Al-Qadisiyah University
College of
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Multiple Drug resistance in gram positive pathogen

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BY

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بِسْمِ ٱللَّهِ ٱلرَّحْمَٰنِ ٱلرَّحِيمِ

فَتَعَٰلَى ٱللَّهُ ٱلْمَلِكُ ٱلْحَقُّ ۗ وَلَا تَعْجَلْ بِٱلْقُرْءَانِ مِن قَبْلِ أَن يُقْضَىٰٓ
إِلَيْكَ وَحْيُهُۥ ۖ وَقُل رَّبِّ زِدْنِى عِلْمًا ﴿١١٤﴾

صَدَقَ اللهُ العَظِيمُ

من سورة طه

DEDICATE TO

To whom my heart is happy to meet. To the kindergarten of love that grows the most beautiful flowers ..

My mother ..

To the symbol of manliness and sacrifice ..

To the one who pushed me to the knowledge and in it increased pride my father ..

To those who are closer To me who is my soul To those who shared with me the embrace of pain and with them I draw my pride and my determination My brothers ..

To whoever forgot me in my studies and shared my concerns Remembrance and appreciation..

My friends ..

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Abstract

The discovery of antibiotics has created a turning point in medical interventions to pathogenic infections, but unfortunately, each discovery was consistently followed by the emergence of resistance.The emergence of the resistance in microbial population is a major threat to both animal and human health**.** Mechanisms of antimicrobial resistance in microbes are of natural as well as acquired origin. There are half dozen molecular mechanisms identified that possibly cause the emergence and transfer of antimicrobial resistance within and between different bacterial genera. Genomic duplication, enzymatic site modification, target alteration, modulation in membrane permeability, and the efflux pump mechanism are the major contributors of multidrug resistance (MDR). Efflux pumps are present in all cells, from human to bacteria and are highly conserved, which indicates that they are ancient elements in the evolution of different organisms. Multidrug efflux pumps can be involved in bacterial resistance to antibiotics at different levels. Some efflux pumps are constitutively expressed at low levels and contribute to intrinsic resistance. In addition, their overexpression may allow higher levels of resistance. This overexpression can be transient, in the presence of an effector (phenotypic resistance), or constitutive when mutants in the regulatory elements of the expression of efflux pumps are selected (acquired resistance).Some important aspect of the multriresistance mechanism in gram positive pathogen will briefly discuss in this report.

Gram positive pathogen

Health professionals need to understand the important difference between gram-positive and gram-negative bacteria. Gram-positive bacteria are bacteria classified by the color they turn in the staining method. Hans Christian Gram developed the staining method in 1884. The staining method uses crystal violet dye, which is retained by the thick peptidoglycan cell wall found in gram-positive organisms. This reaction gives gram-positive organisms a blue color when viewed under a microscope. Although gram-negative organisms classically have an outer membrane, they have a thinner peptidoglycan layer, which does not hold the blue dye used in the initial dying process. Other information used to differentiate bacteria is the shape. Gram-positive bacteria comprise cocci, bacilli, or branching filaments. Gram-positive cocci include Staphylococcus (catalase-positive), which grows clusters, and Streptococcus (catalase-negative), which grows in chains. The staphylococci further subdivide into coagulase-positive (S. aureus) and coagulase-negative (S. epidermidis and S. saprophyticus) species. Streptococcus bacteria subdivide into Strep. pyogenes (Group A), Strep. agalactiae (Group B), enterococci (Group D), Strep viridans, and Strep pneumonia.Gram-positive bacilli (rods) subdivide according to their ability to produce spores. Bacillus and Clostridia are spore-forming rods while Listeria and Corynebacterium are not. Spore-forming rods that produce spores can survive in environments for many years. Also, the branching filament rods encompass Nocardia and actinomyces .Gram-positive organisms have a thick peptidoglycan cell wall compared with gram-negative bacteria. It is a 20 to 80 nm thick polymer while the peptidoglycan layer of the gram-negative cell wall is 2 to 3 nm thick and covered with an outer lipid bilayer membrane (Sizar *et al* ., 2021).

Pathophysiology

Gram-positive cocci:

Staphylococcus aureus is a gram-positive, catalase-positive, coagulase-positive cocci in clusters. *S. aureus* can cause inflammatory diseases, including skin infections, pneumonia, endocarditis, septic arthritis, osteomyelitis, and abscesses. *S. aureus* can also cause toxic shock syndrome (TSST-1), scalded skin syndrome (exfoliative toxin, and food poisoning (enterotoxin). Staphylococcus epidermidis is a gram-positive, catalase-positive, coagulase-negative cocci in clusters and is novobiocin sensitive. S. epidermidis commonly infects prosthetic devices and IV catheters producing biofilms. Staphylococcus saprophyticus is novobiocin resistant and is a normal flora of the genital tract and perineum. S. saprophyticus accounts for the second most common cause of uncomplicated urinary tract infection (UTI).

Streptococcus pneumoniae is a gram-positive, encapsulated, lancet-shaped diplococci, most commonly causing otitis media, pneumonia, sinusitis, and meningitis. Streptococcus viridans consist of Strep. Mutants and Strep mites found in the normal flora of the oropharynx commonly cause dental caries and subacute bacterial endocarditis (Strep. Sanguinis).

Streptococcus pyogenes is a gram-positive group A cocci that can cause pyogenic infections (pharyngitis, cellulitis, impetigo, erysipelas), toxigenic infections (scarlet fever, necrotizing fasciitis), and immunologic infections (glomerulonephritis and rheumatic fever). ASO titer detects S. pyogenes infections.

Streptococcus agalactiae is a gram-positive group B cocci that colonize the vagina and is found mainly in babies. Pregnant women need screening for Group-B Strep (GBS) at 35 to 37 weeks of gestation .

Enterococci is a gram-positive group D cocci found mainly in the colonic flora and can cause biliary tract infections and UTIs. Vancomycin-resistant enterococci (VRE) are an important cause of nosocomial infections .

Gram-positive rods:

Clostridia is a gram-positive spore-forming rod consisting of C. tetani, C. botulinum, C. perfringens, and C. difficile. C. difficile is often secondary to antibiotic use (clindamycin/ampicillin), PPI use, and recent hospitalization. Treatment involves primarily with oral vancomycin.

Bacillus anthracis is a gram-positive spore-forming rod that produces anthrax toxin resulting in an ulcer with a black eschar. Bacillus cereus is a gram-positive rod that can be acquired from spores surviving under-cooked or reheated rice. Symptoms include nausea, vomiting, and watery non-bloody diarrhoea .Corynebacterium diphtheria is a gram-positive club-shaped rod that can cause pseudomembranous pharyngitis, myocarditis, and arrhythmias.

Toxoid vaccines prevent diphtheria.Listeria monocytogenes is a gram-positive rod acquired by the ingestion of cold deli meats and unpasteurized dairy products or by vaginal transmission during birth. Listeria can cause neonatal meningitis, meningitis in immunocompromised patients, gastroenteritis, and septicaemia. Treatment includes ampicillin. (Sizar *et al* ., 2021).

-Molecular Mechanism of Drug Resistance

Antimicrobial compounds include antibiotics as well as many other substances which are used to kill or inhibit the growth (multiplication) of bacteria. But nowadays, many bacteria causing diseases of pandemic (e.g., tuberculosis) importance are increasingly developing the resistance against a myriad of the antimicrobial compounds which, in terms, is leading to the ineffective treatment of many fatal human and animal disease outbreaks (Ashraf *et al*., 2019).

Genomic duplication, enzymatic site modification, target alteration, modulation in membrane permeability, and the efflux pump mechanism are the major contributors of multidrug resistance (MDR) (Figure 1), specific antibiotic tolerance development. MDRs will lead to clinical failures for treatment and pose health crisis. The molecular mechanisms of antimicrobial resistance are diverse as well as complex and still are exploited for new discoveries in order to prevent the surfacing of "superbugs."(Longley & Johnston, 2005)

Genomic duplication

Gene duplication (or chromosomal duplication or gene amplification) is a major mechanism through which new genetic material is generated during molecular evolution. It can be defined as any duplication of a region of DNA that contains a gene. Changes in gene copy number are among the most frequent mutational events in all genomes and were among the mutations for which a physical basis was first known, Yet the mechanisms of gene duplication remain uncertain because formation rates are difficult to measure and mechanisms may vary with position in a genome(Reams & Roth, 2015).

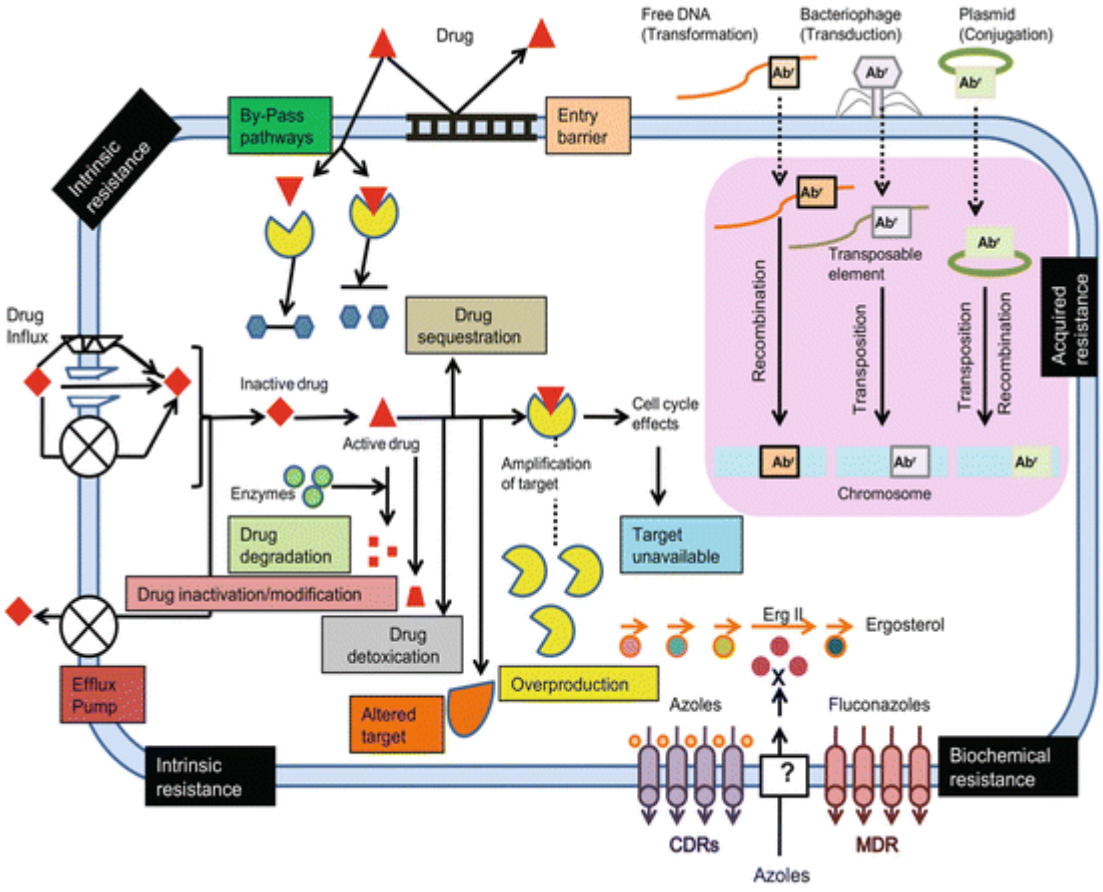


Fig 1 : Schematic presentation of multiple diverse molecular mechanism of microbial resistance

Gene duplication is an event in which one gene gives rise to two genes that cannot be operationally distinguished from each other. The duplicated genes remain in the same genome. Gene duplications are among the oldest and perhaps the most frequent of mutation types . In the bacterial kingdom, gene duplication has been associated with survival in extreme or fluctuating conditions, including exposure to antimicrobial compounds or growth on poor nutrient sources, and may have a role in the Co evolution between host and pathogens. duplication phenomenon has also been observed in S. aureus with respect to methicillin resistance. Genome amplification forms one of the mechanisms of resistance avoiding the boundaries of mutational aspects (Sanchez-Herrero *et al*., 2020).

Enzymatic site modification

Several drugs act by their initial binding to a particular site within bacterial cells in order to initiate their bactericidal/bacteriostatic activity. So when a drug binding target is changed, it may lead to the development of the resistance in bacteria against that drug. This phenomenon has been incriminated in the emergence of the resistance against antibiotics such as the macrolides and phenolics (Chloramphenicol) in which alternations in the binding sites at ribosome drastically reduce the antibacterial activity of these drugs.

Mobile Genetic Elements Associated with Antimicrobial Resistance

Mobile genetic elements MGEs are discrete regions of DNA defined by their ability to move within and/or between bacterial cells(Figure 2). They are categorized into types based on their properties and their genetic layout. Elements capable of integrating into the host DNA are referred to here as integrating MGEs (iMGEs) (Johansson et al., 2021) Insertion sequences (IS) and transposons (Tn) are discrete

DNA segments that are able to move themselves (and associated resistance genes) almost randomly to new locations in the same or different DNA molecules within a single cell. Other elements, such as integrons (In), use site-specific recombination to move resistance genes between defined sites. As these types of MGE are often present in multiple copies in different locations in a genome, they can also facilitate homologous recombination (exchange of sequences between identical or related segments). Intercellular mechanisms of genetic exchange include conjugation/mobilization (mediated by plasmids and integrative conjugative elements [ICE]), transduction (mediated by bacteriophages), and transformation (uptake of extracellular DNA). Interactions between the various types of MGE underpin the rapid evolution of diverse multiresistant pathogens in the face of antimicrobial chemotherapy. Examples of these elements and processes are illustrated in Fig. 1.(Partridge *et al*., 2018)

A survey of 280 geographically and epidemiologically diverse staphylococci (n = 251 S. aureus strains) revealed that three plasmid lineages, represented by pIB485, pMW2, and pUSA300-HOU-MR,encompassed more than half of all the multiresistance plasmids detected (338). pIB485- and pMW2-like plasmids were widely distributed geographically, whereas pUSA300-HOU-MR-like plasmids were found only in isolates from the United States. All three lineages usually carry Tn552-derived β-lactamase genes and genes for cadmium resistance; pUSA300-HOU-MR-like plasmids often also carry genes for resistance to macrolides, aminoglycosides, and bacitracin, while enterotoxin genes are a common feature of pIB485-like plasmids. (Partridge *et al*., 2018)

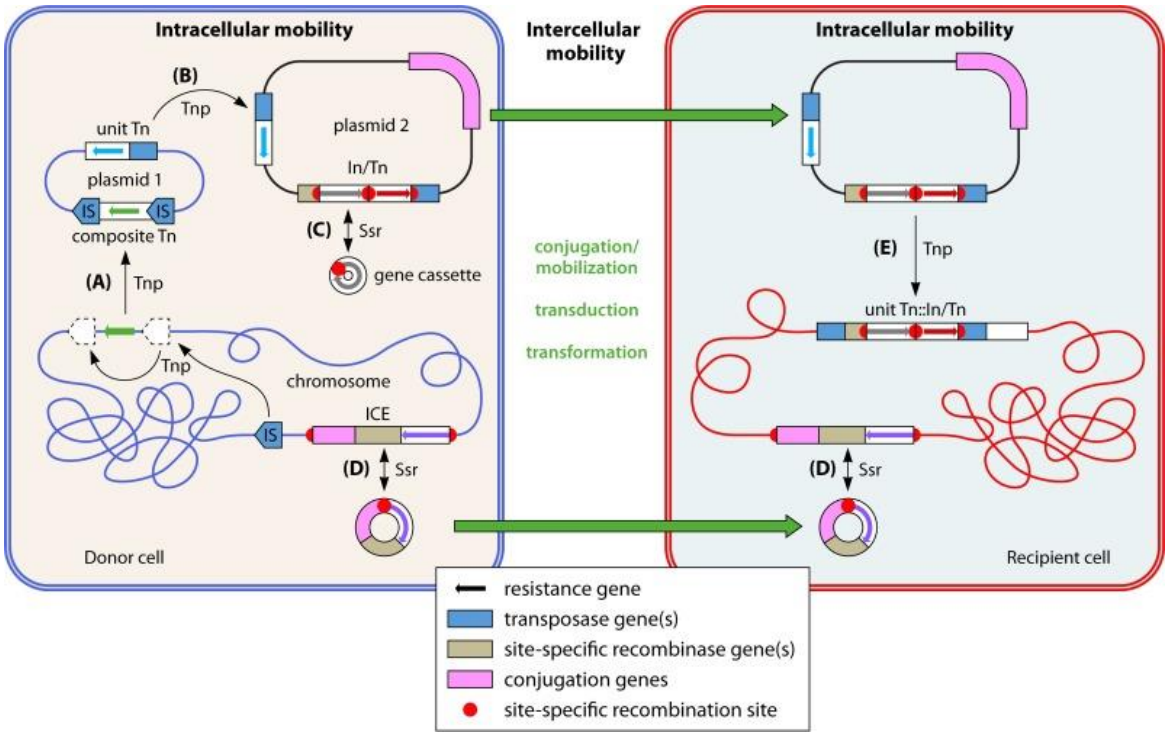


Figure (2) : mobile genetic elements (MGE) and processes involved in intracellular mobility or intercellular transfer of antibiotic resistance genes. Two cells of different strains or species are represented, with one acting as donor (envelope and chromosome shown in blue; contains two plasmids) and the other as recipient (shown in red). Various MGE is shown, with the functions of the genes they carry colour coded as shown in the key. Different resistance genes associated with different MGE are represented by small arrows of various colours. Thin black arrows indicate intracellular processes, with those mediated by a transposase protein labelled Tnp and those mediated by a site-specific recombinase protein labelled Ssr. Thick green arrows represent intercellular (horizontal) transfer. Successive insertions of the same IS on both sides of a resistance gene may allow it to be captured and moved to another DNA molecule (e.g., from the chromosome to a plasmid) as part of a composite ten (A). A unit than carrying a resistance gene may transpose between plasmids (B) or from a plasmid to the chromosome or vice versa. A gene cassette may move between Inside (a class 1 In/ton structure is represented here) via a circular intermediate (C). An ICE can be integrated into the chromosome or excised as a circular element that can then conjugate into a recipient cell and integrate (reversibly) into the chromosome at a specific recombination site (D). A plasmid may be able to mediate its own intercellular transfer by conjugation or, if it lacks a conjugation region, be mobilized by another plasmid (or, alternatively, move horizontally by phage transduction or transformation). Tn and/or In and associated resistance genes on an incoming plasmid may move into the chromosome or other plasmid(s) in the recipient cell (E), as illustrated here for class 1 In/Tn, which are known to target unit Tn. See relevant sections of the text for further details.

Table (1) IS and composite transposons associated with resistance genes in staphylococci and enterococci

IS[a]	Tn	Determinant	Associated resistance(s)	Host[b]
IS*16*	Tn*1547*	*vanB1*	Vancomycin	E
IS*256*		*cfr*	Phenicols/lincosamides/oxazolidinones/pleuromutilins/streptogramin A	S
	Tn*1547*	*vanB1*	Vancomycin	E
	Tn*4001*	*aacA-aphD*	Gentamicin/kanamycin/tobramycin	S
	Tn*5281*	*aacA-aphD*	Gentamicin/kanamycin/tobramycin	E
	Tn*5384*	*aacA-aphD*	Gentamicin/kanamycin/tobramycin	E
	Tn*53*	*erm*(B)	MLS antibiotics	E

	84			
IS*25 7* [c]		*aadD*	Kanamycin/neomycin/paromomycin/tobramycin	S
		aphA-3	Kanamycin/neomycin	S
		bcrAB	Bacitracin	S
		ble	Bleomycin	S
		dfrK	Trimethoprim	S
		erm(C)	MLS antibiotics	S
		fosB5	Fosfomycin	S
		fusB	Fusidic acid	S
		ileS2 (*mupA*)	Mupirocin	S
		qacC	Antiseptics/disinfectants	S
		sat4	Streptothricin	S

		tet(K)	Tetracycline	S
		tet(L)	Tetracycline	S
		vat(A)	Streptogramin A	S
		vga(A)	Streptogramin A/pleuromutilins/lincosamides	S
		vgb(A)	Streptogramin B	S
	Tn*924*	*aacA-aphD*	Gentamicin/kanamycin/tobramycin	E
	Tn*4003*	*dfrA*	Trimethoprim	S
	Tn*6072*	*aacA-aphD*	Gentamicin/kanamycin/tobramycin	S
	Tn*6072*	*spc*	Spectinomycin	S
IS*11*	Tn*54*	*aadE*	Streptomycin	S, E

82	*05*			
	Tn*5405*	*aphA-3*	Kanamycin/neomycin	S, E
	Tn*5405*	*sat4*	Streptothricin	S, E
IS*1216*		*cfr*	Phenicols/lincosamides/oxazolidinones/pleuromutilins/streptogramin A	E
		str	Streptomycin	E
	Tn*5385*	*aacA-aphD*	Gentamicin/kanamycin/tobramycin	E
	Tn*5385*	*aadE*	Streptomycin	E
	Tn*5385*	*blaZ*	Penicillins	E
	Tn*5385*	*erm*(B)	MLS antibiotics	E
	Tn*5385*	*tet*(M)	Tetracycline/minocycline	E

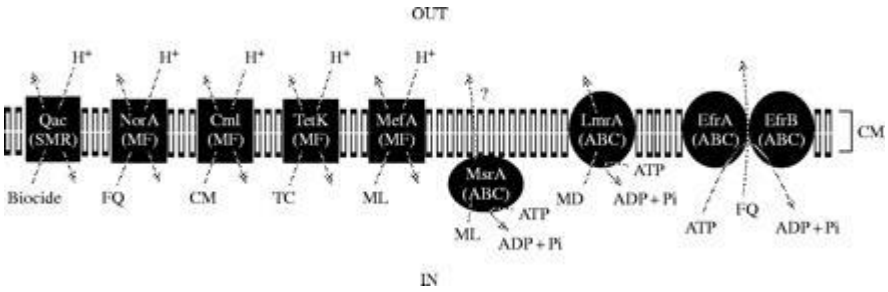
Efflux-mediated antibiotic resistance in Gram-positive bacteria

Bacterial antimicrobial efflux transporters have generally been grouped into four superfamilies, primarily on the basis of amino acid sequence homology. These include the major facilitator superfamily (MFS), the ATP-binding cassette family, the resistance-nodulation-division (RND) family, and the small multidrug resistance protein family. Recently, a fifth family, referred to as the multidrug and toxic compound extrusion (MATE) family, has been identified. Antibiotic efflux pumps fall into the RND, MFS, and MATE groups, with the RND and MATE families so far being unique to gram-negative bacteria. Thus, MFS-type transporters predominate as regards the efflux of antimicrobial agents in gram-positive organisms(Poole, 2000).

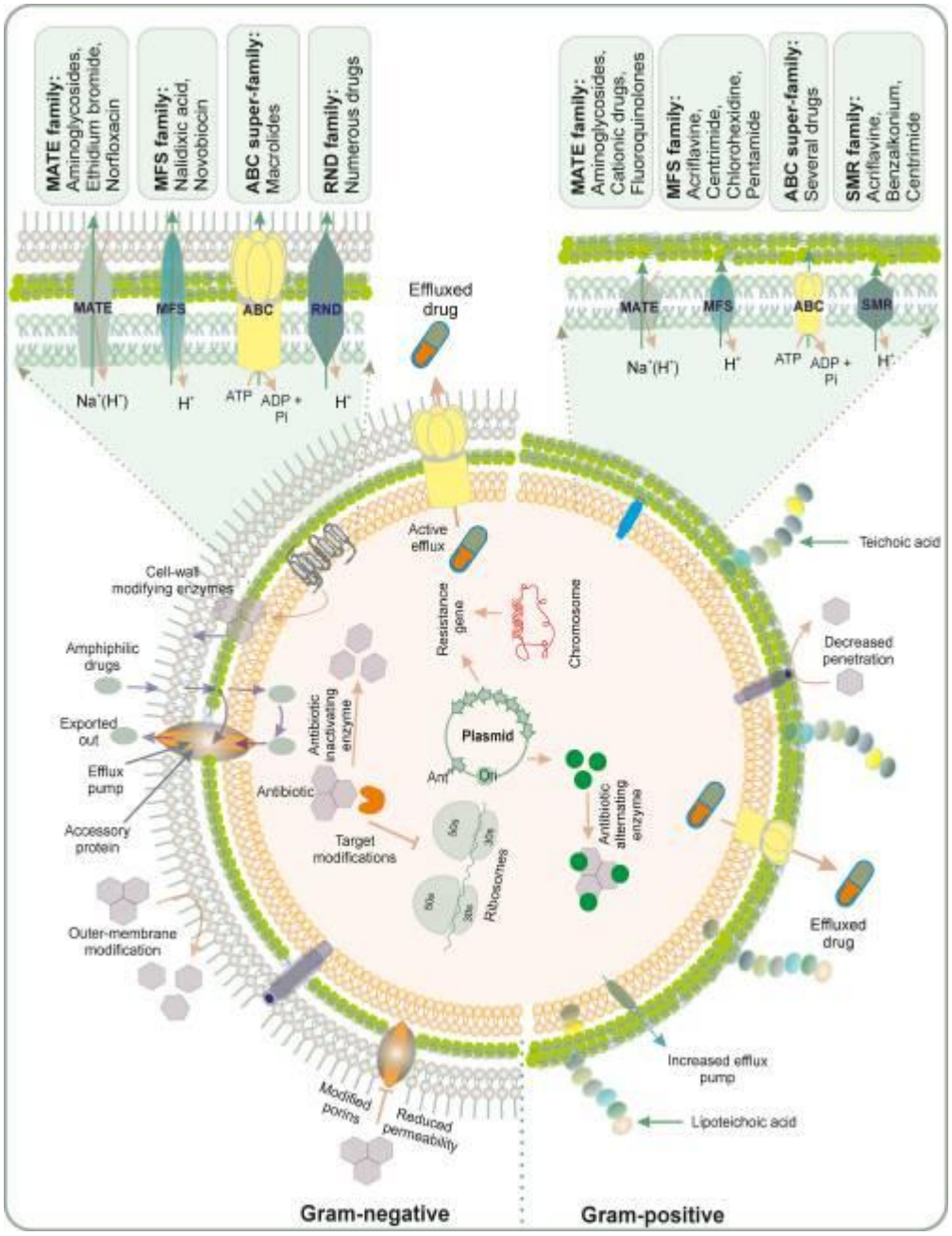
The last of the resistance mechanisms to be identified, efflux was first described as a mechanism of resistance to tetracycline in Escherichia coli, with the plasmid-encoded single component Tet protein export of tetracycline (complexed with Mg2+ it turns out) across the cytoplasmic membrane sufficient for resistance. In the intervening years, numerous plasmid- and chromosome-encoded efflux mechanisms, both agent- or class-specific and multidrug have been described in a variety of organisms where they are increasingly appreciated as important determinants of antimicrobial resistance . The drug efflux pumps in Gram-positive bacteria are usually non-RND pumps and often the singleton protein pumps belonging to the MFS, MATE, SMR or ABC.(Li & Nikaido, 2009).

These multidrug pumps are ubiquitous and are an important class of resistance determinants in pathogens that help to shuttle the substrates across the biological membrane. They recognize varying ranges of antibacterial substrates differing in both valency and structures and are mediated by integral membrane proteins. Antibiotics

such as aminoglycosides, fluoroquinolones, tetracyclines, β-lactams, and macrolides are prone to immediate extracellular efflux.In Gram-positive bacteria, efflux pumps such as cacao, Nor A (Bmr), and Smr pump out solute molecules across a single cytoplasmic membrane layer. The Nora (Bmr) pumps out basic dyes which are lipophilic cations and includes puromycin and fluoroquinolones. In addition, QacA and Smr both export quaternary ammonium compounds and basic dyes. In Gram-negative pathogens, the solutes need to trespass several layers en route to an inner periplasmic membrane. However, efflux pumps from the peptidoglycan or periplasm layer promptly channel substrates extracellularly that enter through the outer -membrane(Baral & Mozafari, 2020)



Schematic diagram of representative drug exporting systems in Gram-positive bacteria, highlighting the different families of pumps involved in resistance. FQ, fluoroquinolone; CM, chloramphenicol; TC, tetracycline; ML, macrolides, MD, multidrug. While NorA is, strictly speaking, a multidrug transporter, it exports only FQs (and biocides) as clinically relevant agents and so it is highlighted here as an MF family efflux determinant of FQ resistance.



Family of different pumps and transporters in Gram-positive and Gram-negative bacteria.

References

Ashraf, M., -Mustafa, B.-E., -Rehman, S.-U., Khalid Bashir, M., & Adnan Ashraf, M. (2019). Emergence of Antimicrobial Resistance, Causes, Molecular Mechanisms, and Prevention Strategies: A Bovine Perspective. In *Bovine Science - A Key to Sustainable Development*. https://doi.org/10.5772/intechopen.79757

Longley, D. B., & Johnston, P. G. (2005). Molecular mechanisms of drug resistance. *Journal of Pathology*. https://doi.org/10.1002/path.1706

Reams, A. B., & Roth, J. R. (2015). Mechanisms of gene duplication and amplification. *Cold Spring Harbor Perspectives in Biology*. https://doi.org/10.1101/cshperspect.a016592

Sanchez-Herrero, J. F., Bernabeu, M., Prieto, A., Hüttener, M., & Juárez, A. (2020). Gene Duplications in the Genomes of Staphylococci and Enterococci. *Frontiers in Molecular Biosciences*. https://doi.org/10.3389/fmolb.2020.00160

Johansson, M. H. K., Bortolaia, V., Tansirichaiya, S., Aarestrup, F. M., Roberts, A. P., & Petersen, T. N. (2021). Detection of mobile genetic elements associated with antibiotic resistance in Salmonella enterica using a newly developed web tool: MobileElementFinder. *Journal of Antimicrobial Chemotherapy*. https://doi.org/10.1093/JAC/DKAA390

Partridge, S. R., Kwong, S. M., Firth, N., & Jensen, S. O. (2018). Mobile genetic elements associated with antimicrobial resistance. *Clinical Microbiology Reviews*. https://doi.org/10.1128/CMR.00088-17

Poole, K. (2000). Efflux-mediated resistance to fluoroquinolones in gram-positive bacteria and the mycobacteria. *Antimicrobial Agents and Chemotherapy*. https://doi.org/10.1128/AAC.44.10.2595-2599.2000

Li, X. Z., & Nikaido, H. (2009). Efflux-mediated drug resistance in bacteria: An update. *Drugs*. https://doi.org/10.2165/11317030-000000000-00000

Baral, B., & Mozafari, M. R. (2020). Strategic Moves of "superbugs" against Available Chemical Scaffolds: Signaling, Regulation, and Challenges. *ACS Pharmacology and Translational Science*. https://doi.org/10.1021/acsptsci.0c00005

Jubeh, B., Breijyeh, Z., & Karaman, R. (2020). Resistance of gram-positive bacteria to current antibacterial agents and overcoming approaches. *Molecules*. https://doi.org/10.3390/molecules25122888

Sizar O, Unakal CG. Gram Positive Bacteria. [Updated 2021 Feb 1]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2021 Jan-. https://www.ncbi.nlm.nih.gov/books/NBK470553/