Acquired inducible antimicrobial resistance in Gram-positive bacteria

Scott T Chancey1,2, Dorothea Zähner1,2, and David S Stephens*,1,2,3
1Division of Infectious Diseases, Department of Medicine, Emory University School of Medicine, Atlanta, GA 30322, USA
2Department of Veterans Affairs Medical Center (Atlanta), Decatur, GA 30033, USA
3Department of Microbiology & Immunology, Emory University School of Medicine, Atlanta, GA 30322, USA

Abstract

A major contributor to the emergence of antibiotic resistance in Gram-positive bacterial pathogens is the expansion of acquired, inducible genetic elements. Although acquired, inducible antibiotic resistance is not new, the interest in its molecular basis has been accelerated by the widening distribution and often ‘silent’ spread of the elements responsible, the diagnostic challenges of such resistance and the mounting limitations of available agents to treat Gram-positive infections. Acquired, inducible antibiotic resistance elements belong to the accessory genome of a species and are horizontally acquired by transformation/recombination or through the transfer of mobile DNA elements. The two key, but mechanistically very different, induction mechanisms are: ribosome-sensed induction, characteristic of the macrolide–lincosamide–streptogramin B antibiotics and tetracycline resistance, leading to ribosomal modifications or efflux pump activation; and resistance by cell surface-associated sensing of β-lactams (e.g., oxacillin), glycopeptides (e.g., vancomycin) and the polypeptide bacitracin, leading to drug inactivation or resistance due to cell wall alterations.

Keywords

antimicrobial resistance; Gram-positive bacteria; inducible resistance; mobile genetic elements; ribosomal stalling; transposon

Antibiotic-resistant Gram-positive bacterial pathogens (e.g., Streptococcus pneumoniae, Streptococcus pyogenes, Streptococcus agalactiae, Staphylococcus aureus, Enterococcus faecalis and Enterococcus faecium) are responsible for community-acquired and hospital-associated infections and are an increasing public health threat. Bacterial resistance has developed to most clinically relevant antibiotics and is typically achieved through one of three mechanisms: target modification, drug inactivation or active drug efflux. These mechanisms can come with a cost to the fitness of the bacteria, but the costs can be mitigated by tight regulatory control of expression of the genes and/or proteins responsible for the resistance.
for resistance in the absence of the antibiotic. Because constitutive expression of resistance determinants can have fitness costs and creates selective pressure against the dissemination of resistance, inducible resistance determinants disseminate more readily and persist longer in bacterial populations. In addition, the often transient nature of expression complicates clinical detection of inducible resistance by phenotypic susceptibility assays. This can lead to treatment failures because isolates may be more resistant \textit{in vivo} than determined \textit{in vitro} or may have a wider spectrum of resistance than expected. An understanding of the molecular basis of induction, including the physiological role and the mechanisms of sensing and responding to antibiotics, allows the evaluation of induction mechanisms as potential antimicrobial targets and aids in the design and discovery of noninducing antimicrobial agents.

While antibiotic resistance mechanisms can be intrinsic to the core genome of a bacterial species or acquired but constitutively expressed due to mutations, this review focuses on the inducible resistance genes that belong to the accessory genome of a species. These genes are often silent unless induced and are horizontally acquired by transformation/recombination or through association with mobile genetic elements (MGEs) that include: insertions sequences (IS elements), integrative and conjugative elements (ICEs, or transposons), bacteriophages and plasmids. In contrast to resistance determinants encoded on the core genome, acquired resistance genes pose a special clinical problem, since they are not present in all strains of a species, and are therefore less predictable. In addition, these resistance determinants may cross species or even genera borders and may thereby contribute to the emergence of new resistance phenomena.

Induction of resistance is defined, for the purpose of this review, as reversibly altered expression of an acquired antibiotic resistance determinant. This definition is in contrast to the sometimes used term ‘induction’ that refers to the occurrence of point mutations that alter the genotype and become a constitutive, integral part of the host phenotype. Also, general stress responses, involving complex global regulatory cascades that result in nonspecific physiological changes that allow bacteria to better tolerate the stress, are not part of this definition. Both of these resistance mechanisms generally involve genes in the bacterial species core genome, which are often essential and therefore would be expected to function in most, if not all, isolates of a bacterial species.

**Gene pool for inducible antibiotic resistance determinants: MGEs**

Inducible antibiotic resistance genes are widely disseminated in Gram-positive genera as genetic cargo on infinitely diverse and increasingly complex MGEs. The predominant mechanism of horizontal transfer varies by genera; however, inducible antibiotic resistance genes have recombined into different classes of MGEs facilitating intra- and inter-species horizontal transfer. Common MGEs in Gram-positives include ICEs, plasmids and bacteriophages. Conjugation is the transfer of MGEs by direct cell-to-cell contact through structures encoded by the MGE in donor cells. ICEs encode the necessary proteins for excision from the host cell chromosome, formation of closed circular intermediates, and transfer by conjugation and integration into the chromosome of recipient cells (reviewed in [1]). ICEs are ubiquitous, having been described in virtually all classifications of bacteria and are promiscuous as the transfer between distantly related bacteria occurs readily [2].

The most widely disseminated ICE in Gram-positives is the Tn916 family of conjugative transposons [3]. Tn916 and Tn916-like elements are characterized by inducible tetracycline resistance, but also frequently harbor inducible macrolide and chloramphenicol resistance as well as constitutively expressed resistance determinants (Figure 1A) [3]. Tn916-like elements have been identified in virtually all Gram-positive genera including
Staphylococcus, Streptococcus, Enterococcus and Clostridium [3]. Inducible vancomycin resistance is disseminated by the Tn1546-like transposons that belong to the Tn3 family, often associated with plasmids (Figure 1B) [4–8]. Also often associated with plasmids is Tn552, which carries inducible β-lactam resistance (Figure 1C). The most clinically relevant resistance-conferring elements in staphylococci are the staphylococcal cassette chromosomes (SCCs). SCC elements carrying methicillin resistance genes (SCCmec) are the determinants of methicillin resistance in all methicillin-resistant S. aureus (MRSA) isolates (Figure 1D). SCCmec elements are extremely diverse and are classified based on the combination of the type of cassette chromosome recombinases (ccr) and class of mec gene complex [9,10].

Plasmids, which are autonomously replicating extrachromosomal elements, can also harbor inducible resistance genes and be horizontally transferred by conjugation (reviewed in [11]) or transformation. Plasmids with inducible antibiotic resistance genes are particularly important in the dissemination of resistance in Staphylococcus and Enterococcus species [10,12]. Bacteriophages can also transfer inducible antibiotic resistance genes [13]. Phages are associated with different inducible macrolide resistance genes and cassettes carried by transposons in S. pneumoniae and other Gram-positive species [14]. β-lactamases can be disseminated in staphylococci by bacteriophages [15]. Antibiotic stress can induce the lysogenic transfer of bacteriophages and therefore promote dissemination of resistance through induction of an SOS-like response [14]. Similarly, horizontal gene transfer can be induced by DNA-damaging compounds in Bacillus subtilis [16] and by antibiotic stress in S. pneumoniae [17].

**Induction of antibiotic resistance mediated by ribosome-associated sensing**

The ribosome is the microbial target for many antibiotics utilized in the treatment of Gram-positive bacterial infections. Antibiotics that bind bacterial ribosomes include the tetracyclines, chloramphenicol and the MLSβ antibiotics: macrolides, lincosamides and streptogramin B. MLSβ antibiotics are commonly used options for the treatment of confirmed Gram-positive infections as well as for empirical treatment of respiratory tract infections, which may be due to Gram-positive bacteria. Macrolides contain 14-, 15- or 16-membered lactone rings with extensive glycosidic substitutions that are involved in binding to the ribosome. Structurally distinct lincosamides and streptogramins bind to similar sites on the ribosome as the macrolides. Binding of MLSβ antibiotics blocks elongation of nascent peptides and inhibits protein translation, but binding of certain macrolides (i.e., 14- and 15-membered) also triggers expression of inducible MLSβ and macrolide resistance genes.

Acquired inducible resistance of Gram-positive bacteria by ribosomal-binding antibiotics occurs primarily by target modification or by active drug efflux. The most common determinant is the class of rRNA methylase genes known as erm (erythromycin resistance methylases) that confer resistance to the MLSβ antibiotics. Erm methylases confer high-level resistance to the MLSβ antibiotics by the transfer of one or two methyl groups to a specific adenine nucleotide of the 23S rRNA subunit of the 50S subunit of the ribosome. This blocks binding of MLSβ antibiotics. Conversely, macrolide efflux pumps confer low-level resistance to 14- and 15-membered but less commonly 16-membered macrolides. Expression of erm and other MLSβ resistance mechanisms likely has a cost in bacterial fitness and resistance gene expression is typically repressed in the absence of the drug.

Induction of methylase and efflux-mediated resistance involves complex molecular interactions between the antibiotics and various components of the ribosomal complex.
MLSB antibiotics bind to the 50S ribosomal subunit in the nascent peptide exit tunnel (NPET), thereby disrupting the incorporation of amino acids into nascent peptides in the peptidyl transferase center (PTC). However, inducible MLSB resistance genes contain a leader peptide that evokes leader peptide-mediated stalling of the ribosome upon binding of a MLSB antibiotic. This permits expression of the resistance determinant and thus loss of susceptibility of the host to the antimicrobial compound. We review recent developments of the mechanisms by which ribosome stalling in the presence of different translation inhibiting antibiotics results in the expression of inducible resistance genes.

Induction of ribosome methylases by MLSB antibiotics

Genes encoding Erm methylases are diverse and widespread in Gram-positive bacteria. To date, 34 erm alleles have been identified and all but two have been reported in Gram-positive pathogens [18]. The classes of erm genes, erm(A), erm(B) and erm(C) are the most widely distributed in Gram-positive pathogens (Table 1) [19]. Distinctions between each erm class are based on bacterial host range and the MLSB resistance phenotype that is conferred. Each erm is predominantly associated with, but not limited to, one or two genera [18,19,201]; erm(A) is often associated with Staphylococcus, but has been reported in four other Gram-positive genera including Streptococcus and Enterococcus [20,21]; erm(B) is the prevalent determinant of MLSB resistance in streptococci but has been observed in 17 additional Gram-positive genera [18,201]. The most widely disseminated and clinically important determinant of MLSB resistance in Gram-positive pathogens is erm(C), which has been identified in at least 13 Gram-positive genera, and is the predominant determinant of MLSB resistance in staphylococci [18]. Even though Erm methylation can confer resistance to all MLSB antibiotics, expression of erm is most often inducible by common 14- and 15-membered macrodilides, such as erythromycin or azithromycin. Thus, isolates expressing inducible MLSB resistance can remain susceptible to 16-membered MLSB antibiotics unless induced by other compounds. Expression of erm(C) is the best-studied example of inducible MLSB resistance and will be discussed as the model for regulation of inducible erm expression.

Induction by translational attenuation: the erm(C) paradigm—Resistance in Gram-positive isolates carrying erm(C) can be induced by common 14- and 15-membered macrodilides. The expression of erm(C) is controlled by translational attenuation and is dependent upon the structure of the 5′ leader sequence of the erm(C) mRNA [22,23]. The leader sequence contains a small open reading frame that encodes a 19 amino acid leader peptide Erm(C)L (MGIFSIFVISTVHYQPNKK), which is separated from the structural gene by a 60-nucleotide spacer region (Figure 2A). The spacer region contains four G/C-rich inverted repeats (IR1–IR4) that are involved in folding of the leader sequence into alternate conformations during inducing and noninducing conditions (Figure 2A). In the absence of inducer, the energetically favorable inactive conformation is formed by annealing of IR1 with IR2 (forming stem-loop 1 + 2) and IR3 with IR4 (stem-loop 3 + 4). Stem-loop 3 + 4 sequesters the erm(C) ribosomal binding site (RBS2) and blocks initiation of erm(C) translation (Figure 2A). The active conformation forms when stem-loop 1 + 2 is disrupted allowing annealing of IR2 and IR3 (stem-loop 2 + 3), which frees RBS2 and activates erm(C) translation (Figure 2A). The formation of the active or inactive conformation is determined by the translational status of the ribosome on the erm(C)L leader cistron. In the absence of inducer, unimpeded ribosomes unwind stem-loop 1 + 2, translate erm(C)L and dissociate from the transcript. This allows refolding of the stem-loop and the inactive conformation [24]. However, when an inducing molecule is bound in the NPET, the ribosomal complex stalls on erm(C)L, blocking reformation of stem-loop 1 + 2 and favoring formation of the active conformation, resulting in translation of the resistance determinant.
Ribosomal stalling on the \textit{erm(C)L} leader peptide is dependent upon complex molecular interactions of ribosomal nucleotides and proteins with the nascent peptide as it travels the length of the ribosome through the NPET [25]. Interactions of the nascent leader peptide carboxyl terminus with nucleotides near the peptidyl transferase center dictate the position of ribosomal stalling in the presence of an inducer. In the presence of erythromycin, ribosomes stall on the amino acid sequence IFVI, the 6–9 positions of the nascent Erm(C)L peptide, and specifically on the leucine codon in the ninth position due to a failure to catalyze peptide bond formation between the leucine and the serine encoded by the tenth codon [26]. The peptidyl tRNA carrying the nine amino acid peptide MGIFSIFVI remains tightly bound to the ribosome and prevents progression of the ribosome. Sequence-independent interactions of the Erm(C)L amino terminus and ribosomal proteins approximately 8–12 amino acids from the PTC are indispensible for ribosome stalling, demonstrating the importance of the length of the leader peptide [24–26].

The ability of different MLS\(_B\) drugs to induce \textit{erm} expression is due to the structural features of the compound that influence ribosome activity, but the sequence of the \textit{erm(C)} leader peptide is also involved in differentiating inducers and noninducers. As stated above, certain macrolides are capable of inducing MLS\(_B\) resistance while others are not. Lincosamides and streptogramin B generally are not inducers. Common features of \textit{erm}\-inducing macrolides include, 14- or 15-membered macrolide rings, cladinose at the C-3 position of the lactone ring and a monosaccharide at C-5. Noninducers typically have 16-membered lactone rings, no C-3 cladinose and a disaccharide at C-5. Ketolides have ring size and C-5 monosaccharide of inducers, but no cladinose and have generally been reported as noninducers suggesting that presence of cladinose is the key structural feature required for induction. However, reports of ketolides failing to induce were based on phenotypic observations, that is their antimicrobial activity, which could reflect their excellent antimicrobial activity even in the presence of ribosomal methylation. Indeed, when measuring methylase activity or \textit{erm(C)} expression as direct indicators for induction, several ketolides including telithromycin and cethromycin were shown to be inducers [27].

Interestingly, mutational analysis of the \textit{erm(C)} leader peptide was able to differentiate between induction by ketolides and cladinose-containing macrolides [27] Deletion of the codons for amino acids 2–4, which shortened the peptide without disturbing the stall sequence IFVI, abolished the induction by erythromycin and its derivatives. However, this had no effect on induction by telithromycin, suggesting that ketolide induction may be by a different mechanism than cladinose-containing macrolides [27]. The differential ability of macrolides to induce the expression of \textit{erm(C)} is likely related to the subtle differences in their mode of action. 14- and 15-membered macrolides and ketolides block the passage of nascent peptides through the NPET resulting in premature peptidyl-tRNA dissociation (i.e., drop off) after incorporation of approximately six to eight amino acids [28]. 14- and 15-membered and 16-membered macrolides bind in a similar location in the NPET but the C-5 disaccharide of 16-membered macrolides extends farther into the NPET and makes direct contact with the PTC, disrupting peptide bond formation and forcing peptidyl-tRNA drop off after only two to four amino acids, preventing ribosome stalling and formation of the active \textit{erm(C)} mRNA conformation [28,29]. Streptococcal isolates constitutively expressing \textit{erm(C)} genes have been reported and in all cases contain mutations that destabilize attenuator structures and lead to unregulated translation of \textit{erm(C)} repression of translation in the absence of inducer [30,31].

\textbf{Variations of translational \& transcriptional attenuation of other ribosome methylases}—The other major Gram-positive methylases \textit{erm(A)} and \textit{erm(B)} are also regulated by translational attenuation. The \textit{erm(A)} gene is preceded by a unique attenuator structure encoding two leader peptides. In the \textit{S. aureus \textit{erm(A)}} allele, the peptides are the
15 amino acid erm(A)L1 (MCTSIAVVEITLSHS) and the 19 amino acid erm(A)L2 (MGTFSFIFVINKVRYQPNON) [32]. Induction of erm(A) occurs due to sequential formation of stalled ribosome complexes on the erm(A)L1 and erm(A)L2 cistrons in the presence of an inducing antibiotic [32]. Stalling at erm(A)L1 induces initiation of translation of erm(A)L2. A second stalling event on erm(A)L2 then induces translation erm(A) structural gene [32]. The sequence encoded by erm(A)L2 is highly conserved with that of erm(C)L and contains the erm(C) stall sequence IFVI. Like with erm(C), stalling occurs on the leucine codon in the ninth position of erm(A)L2 [32]. The erm(A)L1 open reading frame is not as conserved, and contains a different stall sequence (SIAVV) (Table 1).

The translational attenuator of erm(B) allele is similar to erm(C) with a single leader peptide coding sequence. However, the Erm(B)L sequence (MLVFQMRYNDKTSTVLKQTKNSDYADK) bears no resemblance to the leader peptides of erm(A) or erm(C). The Erm(B)L stall sequence is MRYND with stalling occurring on the aspartic acid codon in the tenth position [33]. Constitutive expression of erm(A) and erm(B) isolates has been observed in staphylococci, streptococci and enterococci and involves mutations that disrupt formation of the attenuator structure (Table 1) [29,33].

Erm(K) is a member of the Erm(D) class found in Bacillus species. The regulation of erm(K) is unique for erm genes in that it is controlled by transcriptional attenuation [34]. The erm(K) transcript is prematurely terminated in the absence of inducer due to two rho-independent transcriptional attenuators in the inactive conformation of its mRNA (Figure 2B). Like the translational terminators, activation requires pausing of the ribosomal complex on a leader peptide encoded on the 5’-end of the transcript, leading to formation of the active conformation [34]. The erm(K) leader peptide is 14 amino acids (MTHSMRLRTNR) and the pause sequence is MRLR (Table 1) [34].

**Induction of macrolide efflux**

Active drug efflux is another major mechanism of inducible MLS\(_B\) resistance in Gram-positive pathogens. Inducible efflux-mediated macrolide resistance was first described in *Staphylococcus epidermidis* and is conferred by msr(A) encoding an incomplete ABC transporter [35]. msr(A) has been identified in other staphylococci, including *S. aureus* and has recently been reported in *Streptococcus*, *Enterococcus* and *Corynebacterium* species (Table 1) [36]. msr(A) is inducible by 14- and 15-membered macrolides and confers resistance to 14- and 15-membered macrolides and streptogramins, but not 16-membered macrolides or lincosamides [37]. Msr(A)-mediated resistance to telithromycin is dependent upon prior induction of msr(A) by erythromycin indicating that telithromycin is not an inducer but it is a substrate for msr(A)-mediated efflux (Table 1) [38]. The mechanism of induction of msr(A) has not been confirmed, but analyses of the DNA sequences immediately upstream reveal similarities to MLS\(_B\) attenuators [Chancey ST, Unpublished Data]. The putative leader sequence msr(A)L is predicted to encode a small leader peptide (MTAMRLR) that is almost identical to that of erm(K) [24].

In *S. pneumoniae*, macrolide efflux is encoded by mef(E) and the msr(A) homolog mel, which constitute an inducible operon carried on the mobile macrolide efflux genetic assembly (MEGA) (Figure 1A) [39,40]. mef(E) is predicted to encode a major facilitator family efflux pump and mel is a homolog of the incomplete ABC transporter msr(A). mef(E)/mel confers pneumococcal resistance to 14- and 15-membered macrolides and a modest increase in resistance to telithromycin [20]. Induction of mef(E)/mel is controlled at the level of transcription [39]. A putative leader peptide is encoded 327-bp upstream of mef(E) and is almost identical to that of msr(A) in *Staphylococcus* including a putative leader peptide (MTAMRLR), which is identical to msr(A) and similar to the transcriptionally attenuated erm(K) (Table 1) [34]. These data suggest that both msr(A) and
mef(E)/mel are induced by transcriptional attenuation. Homologous systems include mef(A)/msr(D) primarily found in S. pyogenes and, mef(I)/msr(D) in group C streptococci and S. pneumoniae (Table 1) [41]. The mef(A)/msr(D) operon is also inducible in S. pyogenes, but induction of mef(I)/msr(D) has not been reported [42]. The msr(A) homolog msrC confers a similar inducible resistance phenotype in Enterococcus faecium and, although the putative leader peptide (MTASMKLRFELLNNN) is longer, a transcriptional attenuator mechanism is suggested as well [43].

In addition to being induced by 14- and 15-membered macrolides, mef (E)/mel is induced by telithromycin and the 16-membered macrolides tilmicosin and rosamicin [39]. Telithromycin, tilmicosin and rosamicin have a monosaccharide at C5 but no cladinose, indicating that the cladinose is not required for induction of mef(E)/mel. Furthermore, C5 disaccharide-containing 16-membered macrolides failed to induce mef(E) expression consistent with the model proposed for induction of erm(C) that states that the C5 disaccharide prevents ribosome stalling causing termination of the leader peptide synthesis prior to the stall sequence incorporation. Interestingly, mef(E) on the MEGA element is also induced by LL-37, a cationic antimicrobial peptide produced in human macrophages [40]. This is a significant observation because it suggests that mef(E) may be induced at the site of infections and therefore resistance to macrolides could be higher than predicted by *in vitro* MIC analyses [40]. LL-37 is not believed to bind ribosomes and likely induces mef(E) transcription through an alternate pathway, possibly through a general cell stress response [40].

**Other ribosome-mediated induction mechanisms**

The resistance of Gram-positive bacteria to other antibiotics that act upon the bacterial ribosome (e.g., chloramphenicol and tetracycline) is also inducible and controlled by transcriptional or translational attenuation. Inducible chloramphenicol resistance is similar to *erm* gene regulation and involves chloramphenicol-induced ribosomal stalling during leader peptide synthesis to alleviate translational attenuation. Inducible chloramphenicol resistance due to drug inactivation via expression of the chloramphenicol acetyl transferase has not been a subject of intensive investigation in recent years and will not be covered further here (for review see [24]).

Two distinct mechanisms of inducible tetracycline resistance have been reported in Gram-positive bacteria: translational attenuation of tet(L) and transcriptional attenuation of tet(M) [44,45]. Regulation of the plasmid-encoded tetracycline efflux gene tet(L) is an interesting variation of leader peptide-dependent translational attenuation. In contrast to the regulation of *erm(C)*, the formation of the active conformation of tet(L) mRNA is not dependent upon ribosomal stalling on the leader peptide, but on inhibition of translation of the leader peptide by tetracycline. The distinction between the two mechanisms is evident by mutations that prevent expression of the respective leader peptides. Mutants that do not express the leader peptide of *erm(C)* cannot be induced whereas similar mutation in the *tet(L)* leader sequence system results in constitutively expressed tet(L) [44].

The conjugative transposon-associated tet(M) gene confers tetracycline resistance by ribosomal protection and is regulated by a transcriptional attenuation mechanism similar to *erm(K)*. Transcription of tet(M) is attenuated in the absence of tetracycline by rho-independent terminators, but tetracycline-induced ribosomal stalling resolves these structures and allows completion of transcription. A significant aspect of inducible tetracycline resistance in Gram-positive bacteria is the regulatory link between tet(M) and the mobility of Tn916. Tetracycline induction of tet(M) leads to a regulatory cascade that ultimately activates expression of the genes involved in excision and transfer of the conjugative transposon. Because Tn916-like elements are carriers of genes encoding
resistance to antibiotics in addition to tetracycline, tetracyclines may facilitate the dissemination of antimicrobial resistance in Gram-positive pathogens. For example, a pneumococcal clone, ST320, containing both the \( \text{met}(E)/\text{mei} \) operon and the \( \text{erm}(B) \) methylase has been responsible for a global epidemic of invasive pneumococcal infections [46]. The \( \text{met} \)-carrying element MEGA and an \( \text{erm}(B) \)-element have inserted into separate loci of Tn916 to generate Tn2010 (Figure 1A).

**Induction of antibiotic resistance by cell surface-mediated drug sensing**

Many important antibiotics used in the treatment of human Gram-positive pathogens interfere with bacterial cell wall synthesis. These antibiotics include but are not limited to the \( \beta \)-lactams (e.g., penicillin, oxacillin, cefoxitin), glycopolptides (vancomycin) and the polypeptides (bacitracin). Apart from intrinsic mechanisms that respond to stress and damage of the cell envelope, with sometimes the antibiotic itself serving as the signal (see for review [47]), Gram-positive bacteria have evolved three substantially different inducible and transferable molecular mechanisms to respond to cell wall targeting antibiotics (Figure 3).

**Inducible vancomycin resistance**

**Mechanism of Van-type vancomycin resistance**—Vancomycin and other glycopolptide antibiotics, such as teicoplanin, inhibit peptidoglycan cross-linking by binding to the \( \alpha \)-alanyl-\( \alpha \)-alanine \( (\alpha \text{-Ala} \alpha \text{-Ala}) \) dipeptide terminus of lipid II, the major precursor in peptidoglycan biosynthesis. In streptomyces, enterococci and (so far) rarely in staphyllococci, resistance to glycopolptide antibiotics is achieved by incorporation of modified penta-peptide termini, such as \( \alpha \)-alanyl-\( \alpha \)-lactate \( (\alpha \text{-Ala} \alpha \text{-Lac}) \) or \( \alpha \)-alanyl-\( \alpha \)-serine \( (\alpha \text{-Ala} \alpha \text{-Ser}) \), instead of \( \alpha \text{-Ala} \alpha \text{-Ala} \), into lipid II. Glycopolptide antibiotics bind with less affinity to these modified termini.

The genetic basis for acquired vancomycin resistance is now well understood. High-level \( \text{van} \)-resistance in enterococci is most frequently associated with transposable elements [8], either integrated in the chromosome or located on plasmids (Figure 1 & Table 2). The prototype, the \( \text{van}A \) system, is encoded by five genes: \( \text{vanR} \), \( \text{vanS} \), \( \text{vanH} \), \( \text{vanA} \) and \( \text{vanX} \) (Figure 4A); \( \text{vanR} \) and \( \text{vanS} \) encode a typical two-component signal transducing system (Figure 3A) composed of the sensor kinase (histidine kinase), \( \text{VanS} \), which is capable of autophosphorylation in response to an environmental signal, and the response regulator, \( \text{VanR} \), that interacts with the phosphorylated histidine kinase [48]. \( \text{VanS} \) senses the presence of vancomycin, and Koteva et al. have shown in an elegant experiment based on VanS of *Streptomyces coelicolor* that vancomycin is itself a ligand for VanS [49]. Presence of the inducing compound causes phosphotransfer from VanS to the response regulator VanR, which subsequently activates transcription of the \( \text{vanHAX} \) genes. The \( \text{vanHAX} \) genes are responsible for the synthesis of the modified precursor peptides and the removal of the native \( \alpha \text{-Ala} \alpha \text{-Ala} \) precursor (Figure 3A). The dehydrogenase \( \text{VanH} \) reduces pyruvate to \( \alpha \text{-Lac} \), the ligase \( \text{VanA} \) forms the ester bond between \( \alpha \text{-Ala} \alpha \text{-Lac} \), and the dipeptidase \( \text{VanX} \) cleaves the \( \alpha \text{-Ala} \alpha \text{-Ala} \) bond, thereby eliminating the target of glycopolptide antibiotics. \( \text{vanY} \) encodes a \( \alpha , \alpha \)-carboxypeptidase that may contribute as an accessory protein to elevated glycopolptide resistance, and \( \text{VanZ} \) confers low-level teicoplanin resistance by a yet unknown mechanisms [6].

Several variants of \( \text{van} \) operons have been identified in enterococci and are referred to as different Van-types (Table 2; review see [50]). The most common are the types \( \text{VanA} \) and \( \text{VanB} \). \( \text{VanB} \) differs from \( \text{VanA} \) in that it is only induced by vancomycin, not by teicoplanin. The \( \text{VanS} \) proteins of the \( \text{VanA} \) (\( \text{VanS}_{\text{A}} \)) and \( \text{VanB} \) (\( \text{VanS}_{\text{B}} \)) differ in their N-terminal sensor domains, which results in differential induction by vancomycin and teicoplanin.
respectively [51,52]. The other identified Van-types – VanD, VanC, VanE, VanG, VanL [53], VanM [54] and VanN [4] – have been found far less frequently than VanA and VanB (Table 2). The VanM- and VanD-types are similar to VanA and VanB in regard that they provide β-Ala-β-Lac penta-peptide precursors. However, VanD-type resistance is constitutively expressed due to mutations in VanS or VanR [55] and in addition, mutations in the chromosome-encoded ddl, which encodes β-Ala-β-Ala ligase, result in the absence of the β-Ala-β-Ala precursor. Van-types VanC, VanE, VanG, VanL and VanN encode proteins that synthesize β-Ala-β-Ser precursors instead of β-Ala-β-Lac and express a serine racemase to convert β-Ser to β-Ser (Figure 4B). Vancomycin has a higher affinity for binding β-Ala-β-Ser than for β-Ala-β-Lac; therefore, VanC, VanE, VanG, VanL and VanN confer only low-level resistance to vancomycin, and in addition, the bacteria remain sensitive to teicoplanin. The VanE-, VanG-, VanL- and VanN-types are acquired and inducible, whereas the VanC-type is intrinsically present in the chromosomes of Enterococcus gallinarum and Enterococcus casseliflavus and is constitutively expressed. In some VanE-type strains, inducible vancomycin resistance was observed despite the fact that mutations rendered the histidine kinase VanS inactive. This finding led to the suggestion that VanR may be activated by other histidine-kinases [56]. This cross-talk phenomenon has been observed in an experimental setting between the histidine kinase PhoB and VanR in the absence of VanS (in recombinant E. coli mutants) [57]. However, in the presence of VanS, while not activated by a glycopeptide, VanS acts as a phosphatase maintaining VanR in an unphosphorylated state [58], thereby preventing cross-phosphorylation by other histidine kinases. Hence, certain mutations in VanS may affect either the kinase or phosphatase activity of VanS and thereby modulate the output of the Van system.

In clinical isolates, Van-types from different geographical sources displayed a great variety of point mutations, deletions of regulatory and accessory genes and insertions (mainly IS elements), leading to modified and fragmented operons with different expression patterns. The modifications and rearrangements have best been studied for the transposon-associated VanA- and VanB-types [59–63]. Surprisingly, a partially vancomycin-dependent vancomycin-resistant S. aureus (VRSA) was identified [64], caused by a mutation in the core genome-encoded β-Ala-β-Ala ligase, which rendered the strain dependent on the van gene cluster to provide β-Ala-β-Lac precursor for cell wall biosynthesis. A gene cluster similar to the VanA-type has been described in the Gram-positive insect pathogen Paenibacillus popilliae, which confers inducible vancomycin resistance and has been named VanF [65].

**Distribution of Van-type-mediated vancomycin resistance**—Van-type-mediated vancomycin resistance has been encountered in many enterococcal species (vancomycin-resistant enterococci [VRE]), which are a major source of van-resistance elements. In addition, van gene clusters have been identified in S. aureus and also many coagulase-negative staphylococci, a few streptococcal species, such as S. bovis [66], and in many environmental nonenterococcal isolates, such as those found in the human bowel [67]. Thus far, Van-type vancomycin resistance has not been identified in clinical isolates of the major human pathogens group A and B streptococci and S. pneumoniae. It is commonly accepted that the van gene clusters in the low-GC Gram-positive bacteria, such as enterococci, originated from the high-GC Gram-positive Streptomyces species, which are the producers of glycopeptides, and therefore contain defensive systems for self-protection [58]. The characterization of the van gene pool, the host range and mechanisms affecting their distribution are an area of ongoing investigation [68].

In 1992 the first conjugal transfer of a vancomycin resistance determinant (VanA-type) from VRE to MRSA was described [69], followed by the first detection of a clinical S. aureus strain with high-level (VanA-type) VRSA in 2002 [70]. Since then several VRSA strains
have been identified, all of them are in MRSA strains that are hypothesized to have acquired Tn1546 in the course of a coinfection with VRE. This was confirmed in the first identified VRSA strain [71]. This strain harbored a 60-kb multiresistance-encoding conjugative plasmid that included Tn1546 (VanA) [72].

All Van-types that confer high-level vancomycin resistance are inducible (Table 2), which led to the suspicion that expression of the van gene cluster may come with a fitness cost. Foucault et al. addressed this question by introducing inducible and constitutively expressed versions of the vanB operon in an isogenic background [73]. The authors found that induction and constitutive expression of the van gene cluster had adverse effects on the ability of the strain to colonize and disseminate in mice. They concluded that the tight regulation of resistance expression drastically reduces the biological cost associated with van-mediated vancomycin resistance and represents an efficient evolutionary pathway for resistance determinants to become selectively neutral.

Recent research on vancomycin resistance has been driven by genomics, providing access to an extensive collection of genome and plasmid sequences. Genomics has provided insight into the evolution of the different van gene clusters in VRE originating from animals and human sources that links to data on glycopeptide usage in animals (as growth promoters and antibiotics) and in humans. These studies are complemented by studies that address the mechanisms, restrictions and dynamics of transmission and maintenance of resistance-conferring elements [74]. The van operon-conferring plasmids and transposons frequently encode additional genetic elements, such as other antibiotic resistance determinants, for example, erm(B) or tet(M), and plasmid maintenance systems (toxin–antitoxin systems [75]), which may help preserve the elements in a defined population in the absence of vancomycin. The toxin–antitoxin systems on the other hand may limit host range [76].

Two areas of considerable research interest are the search for (lipidated-)glycopeptides with altered induction profiles, such as telavancin, which, like teicoplanin, induces only the vanA gene cluster, but to lower resistance levels than by vancomycin and teicoplanin [77]; and the search for compounds such as vancomycin-derivatives that recognize D-Ala-D-Ala and also D-Ala-D-Lac as substrates [78].

**Inducible β-lactam resistance**

**Induction through BlaR1/I & MecR1/I**—Resistance to β-lactam antibiotics is a major problem in the treatment of Gram-positive pathogens. The two acquired, inducible β-lactam resistance mechanisms are the β-lactamases (BlaZ and its homologs) and, in S. aureus, the inducible expression of a low-affinity penicillin-binding protein (PBP), PBP2a (also named PBP2´) encoded by mecA. β-lactam antibiotics disrupt peptidoglycan cross-linking by binding PBPs in the cell wall; however, PBP2a allows S. aureus to maintain cell wall biosynthesis in the presence of β-lactams. PBP2a confers resistance to the β-lactam methicillin, and resistant strains are therefore referred to as MRSA. The induction mechanisms of β-lactamase and PBP2a share common features (Figure 5). In the absence of a β-lactam, the resistance-encoding genes mecA and blaZ are controlled by the repressors MecI and BlaI, respectively. When present, β-lactams bind to the transmembrane signal transducers MecR1 and BlaR1, which results in autocatalytic cleavage of the C-terminal domain of MecR1 and BlaR1, respectively, and release of a metalloprotease-containing cytoplasmic domain of the signal-transducing molecule. The metalloproteases initiate cleavage of the repressors MecI and BlaI, a process which may involve additional factors [79]. The cleavage of MecI and BlaI leads to transcription of the divergently transcribed regulatory genes mecI and blaI, respectively, as well as the structural genes mecA and blaZ and hence the production of PBP2a or the β-lactamase. For more details on the BlaR1/I and MecR1/I signal-transducing mechanisms see [80].

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The MecR1 peptidase is specific for MecI, as is the BlaR1 peptidase for BlaI; however, the repressors MecI and BlaI act interchangeably (Figure 3A) [81], such that BlaI bound to the mec regulatory region can be inactivated by BlaR1 in the presence of a β-lactam. The induction kinetics of MecR1/I and BlaR1/I vary greatly, while BlaR1 induces BlaZ as well as PBP2a synthesis within minutes, MecR1 takes hours to induce production of PBP2a [82,83], and this induction has been observed to sometimes not be rapid enough to prevent death [84]. Hence, presence of both resistance determinants may provide a fitness advantage.

MRSA strains with slow PBP2a induction often appear sensitive to oxacillin in routine MIC testing on agar plates. To account for this phenotype, cefoxitin, a potent inducer of mecA, which allows MRSA to grow readily, is widely used as a surrogate marker for detection of PBP2a-mediated β-lactam resistance. However, there is research to more readily and reliably identify mecA-containing S. aureus isolates in routine diagnostic (for review, see [85]).

Staphylococcal cassette chromosome mec—The mecA gene and the mec regulatory genes are encoded on the SCCmec, an accessory genetic region of 21–67 kb (Figure 1D; for review see [86]). Five classes (A–E) of SCCmec elements have been defined based on differences in the mec region (Figure 5B), with some classes further divided into types based on differences in the mec element flanking regions (Figure 5B). SCCmec elements A–C are common in S. aureus (Figure 5B) [9]. Class A is the prototype complex, which contains mecA, and complete mecR and mecI regulatory genes, whereas in SCCmec classes B and C, mecR1 is truncated by IS1272 or IS431. Insertion of the IS elements replaces mecI, which abolishes induction and renders mecA constitutively active. In class D, mecR1 is deleted. Class E encodes the β-lactamase BlaZ upstream of the mec element (mecA, mecR1 and mecI). A mec element containing blaZ was first identified in Macrococcus caseolyticus [87], and most recently in S. aureus [88], designated SCCmec XI (Figure 5B). This finding fostered the speculation that M. caseolyticus may represent the ancestral form of the mec complex in staphylococci and supported earlier speculation that the mec element evolved by integration of mecA into a blaZ locus [89].

Many MRSA strains contain SCCmec and a blaZ locus concomitantly, and while many isolates contain nonfunctional BlaR1/I or MecR1/I regulatory components (Figure 5), presumably one of the two regulatory systems remains functional [90]. In particular, mecA transcription often seems to be under the control of the blaR1/I genes only. In line with these observations, the presence of the blaZ locus has been shown to promote mecA acquisition and stabilization: plasmid-based mecA introduced in S. aureus strains with different bla/mec regulatory backgrounds was only maintained in the presence of either a bla or a mecA regulatory region [91]. Thus the requirement for regulation of regulatory-deficient SCCmec elements by BlaR1/I constitutes a mechanism of host restriction. Furthermore, Rosato et al. observed that constitutive expression of PBP2a or β-lactamase affected bacterial growth [90]. Two later studies verified this finding and described an inverse proportional relationship between oxacillin resistance level and the rate of growth, indicating substantial fitness costs associated with expression of this resistance determinant and underlining the role of tight regulation [92,93]. A more recent study by Milheirico et al. found evidence that clinical isolates with a blaZ locus and SCCmec are under selective pressure not only to maintain functional bla regulatory components, but also an intact β-lactamase [94]. Many clinical isolates of MRSA show, despite the presence of mecA, decreased oxacillin MICs presumably due to secondary mutations and additional regulatory events [83], which may indicate the need to balance the advantages of resistance with a cost in fitness [84].
The curiosity of vancomycin-dependent enterococcal strains has a counterpart in the oxacillin-dependent \textit{S. aureus} strain 2884, which was selected \textit{in vitro} on oxacillin and depends on oxacillin for growth [95]. This strain has a low expression level of native PBP2, due to a loss of function in the PBP2 regulatory two-component system VraSR and an SCCmec element (type I, Figure 5). Thus, the strain depends on oxacillin to allow expression of the now essential PBP2a functions.

**Distribution of mecA & blaZ**—SCCmec elements have also been identified in \textit{S. epidermidis} and other coagulase-negative staphylococci, and they are considered a potential reservoir of SCCmec elements that may facilitate the emergence of new MRSA clones [96,97]. SCCmec IV (Figure 5B) is the most common SCCmec element in \textit{S. epidermidis}, detected in approximately 40% of methicillin-resistant \textit{S. epidermidis} from humans. These strains belong to a wide variety of genetic backgrounds [98], and SCCmec IV is also common among coagulase-negative staphylococci from animals [99]. However, in SCCmec IV, the regulatory genes are disrupted and future work will need to determine if this affects transmission and/or a fitness cost [92,93], which may influence the evolutionary success of this element [15]. Recently, a variety of ‘atypical’ and ‘nontypeable’ SCCmec elements have been described in coagulase-negative staphylococci [100], indicating that identification, spread and evolution of SCCmec elements is still ongoing, and prompts the question of where these novel elements originated from and how they may contribute to the further spread of β-lactam resistance.

In contrast to the chromosome-located SCCmec complex, the blaZ locus has been found on Tn552 and Tn552-like transposons (Tn4002 and a Tn4001-like element; Figure 1C) located on plasmids or are bacteriophage-associated. Even SCCmec type V, which is flanked by two IS431 elements, rendering it potentially transposable, has never been found on a plasmid in clinical isolates [84]. Beyond staphylococci, blaZ homologs have been identified in many bacilli [101]. In addition, genes with homology to blaZ and the regulatory components BlaR1/BlaI have been identified in many bacterial genomes, such as in the \textit{Clostridium difficile} genome of strain A630 [102]; however, future studies will have to provide experimental evidence whether these genetic elements indeed confer the observed β-lactam resistance to the hosting bacterium.

**Antagonism between mecA & vanA in MRSA/VRSA strains**—A methicillin- and vancomycin-resistant strain of \textit{S. aureus} was constructed by introducing the vanA-containing plasmid HIP11714 into the highly oxacillin-resistant strain COL, producing strain COLVA [103]. Although this strain was clearly able to express high-level resistance to oxacillin and vancomycin, the two antibiotics had strong and mutual antagonistic effects on the expression of the drug-resistance phenotypes. As little as 40 µg/ml oxacillin reduced the vancomycin MIC value of this strain from 512 to 12 µg/ml. Similarly, as little as 50 µg/ml vancomycin reduced the oxacillin MIC from 800 to 10 µg/ml. The authors concluded that the decrease in vancomycin resistance by oxacillin suggested that the low-affinity PBP2a, which is the only transpeptidase that remains active in this strain in the presence of oxacillin, is not able to utilize the depsipeptide cell wall precursors [103]. Subsequently the synergistic effect of oxacillin and vancomycin was successfully used to treat experimental endocarditis caused by VRSA [104], and was therefore suggested for treatment of infections with VRSA. However, treatment of COLVA with oxacillin or vancomycin resulted in both cases in a change from a homogenous to a heterogenous resistance phenotype for the noninduced antibiotic [103]. This phenotype was also observed with clinical VRSA isolates and suggested that some bacteria may tolerate the combination of both antibiotics [105]. However, the mecA/vanA antagonism demonstrates the importance of a tightly regulated – that is, inducible – mechanism to alleviate the cost of resistance.
Inducible bacitracin resistance in *E. faecalis*

Bacitracin is a mixture of high-molecular-weight polypeptides produced by *Bacillus licheniformis*. Bacitracin sequesters undecaprenyl pyrophosphate (UPP), the lipid carrier in peptidoglycan synthesis. By preventing dephosphorylation of UPP, bacitracin depletes the lipid carrier, causing disruption of cell wall biosynthesis. Bacitracin is widely used in topical applications in humans and extensively in animal foods, in particular in chicken production. A one-component signal transducing system (Figure 3C) was recently described in *E. faecalis* [106], conferring inducible bacitracin resistance. This system senses Zn\(^{2+}\)-bacitracin via a membrane-bound transcriptional regulator, BcrR, which subsequently induces a bacitracin-specific ABC transporter, BcrAB, and a cotranscribed UPP, BcrD (Figure 6) [106]. BcrD alone has no significant effect on bacitracin resistance [106]. BcrR has an N-terminal helix-turn-helix DNA-binding motif and four C-terminal transmembrane domains, and has been shown to induce transcription of BcrABD in the absence of auxiliary proteins [107]. Thus, BcrR is a one-component signal-transducing system. Expression of bcrABD depends on induction by BcrR and is specific for bacitracin – that is, it is not induced by other cell wall active compounds such as vancomycin or indirectly by the accumulation of cell wall precursors [106].

The BcrRABD system is plasmid-encoded and has been shown to be transferable by conjugation between *E. faecalis* isolates [106]. Acquisition of the bcrRABD system results in an increase in bacitracin MIC from 32 µg/ml to ≥256 µg/ml in the recipient strain. While bcrRABD was first identified on a partial sequence of plasmid pJM01 [106], recent sequence released from enterococcal plasmid pTW9 (85 kb) shows the bcrRABD genes adjacent to the van genes of Tn1546 and erm(B) (Annotation according to GenBank, Acc. no. NC 014726) (Figure 6). Thus, on pTW9, the bcrRABD region is part of a multidrug resistance-encoding conjugative plasmid. A study analyzing the distribution of antimicrobial resistance determinants in enterococci isolated from broiler chickens identified bcrR in all (seven out of seven) *E. faecalis* isolates and in 92% (46 out of 50) *E. faecium* isolates [108]. Further studies are needed to assess the prevalence of this mechanism in enterococci isolated from human sources. However, the past experience with other resistance determinants, such as vanA/B and erm(B)/(C) has taught us that staphylococci and streptococci should be monitored for the emergence of this newly identified resistance mechanism.

**Conclusion**

Acquired, inducible antimicrobial resistance in Gram-positive bacteria represents a widening silent spring that has major diagnostic and clinical implications including accentuating the limitations on available agents to treat Gram-positive infections. Acquired, inducible antibiotic resistance determinants belong to the accessory genome of a species and are horizontally acquired by transformation/recombination or through MGEs, including IS elements, ICEs or transposons, bacteriophages and plasmids. The two major, mechanistically very different, induction mechanisms are: ribosome-sensed induction, characteristic of MLS\(_B\) and tetracycline resistance; and resistance by cell surface-associated sensing of the \(\beta\)-lactams (e.g., oxacillin), the glycopeptides (e.g., vancomycin) and now recently the polypeptide bacitracin. Although there is general conservation of inducibility because of fitness costs, variations and evolution of inducible agents and their regulatory systems is quite striking. An understanding of the molecular basis of induction, including the physiological role and the mechanisms of sensing and responding to antibiotics, allows the evaluation of induction mechanisms for potential new antimicrobial targets and aids in the design and discovery of noninducing antimicrobial agents.
**Future perspective**

Clinically, a major challenge is to reliably and cost-effectively identify infections caused by bacteria with acquired inducible antibiotic resistance [109,110]. Identification is important not only for the implementation of appropriate antibiotic regimens to treat these infections, but also for instituting infection control measures to prevent dissemination of these resistant isolates. While biologic tests are used by clinical microbiology laboratories to detect some inducible resistance, they can be difficult to interpret and are not always reliable. New diagnostic tools such as microarrays or genome sequencing to test for the presence of inducible resistance mechanisms are currently being tested. The development and widespread usage of multilocus sequence typing methods in the last 15 years has greatly improved the ability to assess the distribution and dissemination of inducible resistant and multidrug-resistant isolates. The list of Gram-positive species for which an MLST strategy has been developed continues to grow. However, whole-genome sequencing has revealed surprising genetic variability even between isolates of identical MLST sequence types and clonal complexes. The cost of sequencing the entire genome of a clinical isolate has, or will, become less than the cost of individually sequencing the alleles required to determine the MLST. As more clinical isolate genomes are sequenced, a higher-resolution picture of the evolutionary history of Gram-positive bacteria will become apparent, providing insights into mechanisms of horizontal transfer of inducible resistance mechanisms which could lead to new methodologies for preventing dissemination of the resistance determinants. For example, metagenomics, or sequencing of DNA isolates directly from clinical specimens will allow to address the ecology of microbe–microbe interactions in vivo and will improve our understanding of the mechanisms of inter- and intra-species horizontal gene flow.

Identification of avenues and limitations to gene flow will allow implementation of strategies to prevent dissemination. As resistance mechanisms become more prevalent, matrix-assisted laser desorption/ionization time of flight mass spectrometry technologies that have recently been developed for clinical identification of bacterial species are now being developed to test for resistance determinants [111]. Whole transcriptome analyses will help elucidate complex regulatory mechanisms and identify targets to prevent induction and targets for novel antimicrobials.

**References**

Papers of special note have been highlighted as:

- of interest
- of considerable interest

   - This review of integrative and conjugative elements discusses the core functions of these elements that allow for dynamic lateral gene flow, including regulation of conjugative transfer.


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**Websites**


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Executive summary

Antibiotic resistance mechanisms in Gram-positive pathogens

- Antibiotic-resistant Gram-positive pathogens are responsible for community-acquired and hospital-associated infections and are an increasing public health threat.
- Resistance of Gram-positive pathogens to antibiotics is conferred by three primary mechanisms: target modification, drug inactivation and antimicrobial efflux.
- Expression of resistance mechanisms can come with a cost to the fitness of the bacteria, but the costs can be mitigated by tight regulatory control, in the absence of antibiotics, of expression of the genes and/or proteins responsible for resistance in the absence of the antibiotic.
- Inducible antibiotic resistance is defined, for the purpose of this review, as reversibly altered expression of acquired antibiotic resistance determinants.

Clinical significance of inducible antibiotic resistance in Gram-positive pathogens

- The transient nature of inducible expression complicates clinical detection of inducible resistance by phenotypic susceptibility assays and can lead to treatment failures due to failures to recognize resistance.
- Because of the reduced fitness costs, inducible resistance determinants disseminate more readily and may persist longer in bacterial populations than constitutively expressed resistance determinants.

Dissemination of inducible resistance determinants

- Inducible resistance determinants are widely disseminated in Gram-positive pathogens through transformation/recombination and in association with mobile genetic elements such as transposons, plasmids and bacteriophages.

Inducible resistance to ribosome-targeting antibiotics

- Translation-inhibiting antibiotics such as tetracycline, chloramphenicol and the macrolides–lincosamides–streptogramin B antibiotics bind ribosomes and alter ribosomal activity.
- Acquired inducible resistance of Gram-positive bacteria by ribosomal binding antibiotics occurs primarily by target modification through the activity of Erm methylases or by active drug efflux.
- Expression of \(erm\) or efflux genes is attenuated in the absence of inducing antibiotics by transcript secondary structures that block translation or force premature termination of transcription. Binding of inducers promotes ribosomal stalling and refolding of the transcript into an active conformation leading to resistance expression.
- Inducible efflux-mediated resistance to macrolides is conferred by \(mef\) and \(msr(A)\) genes, which are likely controlled by transcriptional attenuation.

Inducible resistance to cell wall-targeting antibiotics

- Many important antibiotics used in the treatment of Gram-positive pathogens interfere with bacterial cell wall synthesis, including the \(\beta\)-lactams and glycopeptides (vancomycin).
Inducible β-lactam resistance

- The two acquired, inducible β-lactam-resistance mechanisms are the β-lactamases (BlaZ and its homologs) and the inducible expression of a low-affinity penicillin-binding protein PBP2a (also named PBP2’) encoded by mecA in *Staphylococcus aureus*.
- The resistance-encoding genes mecA and blaZ are repressed in the absence of a β-lactam, controlled by the repressors MecI and BlaI. Cleavage of the repressors is mediated by the signal transducing molecules MecR1 and BlaR1 upon binding to a β-lactam.

Inducible Van-type vancomycin resistance

- Vancomycin and other glycopeptide antibiotics inhibit peptidoglycan cross-linking by binding to precursors in peptidoglycan biosynthesis. Resistance results from modification of these precursors resulting in less affinity to the antibiotics.
- Induction of resistance is mediated by a typical two component signal transducing system encoded by vanR and vanS, which sense the presence of inducing glycopeptides and activate transcription of the van gene cluster involved in the synthesis of modified peptidoglycan precursor.

Inducible bacitracin resistance in *Enterococcus faecalis*

- Bacitracin sequesters undecaprenol pyrophosphate, the lipid carrier in peptidoglycan synthesis. Bacitracin resistance is mediated by the ABC transporter BcrAB and the cotranscribed undecaprenol pyrophosphate BcrD. Expression of BcrABD is activated in the presence of bacitracin by a one-component signal transducing system BcrR.
Figure 1. Representative mobile genetic elements carrying inducible resistance genes

(A) Conjugative transposon Tn\textsubscript{916} and Tn\textsubscript{916}-like mosaic elements carrying inducible macrolide resistance (blue) and tetracycline resistance (yellow) genes. (B) Conjugative transposon Tn\textsubscript{1546} encoding inducible vancomycin resistance. (C) Transposon Tn\textsubscript{552} encoding inducible β-lactam resistance. (D) Staphylococcal cassette chromosome encoding resistance to β-lactams. Resistance determinants are indicated in blue; genes encoding regulatory (red) or accessory functions (black) for antibiotic resistance are also indicated. See text for details.
Figure 2. Translational and transcriptional attenuation of *erm* gene expression
(A) Translational attenuation of *erm(C)*. In the absence of inducer, ribosomes translate the leader peptide (Erm(C)L) and dissociates allowing reformation of stem-loop 1 + 2. Stem-loop 3 + 4 (red) forms in the absence of inducer and blocks ribosome binding to the *erm(C)* RBS2 thus preventing translation. In the presence of inducer, the ribosome stalls on *erm(C)L*, disrupting stem-loop 1 + 2 and through formation of 2 + 3, prevents formation of 3 + 4 and promotes translation of the Erm(C).
(B) Transcriptional attenuation of *erm(K)*. In the absence of inducer, four stem-loop structures, including two Rho-independent transcriptional terminators (red) that prematurely terminate the *erm(K)* transcript. In the presence of inducer, refolding of the transcript disrupts both terminators allowing completion of the *erm(K)* transcript and subsequent translation of Erm(K).

RBS: Ribosomal binding site.
Figure 3. Induction of antibiotic resistance via signal transduction systems
(A) Vancomycin, (B) β-lactams and (C) bacitracin. See text for details.
P: Phosphate; TCS: Two-component system.
Figure 4. Organization of transferable van gene clusters
Produced precursors are (A) D-Ala-D-Lac and (B) D-Ala-D-Ser. The *Streptomyces coelicolor* van gene cluster (C) is shown for comparison.
†Constitutive mecA expression; however, in the presence of the bla regulatory elements BlaI and BlaR1 mecA expression may be under regulatory control of these elements.

ermA ars: Arsenic resistance.

The classification is based on the information provided by the SCCmec website curated by the International Working Group on the Classification of Staphylococcal Cassette Chromosome Elements [202]. See text for further details.
Figure 6. Schematic organization of the bcrABD region on Enterococcus faecalis AR01/DGVS plasmid pJM01 (GenBank AY496968) and on the bcrABD and additional resistance determinant-containing part of E. faecalis plasmid TW9 (GenBank NC_014726) Bacitracin resistance-associated genes are indicated in black, genes for other resistance determinants and their associated regulatory genes are indicated in dark gray, all other genes are indicated in light gray.
Table 1


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</tr>
<tr>
<td>erm(C)</td>
<td>S. aureus</td>
<td>M(_{14/15})</td>
<td>M(_{14/15}) MLSK</td>
<td>MGIFSIFVISTVHYQPNKK [31,38]</td>
</tr>
<tr>
<td></td>
<td>S. agalactiae</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>S. pneumoniae</td>
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<tr>
<td></td>
<td>B. subtilis</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>CoNS</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Constitutive</strong></td>
<td></td>
<td></td>
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<tr>
<td>erm(A)</td>
<td>S. aureus</td>
<td>n/a</td>
<td>MLSK(^*)</td>
<td>Deletions in the 5' UTR disrupt attenuator structure [20,30,112,114]</td>
</tr>
<tr>
<td></td>
<td>S. agalactiae</td>
<td>n/a</td>
<td>MLSK(^*)</td>
<td>Deletions in the 5' UTR disrupt attenuator structure [20,30,112,114]</td>
</tr>
<tr>
<td></td>
<td>S. pneumoniae</td>
<td>n/a</td>
<td>MLSK(^*)</td>
<td>Deletions in the 5' UTR disrupt attenuator structure [20,30,112,114]</td>
</tr>
<tr>
<td>erm(B)</td>
<td>S. pyogenes</td>
<td>M(_{14/15})</td>
<td>M(_{14/15}) MLSK</td>
<td>MTHSMR1FLPFLNQ [34]</td>
</tr>
<tr>
<td></td>
<td>S. agalactiae</td>
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<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>E. faecalis</td>
<td></td>
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<tr>
<td></td>
<td>E. faecalis</td>
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<tr>
<td></td>
<td>Streptococcus pneumoniae</td>
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<td>Bacillus subtilis</td>
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<tr>
<td></td>
<td>Clostridium perfringens</td>
<td></td>
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<td></td>
</tr>
<tr>
<td><strong>Inducible</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>mef(E)/mel</td>
<td>S. pyogenes</td>
<td>M(<em>{14/15})-M(</em>{15})^(\dagger) M(<em>{14/15}) M(</em>{14/15})M(_{14/15})</td>
<td>MTASMRLK(^\ddagger)</td>
<td>[20,39,40,114]</td>
</tr>
<tr>
<td></td>
<td>E. faecium</td>
<td></td>
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<td></td>
</tr>
<tr>
<td></td>
<td>S. pneumoniae</td>
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<tr>
<td></td>
<td>C. perfringens</td>
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</tr>
<tr>
<td>msr(A)</td>
<td>S. aureus</td>
<td>M(_{14/15})</td>
<td>M(<em>{14/15}) LM(</em>{16})</td>
<td>MTASMRLK(^\ddagger)</td>
</tr>
<tr>
<td></td>
<td>S. epidermidis</td>
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</tr>
</tbody>
</table>

\(^{\dagger}\) Resistance conferred only after exposure to inducing antibiotics.

\(^{\ddagger}\) Ribosome stall sites indicated in bold.

\(^{\ddagger\ddagger}\) Resistance 16-membered macrolides induce when possessing a monosaccharide at C-5. Disaccharide 16-membered macrolides do not induce.

\(^{\ddagger\ddagger\ddagger}\) Predicted.
AMP: Antimicrobial peptide (i.e., LL-37); CoNS: Catalase-negative staphylococci; K: Ketolides; L: Lincomycin; M: All macrolides; M$_{14/15}$: 14- and 15-membered macrolides; M$_{16}$: 16-membered macrolides; n/a: Not applicable; S: Streptogramin B; UTR: Untranslated region.
Table 2

Characteristics of acquired vancomycin resistance systems.

<table>
<thead>
<tr>
<th>System</th>
<th>Resistance level</th>
<th>MIC [µg/ml]</th>
<th>Conjugation</th>
<th>Mobility</th>
<th>Expression</th>
<th>Location</th>
<th>Modification</th>
<th>Organisms†</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Vancomycin</td>
<td>Teicoplanin</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>VanB</td>
<td>Variable</td>
<td>4–1000</td>
<td>0.5–1</td>
<td>Positive</td>
<td>Tn1547/Tn1549/Tn5382</td>
<td>Inducible</td>
<td>Plasmid or chromosome</td>
<td>D-Ala-, D-Lac</td>
</tr>
<tr>
<td>VanM</td>
<td>High</td>
<td>&gt;256</td>
<td>&gt;96</td>
<td>Positive</td>
<td>Unknown</td>
<td>Inducible</td>
<td>Plasmid</td>
<td>D-Ala-, D-Lac</td>
</tr>
<tr>
<td>VanD</td>
<td>Moderate</td>
<td>64–128</td>
<td>4–64</td>
<td>Negative</td>
<td>Unknown</td>
<td>Constitutive</td>
<td>Chromosome</td>
<td>D-Ala-, D-Lac</td>
</tr>
<tr>
<td>VanE</td>
<td>Low</td>
<td>8–32</td>
<td>0.5</td>
<td>Negative</td>
<td>Unknown</td>
<td>Variable</td>
<td>Chromosome</td>
<td>D-Ala-, D-Ser</td>
</tr>
<tr>
<td>VanG</td>
<td>Low</td>
<td>16</td>
<td>0.5</td>
<td>Positive</td>
<td>Unknown</td>
<td>Inducible</td>
<td>Chromosome</td>
<td>D-Ala-, D-Ser</td>
</tr>
<tr>
<td>VanL</td>
<td>Low</td>
<td>8</td>
<td>n.d.</td>
<td>Negative</td>
<td>Unknown</td>
<td>Variable</td>
<td>Chromosome</td>
<td>D-Ala-, D-Ser</td>
</tr>
<tr>
<td>VanN</td>
<td>Low</td>
<td>16</td>
<td>0.5</td>
<td>Positive</td>
<td>Unknown</td>
<td>Constitutive</td>
<td>Chromosome</td>
<td>D-Ala-, D-Ser</td>
</tr>
<tr>
<td>VanC</td>
<td>Low</td>
<td>2–32</td>
<td>0.5–1</td>
<td>Negative</td>
<td>–</td>
<td>Constitutive</td>
<td>Chromosome</td>
<td>D-Ala-, D-Ser</td>
</tr>
</tbody>
</table>

†Limited to clinically relevant species.


Adapted from [50].