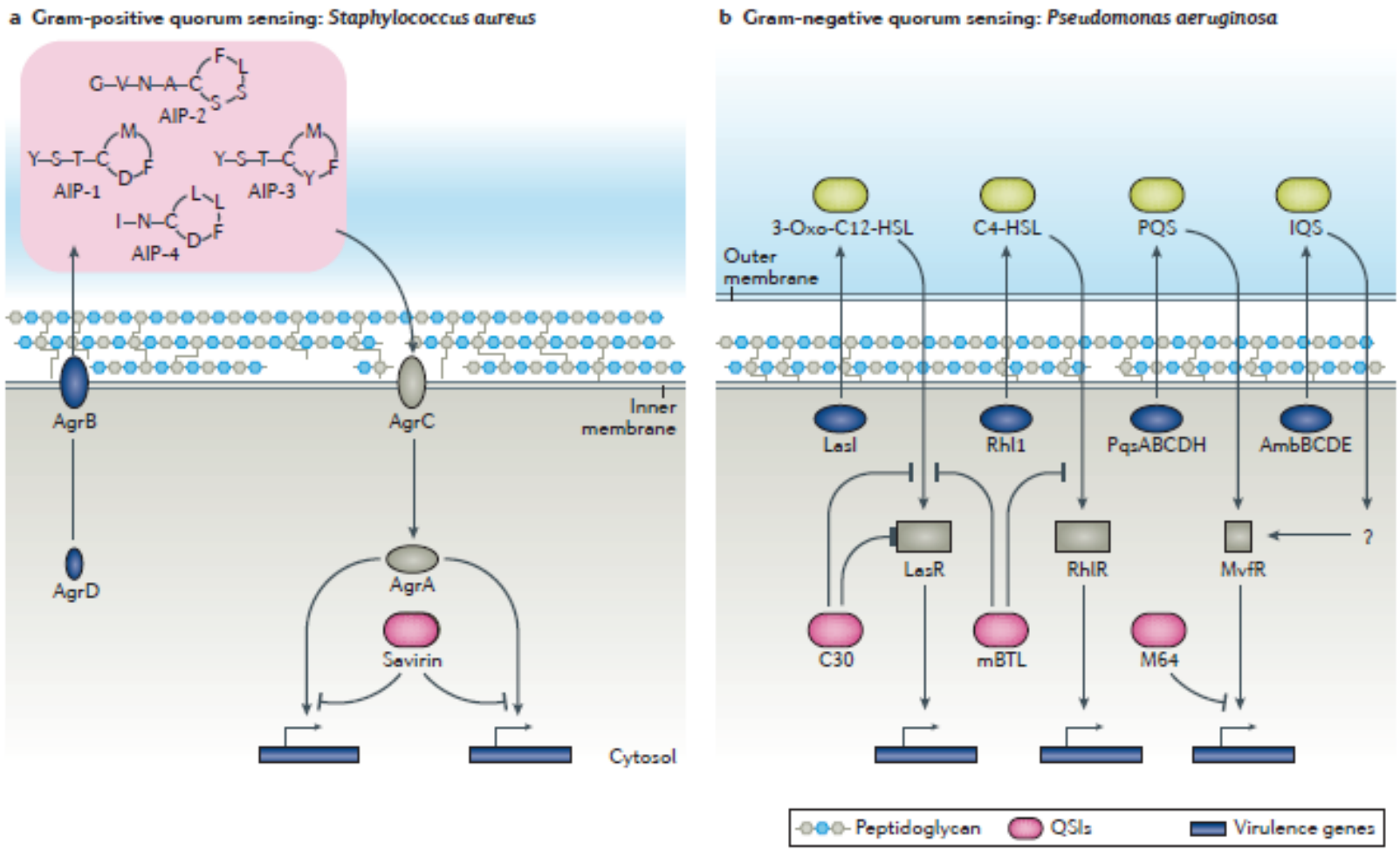
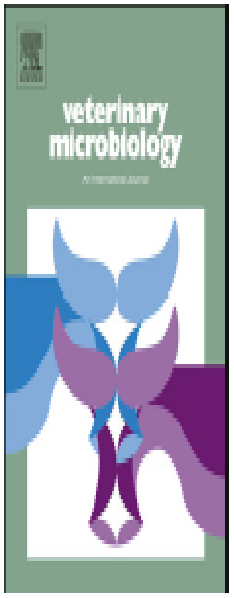


Figure 3 | **Quorum sensing and inhibition in model Gram-positive and Gram-negative pathogens.** Quorum-sensing pathways and inhibition in the model Gram-positive bacterium *Staphylococcus aureus* (part **a**) and the model Gram-negative bacterium *Pseudomonas aeruginosa* (part **b**). Synthases and exporters (dark blue) produce auto-inducers (AIP-1, AIP-2, AIP-3 and AIP-4) that signal through receptors (grey). Activated receptors globally modulate gene expression, including that of many virulence factors. Selected examples of quorum-sensing inhibitors (QSIs) that block receptors are shown. QSIs can block ligand binding (C30 and presumably meta-bromo-thiolactone (mBTL)), promote receptor degradation (C30 (REF. 183)) or block promoter binding (savirin and M64). Note that *P. aeruginosa* produces homoserine lactone (HSL) and quinolone-based auto-inducers and *S. aureus* produces cyclic peptide-based auto-inducers. Quorum-sensing feedback loops and crosstalk between pathways are omitted for simplicity. Adapted with permission from REF. 174, Macmillan Publishers Limited.

Contents lists available at ScienceDirect

Veterinary Microbiology

journal homepage: www.elsevier.com/locate/vetmic



Functional metagenomic analysis reveals rivers are a reservoir for diverse antibiotic resistance genes

G.C.A. Amos [a], L. Zhang [a,1], P.M. Hawkey [b,c], W.H. Gaze [a,1,2], E.M. Wellington [a,2,*]

[a] School of Life Sciences, University of Warwick, Coventry CV4 7AL, United Kingdom
[b] Health Protection Agency, West Midlands Public Health Laboratory, Heart of England NHS Foundation Trust, Bordesley Green East, Birmingham, United Kingdom
[c] Institute of Microbiology and Infection, Biosciences, University of Birmingham, Birmingham, United Kingdom

ARTICLE INFO

Keywords:
Functional metagenomics
Antibiotic resistance
Waste water
Environmental resistance
Sewage

ABSTRACT

The environment harbours a significant diversity of uncultured bacteria and a potential source of novel and extant resistance genes which may recombine with clinically important bacteria disseminated into environmental reservoirs. There is evidence that pollution can select for resistance due to the aggregation of adaptive genes on mobile elements. The aim of this study was to establish the impact of waste water treatment plant (WWTP) effluent disposal to a river by using culture independent methods to study diversity of resistance genes downstream of the WWTP in comparison to upstream. Metagenomic libraries were constructed in *Escherichia coli* and screened for phenotypic resistance to amikacin, gentamicin, neomycin, ampicillin and ciprofloxacin. Resistance genes were identified by using transposon mutagenesis. A significant increase downstream of the WWTP was observed in the number of phenotypic resistant clones recovered in metagenomic libraries. Common β-lactamases such as bla_{TEM} were recovered as well as a diverse range of acetyltransferases and unusual transporter genes, with evidence for newly emerging resistance mechanisms. The similarities of the predicted proteins to known sequences suggested origins of genes from a very diverse range of bacteria. The study suggests that waste water disposal increases the reservoir of resistance mechanisms in the environment either by addition of resistance genes or by input of agents selective for resistant phenotypes.

Table 3
Identities of resistance genes and predicted proteins from clones analysed by transposon mutagenesis.

Antibiotic resistance conferred (library)	Predicted size of protein (amino acids)	Predicted domains	Nearest sequence identity (bacteria identity)
Gentamicin clone 1 (US library)	420	Potassium transporter superfamily	77% Potassium transport protein (*Janthinobacterium* sp.)
Gentamicin clone 2 (US library)	58	None	75% Hypothetical protein (*Escherichia coli*)
Gentamicin clone 3 (US library)	264	Aminoglycoside 3-N-acetyltransferase	59% Aminoglycoside-(3)-N-acetyltransferase (*Escherichia coli*)
Gentamicin clone 4 (US library)	329	Thiamine pyrophosphate family	88% Pyruvate dehydrogenase subunit E1 (*Janthinobacterium* sp.)
Gentamicin clone 5 (DS library)	178	Aminoglycoside 3-N-acetyltransferase	36% Acetyltransferase (GNAT) family protein (*Providencia rettgeri*)
Gentamicin clone 6 (DS library	77	DUF4111	90% Aminoglycoside 3′-adenylytransferase (*Yersinia pestis*)
Gentamicin clone 7 (DS library)	88	Aminoglycoside 3′-phosphotransferase (APH)	100% Aminoglycoside 3′-phosphotransferase (*Pseudomonas putida*)
Amikacin clone 1 (DS library	185	Aminoglycoside 3-N-acetyltransferase	58% Aminoglycoside N(6′)-acetyltransferase (*Gloeocapsa* sp.)
Amikacin 2 (DS library)	119	Nucleotidyl transferase superfamily	62% Methionyl-tRNA synthetase (*Haliscomenobacter hydrossis*)
Ampicillin clone 1 (US library	289	Beta-lactamase2 superfamily	99% Beta-lactamase TEM (*Bacillus subtilis*)
Ampicillin clone 2 (DS library)	289	Beta-lactamase2 superfamily	99% Beta-lactamase TEM (*Bacillus subtilis*)
Ampicillin clone 3 (DS library)	289	Beta-lactamase2 superfamily	99% Beta-lactamase TEM (*Bacillus subtilis*)
Neomycin clone 1 (DS library)	107	TroA like superfamily FepB BC-type Fe3+ -hydroxamate transport system, periplasmic component	48% Hypothetical protein (*Streptomyces* sp.)
Neomycin clone 2 (DS library)	162	Glycosyltransferase family 25	36% Glycosyltransferase 25 family member 1 (*Agrobacterium* sp.)
Ciprofloxacin clone 1 (DS library)	145 154	RecX (recombination regulator) RecA (bacterial DNA recombination protein)	33% Regulatory protein RecX (*Listeria seeligeri*) 74% Recombinase A (*Geobacter lovleyi*)

Review

Antibiotic-Resistance Genes in Waste Water

Antti Karkman,[1,2,3] Thi Thuy Do,[4] Fiona Walsh,[4] and Marko P.J. Virta[5,*]

Waste water and waste water treatment plants can act as reservoirs and environmental suppliers of antibiotic resistance. They have also been proposed to be hotspots for horizontal gene transfer, enabling the spread of antibiotic resistance genes between different bacterial species. Waste water contains antibiotics, disinfectants, and metals which can form a selection pressure for antibiotic resistance, even in low concentrations. Our knowledge of antibiotic resistance in waste water has increased tremendously in the past few years with advances in the molecular methods available. However, there are still some gaps in our knowledge on the subject, such as how active is horizontal gene transfer in waste water and what is the role of the waste water treatment plant in the environmental resistome? The purpose of this review is to briefly describe some of the main methods for studying antibiotic resistance in waste waters and the latest research and main knowledge gaps on the issue. In addition, some future research directions are proposed.

Key Figure

Selection and Transfer of Antibiotic Resistance in Waste Water

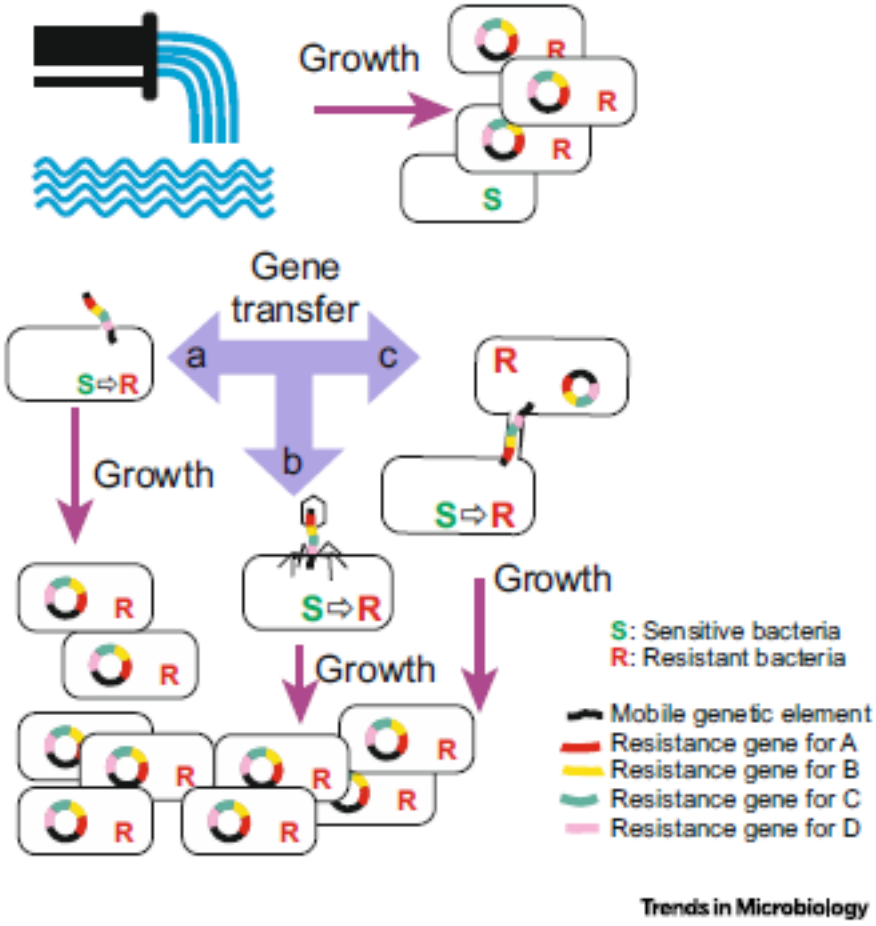


Figure 1. When there is selection pressure for antibiotic-resistant bacteria (ARB) (R), they overgrow the sensitive bacteria (S). The sensitive bacteria can become resistant by acquiring a resistance gene by transformation (a), transduction (b), or conjugation (c). Selection pressure can be caused by antibiotics, metals, or biocides present in the waste water. Selection pressure against one resistance gene can select other resistance genes also by coselection, as indicated by different resistance genes.

Wastewater Treatment Plants Release Large Amounts of Extended-Spectrum β-Lactamase–Producing *Escherichia coli* Into the Environment

Caroline Bréchet,[1] Julie Plantin,[1] Marlène Sauget,[1] Michelle Thouverez,[1] Daniel Talon,[1] Pascal Cholley,[1] Christophe Guyeux,[2] Didier Hocquet,[1] and Xavier Bertrand[1]

[1]Service d'Hygiène Hospitalière, UMR 6249 Chrono-environnement, Centre Hospitalier Régional Universitaire, Université de Franche-Comté, Besançon; and [2]Département DISC, Institut FEMTO-ST, UMR 6174 CNRS, Université de Franche-Comté, Belfort, France

(See the Editorial Commentary by Griffiths and Barza on pages 1666–7.)

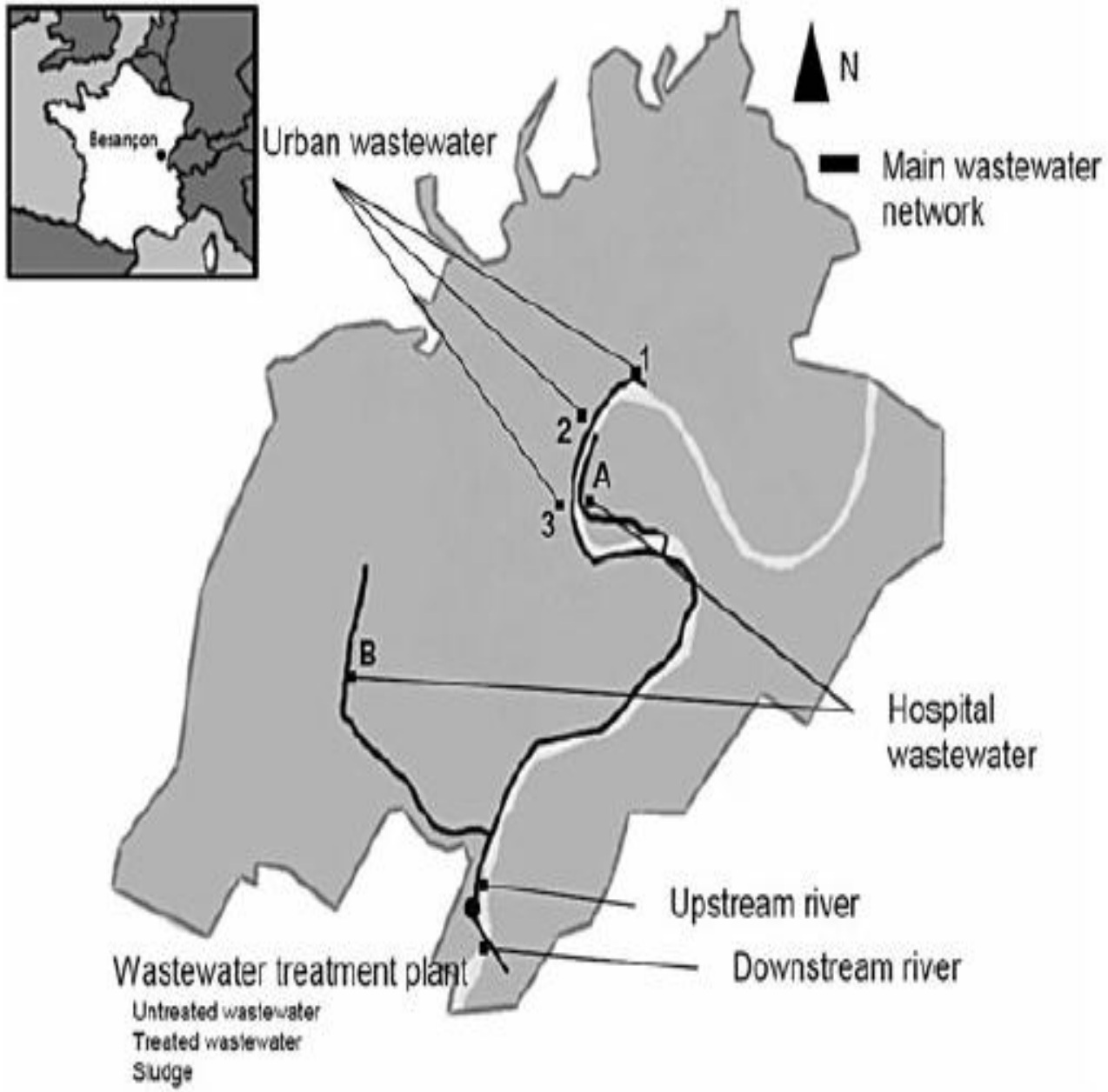
Background. The determinants of the spread of extended-spectrum β-lactamase–producing *Escherichia coli* (ESBLEC) in the community remain unclear. To evaluate its dissemination in the environment, we analyzed the ESBLEC population throughout an urban wastewater network.

Methods. Samples were collected weekly, over a 10-week period, from 11 sites throughout the wastewater network of Besançon city (France). Total *E. coli* and ESBLEC loads were determined for each sample. As a control, we analyzed 51 clinical ESBLEC isolates collected at our hospital. We genotyped both environmental and clinical ESBLEC by pulsed-field gel electrophoresis and multilocus sequence typing and identified their bla_{ESBL} genes by sequencing.

Results. The *E. coli* load was higher in urban wastewater than in hospital wastewater (7.5×10^5 vs 3.5×10^5 CFU/mL, respectively). ESBLEC was recovered from almost all the environmental samples and accounted for 0.3% of total *E. coli* in the untreated water upstream from the wastewater treatment plant (WWTP). The ESBLEC load was higher in hospital wastewater than in community wastewater (27×10^3 vs 0.8×10^3 CFU/mL, respectively). Treatment by the WWTP eliminated 98% and 94% of total *E. coli* and ESBLEC, respectively. The genotyping revealed considerable diversity within both environmental and clinical ESBLEC and the overrepresentation of some clonal complexes. Most of the sequence types displayed by the clinical isolates were also found in the environment. CTX-M enzymes were the most common enzymes whatever the origin of the isolates.

Conclusions. The treatment at the WWTP led to the relative enrichment of ESBLEC. We estimated that >600 billion of ESBLEC are released into the river Doubs daily and the sludge produced by the WWTP, used as fertilizer, contains 2.6×10^5 ESBLEC per gram.

Keywords. sequence types; WWTP; multidrug-resistant bacteria; environmental risk; sludge.


Besançon
N
Urban wastewater
Main wastewater network
1
2
3
A
B
Hospital wastewater
Upstream river
Downstream river
Wastewater treatment plant
Untreated wastewater
Treated wastewater
Sludge

Insects Represent a Link between Food Animal Farms and the Urban Environment for Antibiotic Resistance Traits

Ludek Zurek,[a,b] Anuradha Ghosh[a]

Department of Diagnostic Medicine and Pathobiology, College of Veterinary Medicine,[a] and Department of Entomology, College of Agriculture,[b] Kansas State University, Manhattan, Kansas, USA

Antibiotic-resistant bacterial infections result in higher patient mortality rates, prolonged hospitalizations, and increased health care costs. Extensive use of antibiotics as growth promoters in the animal industry represents great pressure for evolution and selection of antibiotic-resistant bacteria on farms. Despite growing evidence showing that antibiotic use and bacterial resistance in food animals correlate with resistance in human pathogens, the proof for direct transmission of antibiotic resistance is difficult to provide. In this review, we make a case that insects commonly associated with food animals likely represent a direct and important link between animal farms and urban communities for antibiotic resistance traits. Houseflies and cockroaches have been shown to carry multidrug-resistant clonal lineages of bacteria identical to those found in animal manure. Furthermore, several studies have demonstrated proliferation of bacteria and horizontal transfer of resistance genes in the insect digestive tract as well as transmission of resistant bacteria by insects to new substrates. We propose that insect management should be an integral part of pre- and postharvest food safety strategies to minimize spread of zoonotic pathogens and antibiotic resistance traits from animal farms. Furthermore, the insect link between the agricultural and urban environment presents an additional argument for adopting prudent use of antibiotics in the food animal industry.

TABLE 1 Insects with antibiotic-resistant bacteria from food animal production farms and surrounding urban environments

Insect	Bacterial species	Antibiotic resistance profile[a]	Environment(s)	Reference
Cockroaches (Dictyoptera)				
German cockroach (*Blattella germanica*)	*Enterococcus faecalis, Enterococcus faecium, Enterococcus hirae, Enterococcus casseliflavus*	AMP, CHL, CIP, ERY, KAN, STR, TET	Swine farms	56
Flies (Diptera)				
Housefly (*Musca domestica*)	*Enterococcus faecalis, Enterococcus faecium, Enterococcus casseliflavus*	CIP, ERY, KAN, STR, TET	Fast-food restaurants	59
Housefly (*Musca domestica*) Blowfly (*Lucilia* spp.) Bottle fly (*Phaenicia* spp.)	*Enterococcus faecalis, Enterococcus faecium, Staphylococcus* spp.	CLN, ERY, PEN, SYN, TET	Poultry farms	55
Housefly (*Musca domestica*)	*Enterococcus faecalis, Enterococcus faecium, Enterococcus hirae, Enterococcus casseliflavus*	AMP, CHL, CIP, ERY, KAN, STR, TET	Swine farms	56
Housefly (*Musca domestica*)	*Enterococcus faecalis, Enterococcus faecium*	DOX, ERY, GEN, STR, TET	Wastewater treatment facilities	61
Housefly (*Musca domestica*)	*Escherichia coli* O157:H7	AMP, CER, CTE, GEN, NEO, OXY, SPC, SXT	Cattle farm	46
Housefly (*Musca domestica*)	*Escherichia coli*	AMP, STR, SUL, TET	Swine farms	51
Housefly (*Musca domestica*) Stable fly (*Stomoxys calcitrans*)	*Escherichia coli*	AMP, AMX, CHL, CEP, CIP, GEN, NAL, SUL, STR, SXT, TET	Dairy cattle farm	52
Housefly (*Musca domestica*) False stable fly (*Muscina stabulans*)	*Escherichia coli*	AMP, CED, CEZ, STR, TET, TRM	Cattle farm	53
Housefly (*Musca domestica*) Blowfly (*Lucilia* spp.)	*Escherichia coli*	CAZ, CEF	Poultry farms	54
Australian bush fly (*Musca vetustissima*)	*Escherichia coli, Salmonella* spp., *Shigella* spp.	AMX, CLR, ROX	Cattle farm, urban area, outdoor eateries	50

Journal of Hospital Infection (2009) 73, 378–385

Available online at www.sciencedirect.com

ScienceDirect

www.elsevierhealth.com/journals/jhin

REVIEW

The role of environmental cleaning in the control of hospital-acquired infection

S.J. Dancer*

Summary Increasing numbers of hospital-acquired infections have generated much attention over the last decade. The public has linked the so-called 'superbugs' with their experience of dirty hospitals but the precise role of environmental cleaning in the control of these organisms remains unknown. Until cleaning becomes an evidence-based science, with established methods for assessment, the importance of a clean environment is likely to remain speculative. This review will examine the links between the hospital environment and various pathogens, including meticillin-resistant *Staphylococcus aureus*, vancomycin-resistant enterococci, norovirus, *Clostridium difficile* and acinetobacter. These organisms may be able to survive in healthcare environments but there is evidence to support their vulnerability to the cleaning process. Removal with, or without, disinfectants, appears to be associated with reduced infection rates for patients. Unfortunately, cleaning is often delivered as part of an overall infection control package in response to an outbreak and the importance of cleaning as a single intervention remains controversial. Recent work has shown that hand-touch sites are habitually contaminated by hospital pathogens, which are then delivered to patients on hands. It is possible that prioritising the cleaning of these sites might offer a useful adjunct to the current preoccupation with hand hygiene, since hand-touch sites comprise the less well-studied side of the hand-touch site equation. In addition, using proposed standards for hospital hygiene could provide further evidence that cleaning is a cost-effective intervention for controlling hospital-acquired infection.

What is 'clean'?

If we state that a hospital is clean, we assume that it looks clean and that it is safe for patients. Unfortunately, the microbes responsible for HAI are invisible to the naked eye. This means that visual assessment is insufficient for defining cleanliness, nor will it accurately predict the infection risk for patients.[2] Cleanliness should not actually be defined without indicating how it is assessed. A recent study compared visual assessment against both biochemical (ATP bioluminescence) and microbiological screening of the hospital environment.[3] The results showed that whereas most surfaces looked clean, less than a quarter were free from organic soil (ATP) and less than half were microbiologically clean.[3] Given the risk of acquiring hospital pathogens from a hospital ward, visual assessment is outdated, inadequate and scientifically obsolete. The only benefit from a visual inspection is to appease aesthetic obligations.

There has been suggestion that hospitals would benefit from cleaning standards emulating those implemented in the food industry.[2,48] Food prep-

Where to clean?

There is increasing evidence regarding the importance of hand-touch sites in the transmission of pathogens to healthy persons, as well as to patients.[51,52] It is also becoming apparent that the sites closer to the patient are more likely to furnish an infection risk than those situated further away.[7,8] The role of these near-patient hand-touch sites in MRSA transmission and, indeed, other hospital pathogens, has not been given the priority that it deserves. Ward cleaners work to a set specification that encompasses general surfaces and bathrooms, with emphasis on the cleaning of floors and toilets.[53] These are not necessarily near-patient hand-touch sites. Examples of the latter include bed rails, bedside lockers, infusion pumps, door handles and various switches,

How to clean?

Most of the studies describing the benefits from cleaning in this review used disinfectants to clean the hospital environment. Virtually all were reported as part of the response to an outbreak. Only a few UK-based studies used detergent and water, and even fewer reported cleaning benefits in the absence of an outbreak.[18,30] It appears that when reviewing the evidence for the role of cleaning in the control of HAI, there are several issues which still require clarification. First, is there any difference between the quantity, quality and methods for routine cleaning compared with what is needed in the event of an outbreak; and second, is it sufficient to proclaim the benefits from cleaning with disinfectants without establishing what can be achieved using soap and water alone? These questions require an evidence-based approach before we can set the best specification for cleaning in our hospitals. In addition, no one has yet modelled different cleaning methods against the infection risk for patients, their degree of vulnerability and the clinical area in which they are exposed.

The role of the surface environment in healthcare-associated infections

David J. Weber[a,b], *Deverick Anderson*[c], *and William A. Rutala*[a,b]

Purpose of review
This article reviews the evidence demonstrating the importance of contamination of hospital surfaces in the transmission of healthcare-associated pathogens and interventions scientifically demonstrated to reduce the levels of microbial contamination and decrease healthcare-associated infections.

Recent findings
The contaminated surface environment in hospitals plays an important role in the transmission of methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant *Enterococcus* spp. (VRE), *Clostridium difficile*, *Acinetobacter* spp., and norovirus. Improved surface cleaning and disinfection can reduce transmission of these pathogens. 'No-touch' methods of room disinfection (i.e., devices which produce ultraviolet light or hydrogen peroxide) and 'self-disinfecting' surfaces (e.g., copper) also show promise to decrease contamination and reduce healthcare-associated infections.

Summary
Hospital surfaces are frequently contaminated with important healthcare-associated pathogens. Contact with the contaminated environment by healthcare personnel is equally as likely as direct contact with a patient to lead to contamination of the healthcare provider's hands or gloves that may result in patient-to-patient transmission of nosocomial pathogens. Admission to a room previously occupied by a patient with MRSA, VRE, *Acinetobacter,* or *C. difficile* increases the risk for the subsequent patient admitted to the room to acquire the pathogen. Improved cleaning and disinfection of room surfaces decreases the risk of healthcare-associated infections.

Keywords
copper, environment, healthcare-associated infections, hospital surfaces, hydrogen peroxide systems, surface disinfection, ultraviolet light

Figure 1. Classes of biocides based on the functional groups.

TABLE 1. Chemical structures and uses of biocides in antiseptics and disinfectants

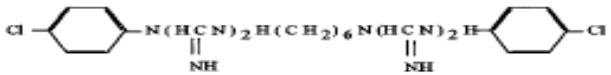
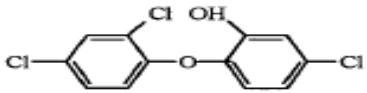
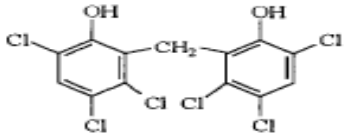
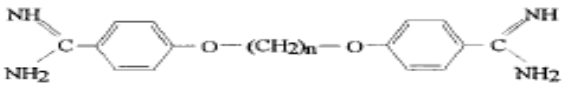
Alcohols	Ethanol	$CH_3—CHOH$	Antisepsis
	Isopropanol	$CH_3—CHOH—CH_3$	Disinfection
			Preservation
Aldehydes	Glutaraldehyde	$OH—CCH_2CH_2CH_2C—HO$	Disinfection
	Formaldehyde	$H—C—HO$	Sterilization
			Preservation
Anilides	General structure	$C_6H_5.NH.COR$	Antisepsis
	Triclocarban		
Biguanides	Chlorhexidine		Antisepsis
			Antiplaque agents
	Alexidine, polymeric biguanides		Preservation
			Disinfection
Bisphenols	Triclosan		Antisepsis
			Antiplaque agents
	Hexachlorophene		Deodorants
			Preservation
Diamidines	Propamidine		Antisepsis
			Preservation
	Dibromopropamidine		

TABLE 1—*Continued*

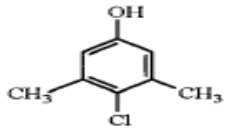
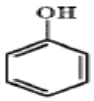
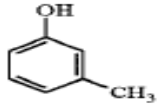
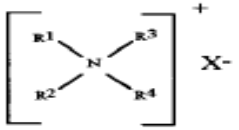
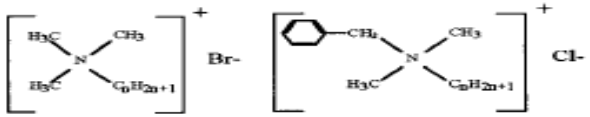
Halogen-releasing agents	Chlorine compounds	⇨OCl-, HOCl, Cl_2	Disinfection Antisepsis
	Iodine compounds	⇨I_2	Cleaning
Halophenols	Chloroxylenol (PCMX)		Antisepsis Preservation
Heavy metal derivatives	Silver compounds	Ag	Preservation Antisepsis
	Mercury compounds	Hg	Disinfection
Peroxygens	Hydrogen peroxide	H_2O_2	Disinfection
	Ozone	O_3	Sterilization
	Peracetic acid	$CH_3.COOOH$	
Phenols and cresols	Phenol		Disinfection Preservation
	Cresol		
Quaternary ammonium compounds	General structure		Disinfection Antisepsis Preservation Cleaning
	Cetrimide, benzalkonium chloride		

TABLE 1—*Continued*

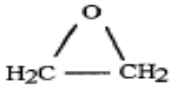
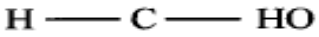
Vapor phase	Ethylene oxide		Sterilization Disinfection
	Formaldehyde		
	Hydrogen peroxide	H_2O_2	

TABLE 2. Summary of mechanisms of antibacterial action of antiseptics and disinfectants

Target	Antiseptic or disinfectant	Mechanism of action
Cell envelope (cell wall, outer membrane)	Glutaraldehyde	Cross-linking of proteins
	EDTA, other permeabilizers	Gram-negative bacteria: removal of Mg^{2+}, release of some LPS
Cytoplasmic (inner) membrane	QACs	Generalized membrane damage involving phospholipid bilayers
	Chlorhexidine	Low concentrations affect membrane integrity, high concentrations cause congealing of cytoplasm
	Diamines	Induction of leakage of amino acids
	PHMB, alexidine	Phase separation and domain formation of membrane lipids
	Phenols	Leakage; some cause uncoupling
Cross-linking of macromolecules	Formaldehyde	Cross-linking of proteins, RNA, and DNA
	Glutaraldehyde	Cross-linking of proteins in cell envelope and elsewhere in the cell
DNA intercalation	Acridines	Intercalation of an acridine molecule between two layers of base pairs in DNA
Interaction with thiol groups	Silver compounds	Membrane-bound enzymes (interaction with thiol groups)
Effects on DNA	Halogens	Inhibition of DNA synthesis
	Hydrogen peroxide, silver ions	DNA strand breakage
Oxidizing agents	Halogens	Oxidation of thiol groups to disulfides, sulfoxides, or disulfoxides
	Peroxygens	Hydrogen peroxide: activity due to from formation of free hydroxy radicals (˙OH), which oxidize thiol groups in enzymes and proteins; PAA: disruption of thiol groups in proteins and enzymes

TABLE 3. Mechanism of antimicrobial action of glutaraldehyde

Target microorganism	Glutaraldehyde action
Bacterial spores	Low concentrations inhibit germination; high concentrations are sporicidal, probably as a consequence of strong interaction with outer cell layers
Mycobacteria	Action unknown, but probably involves mycobacterial cell wall
Other nonsporulating bacteria	Strong association with outer layers of gram-positive and gram-negative bacteria; cross-linking of amino groups in protein; inhibition of transport processes into cell
Fungi	Fungal cell wall appears to be a primary target site, with postulated interaction with chitin
Viruses	Actual mechanisms unknown, but involve protein-DNA cross-links and capsid changes
Protozoa	Mechanism of action not known

TABLE 4. Mechanisms of antimicrobial action of chlorhexidine

Type of microorganism	Chlorhexidine action
Bacterial spores	Not sporicidal but prevents development of spores; inhibits spore outgrowth but not germination
Mycobacteria	Mycobacteristatic (mechanism unknown) but not mycobactericidal
Other nonsporulating bacteria	Membrane-active agent, causing protoplast and spheroplast lysis; high concentrations cause precipitation of proteins and nucleic acids
Yeasts	Membrane-active agent, causing protoplast lysis and intracellular leakage; high concentrations cause intracellular coagulation
Viruses	Low activity against many viruses; lipid-enveloped viruses more sensitive than nonenveloped viruses; effect possibly on viral envelope, perhaps the lipid moieties
Protozoa	Recent studies against *A. castellanii* demonstrate membrane activity (leakage) toward trophozoites, less toward cysts

Table 1. Classes of biocide based on target of action.

Biocides that act on membrane	Biocides that act on proteins	Biocides that act on nucleic acid	Biocides that act on cell wall
QACs [14,133,134]	Alcohols [134]	Alcohols [133]	Alcohols
Biguanides [14,133,134]	Phenols	Acids (parabens) [133]	Phenols [136]
Phenols [14,133,134]	Phenylethers [133]	Antimicrobial dyes [133]	Aldehydes [134,136]
Phenylethers [14,133]	Aldehydes [14,134]	Acridines [14]	Chlorine releasing compounds [136]
Acids [14]	Heavy-metal derivatives [133]	Biguanides [133]	Heavy-metal derivatives (mercurials) [136]
Terpenes [6]	Isothiazolones [133]	Aldehydes [134]	
Alcohols [14,133,134]	Acids (parabens) [133]	Diamidines [135]	
Anilides [134]	Peroxygens [14]	Chlorine compounds [134]	
Peroxygens [134]	Chlorine compounds [14]	Heavy-metal derivatives [133,134]	
Parabens [14]	Biguanides [134]	Peroxygens [134]	
Isothiazolones [14]	Vapor-phase disinfectant [134]	Halogens [134]	
Anionic surfactant [14]		Vapor-phase disinfectant [134]	

Review

Biocide tolerance in bacteria

Elena Ortega Morente, Miguel Angel Fernández-Fuentes, Maria José Grande Burgos, Hikmate Abriouel, Rubén Pérez Pulido, Antonio Gálvez *

Área de Microbiología, Departamento de Ciencias de la Salud, Facultad de Ciencias Experimentales, Universidad de Jaén, 23071-Jaén, Spain

ARTICLE INFO

Article history:
Received 25 June 2012
Received in revised form 24 October 2012
Accepted 15 December 2012
Available online 7 January 2013

Keywords:
Biocide
Antibiotic
Disinfection
Resistance
Tolerance

ABSTRACT

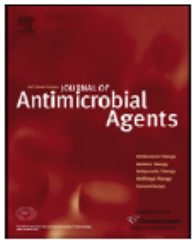
Biocides have been employed for centuries, so today a wide range of compounds showing different levels of antimicrobial activity have become available. At the present time, understanding the mechanisms of action of biocides has also become an important issue with the emergence of bacterial tolerance to biocides and the suggestion that biocide and antibiotic resistance in bacteria might be linked. While most of the mechanisms providing antibiotic resistance are agent specific, providing resistance to a single antimicrobial or class of antimicrobial, there are currently numerous examples of efflux systems that accommodate and, thus, provide tolerance to a broad range of structurally unrelated antimicrobials, both antibiotics and biocides. If biocide tolerance becomes increasingly common and it is linked to antibiotic resistance, not only resistant (even multi-resistant) bacteria could be passed along the food chain, but also there are resistance determinants that can spread and lead to the emergence of new resistant microorganisms, which can only be detected and monitored when the building blocks of resistance traits are understood on the molecular level. This review summarizes the main advances reached in understanding the mechanism of action of biocides, the mechanisms of bacterial resistance to both biocides and antibiotics, and the incidence of biocide tolerance in bacteria of concern to human health and the food industry.

International Journal of Antimicrobial Agents 39 (2012) 381–389

Contents lists available at SciVerse ScienceDirect

International Journal of Antimicrobial Agents

journal homepage: http://www.elsevier.com/locate/ijantimicag



Review

Emergence of resistance to antibacterial agents: the role of quaternary ammonium compounds—a critical review

Sylvie Buffet-Bataillon[a,b], Pierre Tattevin[c], Martine Bonnaure-Mallet[b], Anne Jolivet-Gougeon[b,*]

[a] *Unité d'Hygiène Hospitalière, Pôle Microorganismes, CHU Pontchaillou, 2 avenue du Professeur Léon Bernard, 35043 Rennes, France*
[b] *Equipe de Microbiologie, EA 1254, Université de Rennes 1, Université Européenne de Bretagne, CHU Rennes, Rennes, France*
[c] *Infectious Diseases and Intensive Care Unit, Pontchaillou University Hospital, Rennes, France*

ABSTRACT

Quaternary ammonium compounds (QACs) are widely distributed in hospitals, industry and cosmetics. Little attention has been focused on the potential impact of QACs on the emergence of antibiotic resistance in patients and the environment. To assess this issue, we conducted a literature review on QAC chemical structure, fields of application, mechanism of action, susceptibility testing, prevalence, and co- or cross-resistance to antibiotics. Special attention was paid to the effects of QACs on microflora; in particular, the issue of the potential of QACs for applying selective pressure on multiple-antibiotic-resistant organisms was raised. It was found that there is a lack of standardised procedures for interpreting susceptibility test results. QACs have different impacts on the minimum inhibitory concentrations of antibacterials depending on the antibacterial compound investigated, the resistance genes involved, the measuring methodology and the interpretative criteria. The unmet needs for adequate detection of reduced susceptibility to QACs and antibiotics include (i) a consensus definition for resistance, (ii) epidemiological cut-off values and (iii) clinical resistance breakpoints. This review advocates the design of international guidelines for QAC use.

Table 1
Minimum inhibitory concentrations (MICs) of different quaternary ammonium compounds (QACs) according to the literature.

QAC	Bacteria	MIC (mg/L)	Reference
Benzalkonium chloride	*Pseudomonas aeruginosa* NCIMB 10421	25.4	Joynson et al. [142]
10% w/v benzalkonium chloride, monoquaternary mixture of alkyldimethylbenzylammonium chlorides	*Bacillus stearothermophilus* ATCC 7953	156	Penna et al. [143]
	Escherichia coli ATCC 25922	59	
	Enterobacter cloacae IAL 1976	78	
	Serratia marcescens IAL 1478	59	
	Staphylococcus aureus ATCC 25923	59	
Didecyldimethylammonium chloride (DDDMAC or DDAC)	*S. aureus* ATCC 9518	5	Walsh et al. [144]
	E. coli ATCC 10536	5	
	P. aeruginosa ATCC 15442	500	
	S. aureus ATCC 6538	0.4	Ioannou et al. [20]
N-alkyldimethylbenzylammonium chloride [blend of C_{14} (50%), C_{12} (40%) and C_{16} (10%) homologues]	*S. aureus* ATCC 6538	0.7	Ioannou et al. [20]
N-alkyltrimethylammonium bromide ($C_8/C_{10}/C_{12}/C_{14}/C_{16}/C_{18}$)	*S. aureus* ATCC 6538	594/79.4/7.9/1.22/0.51/1.02	Lambert and Pearson [145]
	P. aeruginosa ATCC 2730	4844/1462/346/83.7/>1000/>1000	
Bardac (commercial twin-chain dimethyl ammonium chloride)	*Aeromonas hydrophila* MBRG 4.3	15.6	McBain et al. [135]
	Pseudomonas sp. strain MBRG 4.7	15.6	
	Enterococcus saccharolyticus MBRG 20.4	31.2	
	Citrobacter sp. strain MBRG 20.9	7.8	
	Sphingobacterium multivorum MBRG 30.1	3.9	

Antiseptic "Resistance": Real or Perceived Threat?

Albert T. Sheldon, Jr.
Antibiotic and Antiseptic Consultants, Silver Spring, Maryland

Biocides (antiseptics, disinfectants, preservatives, and sterilants) are critical components of intervention strategies used in clinical medicine for preventing the dissemination of nosocomial diseases. Biocides are also used in community environments for personal hygiene and to prevent cross-contamination with foodborne pathogens. In vitro studies suggest that exposure to biocides results in reduced susceptibility to antibiotics and biocides by intrinsic or acquired mechanisms of resistance. In addition, microorganisms have adapted to biocide exposure by acquiring plasmids and transposons that confer biocide resistance, the same survival strategies to disseminate acquired mechanisms of resistance to biocides as they have for resistance to antibiotics. The scientific community must weigh the risks and benefits of using biocides in clinical and community environments, to determine whether additional precautions are needed to guide biocide development and use. At present, insufficient scientific evidence exists to weigh these risks, and additional research is needed to allow appropriate characterization of risks in clinical and community environments.

Table 1. Typical characteristics and intended use of agents within biocide classes.

Biocide class	Typical characteristics and uses
Antiseptic	Chemical applied to skin or living tissue that kills or inhibits the growth of vegetative microorganisms. Uses include surgical hand scrubs, health care personnel hand washes, preoperative skin prep solutions, and skin prepping before injection.
Disinfectant	Chemical applied to inanimate surfaces that kills or inactivates vegetative forms of bacteria. Uses include noncritical patient-care instruments and house-keeping surfaces.
Preservative	Chemical used to prevent the growth of organisms resulting in product deterioration. Used on multiuse medical products.
Sterilant	Chemical used to kill vegetative and spore-forming bacteria. Used primarily on multiuse medical devices, such as endoscopes.

Biocides – resistance, cross-resistance mechanisms and assessment

Divya Prakash Gnanadhas, Sandhya Amol Marathe & Dipshikha Chakravortty†

Department of Microbiology and Cell Biology, Centre for Infectious Disease Research and Biosafety Laboratories, Indian Institute of Science, Bangalore, India

Importance of the field: Antibiotic resistance in bacterial pathogens has increased worldwide leading to treatment failures. Concerns have been raised about the use of biocides as a contributing factor to the risk of antimicrobial resistance (AMR) development. *In vitro* studies demonstrating increase in resistance have often been cited as evidence for increased risks. It is therefore important to understand the mechanisms of resistance employed by bacteria toward biocides used in consumer products and their potential to impart cross-resistance to therapeutic antibiotics.

Areas covered: In this review, the mechanisms of resistance and cross-resistance reported in the literature toward biocides commonly used in consumer products are summarized. The physiological and molecular techniques used in describing and examining these mechanisms are reviewed and application of these techniques for systematic assessment of biocides for their potential to develop resistance and/or cross-resistance is discussed.

Expert opinion: The guidelines in the usage of biocides in household or industrial purpose should be monitored and regulated to avoid the emergence of any MDR strains. The genetic and molecular methods to monitor the resistance development to biocides should be developed and included in preclinical and clinical studies.

Keywords: biguanides, biocide resistance, biocides, cross-resistance, disinfectant, quaternary ammonium compound, resistance detection

Table 1. Examples of clinically relevant efflux pumps in Gram-negative bacteria.

Organism	Efflux pump	Substrates	Ref.
Escherichia coli	AcrAB-TolC	Aromatic hydrocarbons, benzalkonium, β-lactams, novobiocin, erythromycin, fusaric acid, fluoroquinolones, tetracycline, chloramphenicol, ethidium bromide, acriflavine, crystal violet, SDS, Triton X-100, bile salts, triclosan, fatty acids, methotrexate, linezolid	[3–5]
Salmonella enterica	AcrAB-TolC	Bile salts, SDS, deoxycholate, acriflavine, fatty acids, novobiocin, erythromycin, chloramphenicol, Triton X-100, crystal violet, rifampicin, tetracycline, cholate, norfloxacin, nalidixic acid, β-lactams, fluoroquinolones	[6–8]
Pseudomonas aeruginosa	MexAB-OprM	Quinolones, macrolides, tetracyclines, lincomycin, chloramphenicol, novobiocin, β-lactams except imipenem	[9–11]
	MexCD-OprJ	Quinolones, macrolides, tetracyclines, lincomycin, chloramphenicol, novobiocin, penicillins except carbenicillin and sulbenicillin, cephems except ceftazidime, flomoxef, meropenem, S-4661	[9–11]
	MexXY-OprM	Quinolones, macrolides, tetracyclines, lincomycin, chloramphenicol, aminoglycosides, penicillins except carbenicillin and sulbenicillin, cephems except cefsulodin and ceftazidime, meropenem, S-4661	[9–11]
Acinetobacter baumannii	AdeABC	Aminoglycosides, cefotaxime, fluoroquinolones, tetracyclines, chloramphenicol, erythromycin and trimethoprim	[12]
Campylobacter jejuni	CmeABC	Fluoroquinolones, erythromycin, β-lactams, rifampicin, tetracycline, ethidium bromide, SDS, deoxycholate, chloramphenicol, gentamicin, acridines	[13,14]
Neisseria gonorrhoeae	MtrCDE	Capric acid, palmitic acid, cholic acid, crystal violet, Triton X-100, erythromycin	[15]

Table 3. Biocide and antibiotic cross-resistance.

S. No.	Organism	Biocide resistance	Altered resistance to antibiotics	Mechanism
1	*P. aeruginosa*	Triclosan	Ciprofloxacin	Mutation in *nfxG* gene [79]
2	*E. coli*	BC, didecyl dimethyl ammonium chloride, dioctyl dimethyl ammonium chloride	Ceftazidime, cefotaxime, chloramphenicol, florfenicol	Enhanced efflux system [81]
3	*Salmonella*	Triclosan	Chloramphenicol, erythromycin, imipenem, tetracycline	Active efflux pumps [84]
4	*Mycobacterium*	Triclosan	Isoniazid	*inhA* mutations [157]
5	*S. aureus*	Triclosan	Ciprofloxacin	Alteration in cell membrane structure and function [158]
6	*Campylobacter jejuni* and *Campylobacter coli*	Triclosan, BC	Erythromycin and Ciprofloxacin	Efflux pumps [40]
7	*Citrobacter freundii*	Triclosan	Erythromycin	Outer membrane adaptation [159]

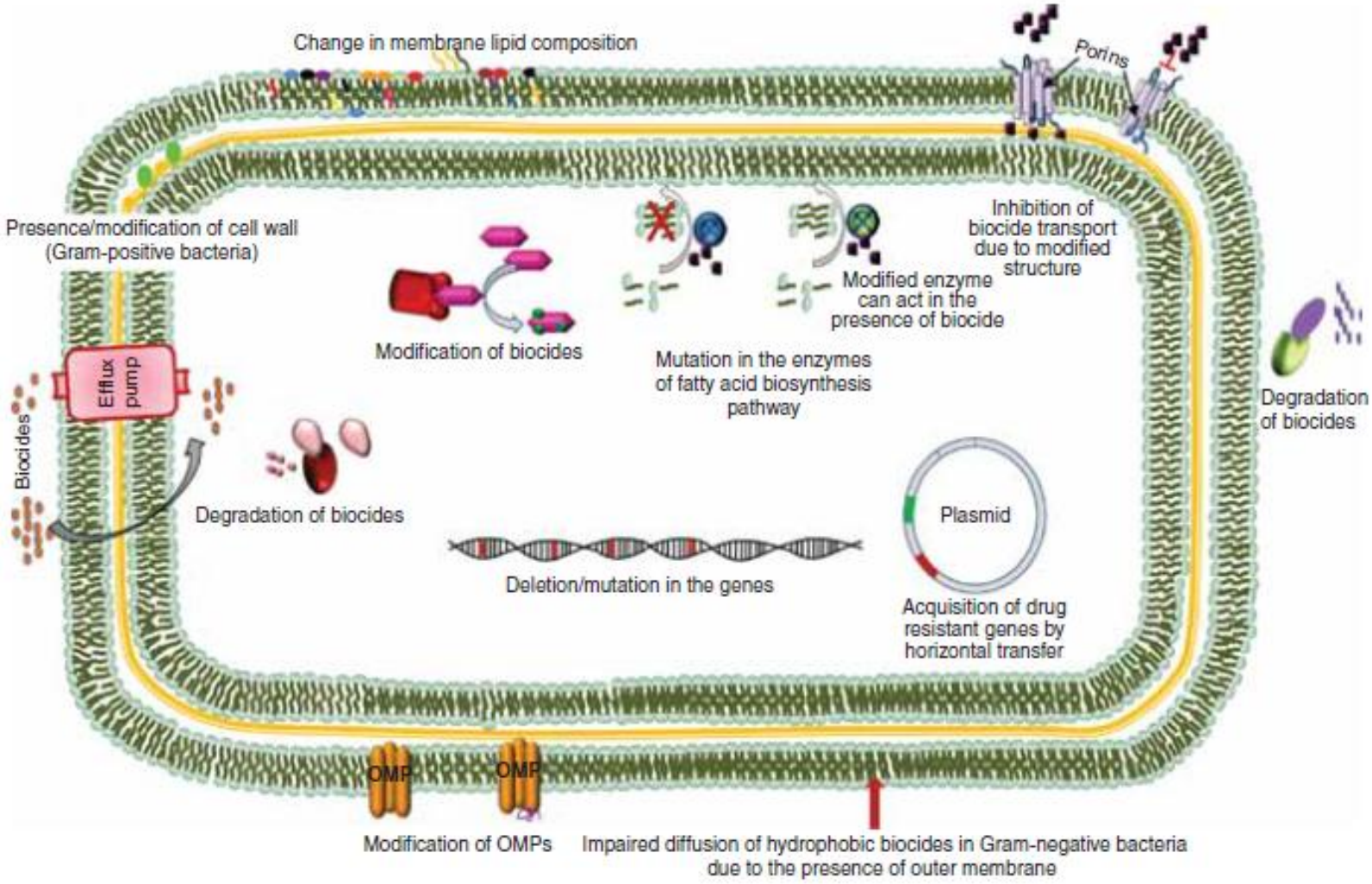


Figure 2. Various mechanisms of resistance against biocides in bacteria. Bacteria are inherently resistant to biocide (intrinsic resistance) or can gain resistance to different biocides (acquired resistance) via different mechanism. Intrinsic resistance is achieved through presence of cell wall, efflux system, etc. The resistance can also be achieved through mutation in genes that are responsible for the formation of cell wall, membrane lipid, porins or OMPs. Acquisition of mobile genetic elements like plasmids through horizontal transfer is another mechanism by which the bacteria gain resistance. Certain genes that encode for protein that can modify or degrade the biocide can be formed either through alteration in preexisting genes or through genes acquired through horizontal transfer.

TABLE 5. Intrinsic resistance mechanisms in bacteria to antiseptics and disinfectants

Type of resistance	Example(s)	Mechanism of resistance
Impermeability		
Gram-negative bacteria	QACs, triclosan, diamines	Barrier presented by outer membrane may prevent uptake of antiseptic or disinfectant; glycocalyx may also be involved
Mycobacteria	Chlorhexidine, QACs Glutaraldehyde	Waxy cell wall prevents adequate biocide entry Reason for high resistance of some strains of *M. chelonae*(?)
Bacterial spores	Chlorhexidine, QACs, phenolics	Spore coat(s) and cortex present a barrier to entry of antiseptics and disinfectants
Gram-positive bacteria	Chlorhexidine	Glycocalyx/mucoexopolysaccaride may be associated with reduced diffusion of antiseptic
Inactivation (chromosomally mediated)	Chlorohexidine	Breakdown of chlorhexidine molecule may be responsible for resistance

TABLE 6. MIC of some antiseptics and disinfectants against gram-positive and gram-negative bacteria[a]

Chemical agent	MIC (μg/ml) for:		
	S. aureus[b]	*E. coli*	*P. aeruginosa*
Benzalkonium chloride	0.5	50	250
Benzethonium chloride	0.5	32	250
Cetrimide	4	16	64–128
Chlorhexidine	0.5–1	1	5–60
Hexachlorophene	0.5	12.5	250
Phenol	2,000	2,000	2,000
o-Phenylphenol	100	500	1,000
Propamine isethionate	2	64	256
Dibromopropamidine isethionate	1	4	32
Triclosan	0.1	5	>300

[a] Based on references 226 and 440.
[b] MICs of cationic agents for some MRSA strains may be higher (see Table 10).

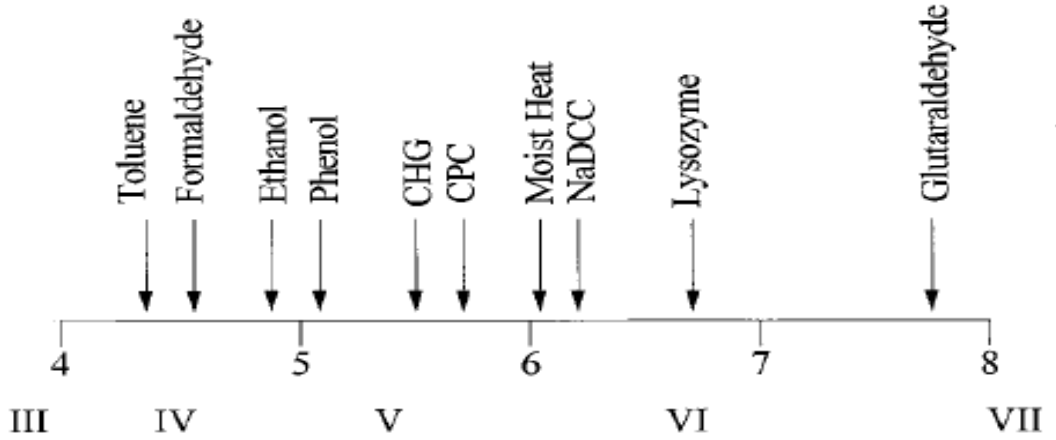


FIG. 2. Development of resistance of *Bacillus subtilis* during sporulation. Roman numerals indicate the sporulation stage from III (engulfment of the forespore) to VII (release of the mature spore). Arabic numbers indicate the time (hours) following the onset of sporulation and the approximate times at which resistance develops against biocides (262). CHG, chlorhexidine; CPC, cetylpyridinium chloride; NaDCC, sodium dichloroisocyanurate.

TABLE 8. Biofilms and microbial response to antimicrobial agents

Mechanism of resistance associated with biofilms	Comment
Exclusion or reduced access of antiseptic or disinfectant to underlying cell	Depends on (i) nature of antiseptic/disinfectant, (ii) binding capacity of glycocalyx toward antiseptic or disinfectant, and (iii) rate of growth of microcolony relative to diffusion rate of chemical inhibitor
Modulation of microenvironment	Associated with (i) nutrient limitation and (ii) growth rate
Increased production of degradative enzymes by attached cells	Mechanism unclear at present
Plasmid transfer between cells in biofilm?	Associated with enhanced tolerance to antiseptics and disinfectants?

TABLE 9. Possible mechanisms of plasmid-encoded resistance to antiseptics and disinfectants

Chemical agent	Examples	Mechanisms
Antiseptics or disinfectants	Chlorhexidine salts	(i) Inactivation: not yet found to be plasmid mediated; chromosomally mediated inactivation; (ii) efflux: some *S. aureus*, some *S. epidermidis*; (iii) Decreased uptake(?)
	QACs	(i) Efflux: some *S. aureus*, some *S. epidermidis*; (ii) Decreased uptake(?)
	Silver compounds	Decreased uptake; no inactivation (cf. mercury compounds)
	Formaldehyde	(i) Inactivation by formaldehyde dehydrogenase; (ii) Cell surface alterations (outer membrane proteins)
	Acridines[a]	Efflux: some *S. aureus*, some *S. epidermidis*
	Diamidines	Efflux: some *S. aureus*, some *S. epidermidis*
	Crystal violet[a]	Efflux: some *S. aureus*, some *S. epidermidis*
Other biocides	Mercurials[b]	Inactivation (reductases, lyases)
	Ethidium bromide	Efflux: some *S. aureus*, some *S. epidermidis*

[a] Now rarely used for antiseptic or disinfectant purposes.
[b] Organomercurials are still used as preservatives.

TABLE 11. *qac* genes and resistance to quaternary ammonium compounds and other antiseptics and disinfectants

Multidrug resistance determinant[a]	Gene location	Resistance encoded to
qacA	pSK1 family of multiresistant plasmids, also β-lactamase and heavy-metal resistance families	QACs, chlorhexidine salts, diamidines, acridines, ethidium bromide
qacB	β-Lactamase and heavy-metal resistance plasmids	QACs, acridines, ethidium bromide
qacC[b]	Small plasmids (<3 kb) or large conjugative plasmids	Some QACs, ethidium bromide
qacD[b]	Large (50-kb) conjugative, multiresistance plasmids	Some QACs, ethidium bromide

[a] The *qacK* gene has also been described, but it is likely to be less significant than *qacAB* in terms of antiseptic or disinfectant tolerance.
[b] These genes have identical target sites and show restriction site homology.

TABLE 12. Possible mechanisms of fungal resistance to antiseptics and disinfectants

Type of resistance	Possible mechanism	Example(s)
Intrinsic	Exclusion	Chlorhexidine
	Enzymatic inactivation	Formaldehyde
	Phenotypic modulation	Ethanol
	Efflux	Not demonstrated to date[a]
Acquired	Mutation	Some preservative
	Inducible efflux	Some preservatives[a]
	Plasmid-mediated responses	Not demonstrated to date

[a] Efflux is now known to be one mechanism of fungal resistance to antibiotics (531).

TABLE 13. Parameters affecting the response of *S. cerevisiae* to chlorhexidine[a]

Parameter	Role in susceptibility of cells to chlorhexidine
Cell wall composition	
Mannan	No role found to date
Glucan	Possible significance: at concentrations below those active against whole cells, chlorhexidine lyses protoplasts
Cell wall thickness	Increases in cells of older cultures: reduced chlorhexidine uptake responsible for decreased activity(?)
Relative porosity	Decreases in cells of older cultures: reduced chlorhexidine uptake responsible for decreased activity(?)
Plasma membrane	Changes altering CHG susceptibility(?); not investigated to date

[a] Data from references 204 to 208 and 436.

TABLE 14. Lethal concentrations of antiseptics and disinfectants toward some yeasts and molds[a]

Antimicrobial agent[b]	Lethal concn (μg/μl) toward:		
	Yeast (*Candida albicans*)	Molds	
		Penicillium chrysogenum	*Aspergillus niger*
QACs			
Benzalkonium chloride	10	100–200	100–200
Cetrimide/CTAB	25	100	250
Chlorhexidine	20–40	400	200

[a] Derived in part from data in reference 525.
[b] CTAB, cetyltrimethylammonium bromide.

TABLE 16. Viral classification and response to some disinfectants[a]

Viral group	Lipid envelope[b]	Examples of viruses	Effects of disinfectants[c]	
			Lipophilic	Broad-spectrum
A	+	HSV, HIV, Newcastle disease virus, rabies virus, influenza virus	S	S
B	−	Non-lipid picornaviruses (poliovirus, Coxsackie virus, echovirus)	R	S
C	−	Other larger nonlipid viruses (adenovirus, reovirus)	R	S

[a] Data from reference 259; see also reference 444. For information on the inactivation of poliovirus, see reference 514.

[b] Present (+) or absent (−).

[c] Lipophilic disinfectants include QACs and chlorhexidine. S, sensitive; R, resistant.

TABLE 15. Kinetic approach: *D*-values at 20°C of phenol and benzalkonium chloride against fungi and bacteria[a]

Antimicrobial agent	pH	Concn (%, wt/vol)	*D*-value (h)[b] against:				
			Aspergillus niger	*Candida albicans*	*Escherichia coli*	*Pseudomonas aeruginosa*	*Staphylococcus aureus*
Phenol	5.1	0.5	20	13.5	0.94	—[c]	0.66
	6.1	0.5	32.4	18.9	1.72	0.17	1.9
Benzalkonium chloride	5.1	0.001	—[d]	9.66	0.06	3.01	3.12
	6.1	0.002	—[d]	5.5	—[c]	0.05	0.67

[a] Abstracted from the data in references 244 and 245.
[b] *D*-values are the times to reduce the viable population by 1 log unit.
[c] Inactivation was so rapid that the *D*-values could not be measured.
[d] No inactivation: fungistatic effect only.

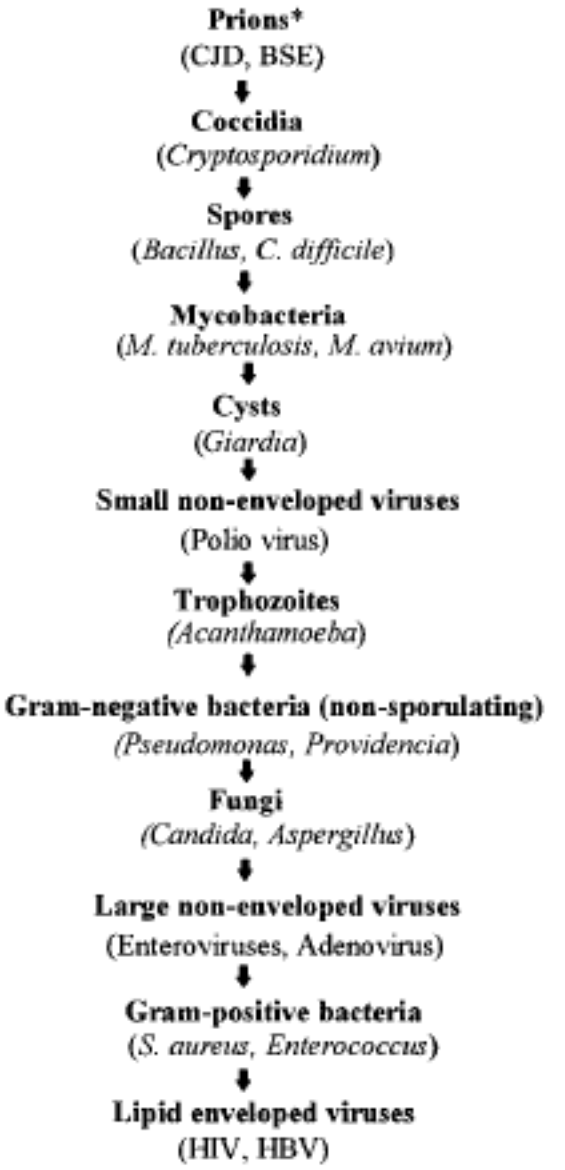


FIG. 1. Descending order of resistance to antiseptics and disinfectants. The asterisk indicates that the conclusions are not yet universally agreed upon.

Muchas gracias!

Roberto Ricardo Grau

Universidad Nacional de Rosario – Facultad de Ciencias Bioquímicas y Farmacéuticas
Pew Latin American Fellow – Philadelphia- EE.UU.
Fulbright International Scholar – Washignton – EE.UU.
CONICET – Argentina
Teléfono: 54-341-4353377 /4804592 (INTERNO 286)
robertograu.docencia@gmail.com
WWW.MICROBIOLOGIAROSARIO.ORG / WWW.MICROBIOLOGYROSARIO.ORG