MiniReview

Staphylococcal methicillin resistance: fine focus on folds and functions

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Abstract

Globalisation has entailed a massive increase in trade and human mobility facilitating the rapid spread of infectious agents, including those that are drug resistant. A particularly serious threat to human health is posed by methicillin-resistant staphylococcal strains which have acquired molecular mechanisms to evade the action of β-lactam antibiotics (BLAs). Full expression of high-level methicillin resistance involves a complex network of molecules and depends primarily on sufficient expression of a penicillin-binding protein with low sensitivity towards BLAs. Other factors include the fine-tuned regulation of autolytic activity of cell-wall components, as well as an optimal rate of peptidoglycan precursor formation and a highly specific peptidoglycan precursor structure. Three-dimensional structural data are available on several of the pieces involved in the jigsaw puzzle and provide a molecular basis for the understanding of methicillin resistance and for the design of new therapeutic strategies.

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1. Staphylococcal resistance towards methicillin

Resistance (Arzneifestigkeit or fastness) is a natural response to antimicrobial stress based on selection which weakens the effect of chemotherapy. The introduction of large numbers of chemotherapeutic agents into clinical practice has generated strains of microorganisms that survive and multiply in vivo in the presence of high drug concentrations. Drug-resistance had already been encountered in trypanosomes by Paul Ehrlich and co-workers before 1908 [1] and was documented in humans in 1917 [2]. It cannot be eradicated-it can only be delayed.

β-lactam antibiotics (BLAs) are highly specific inhibitors of eubacterial peptidoglycan metabolism and among the most popular chemotherapeutic agents due to their selective toxicity and generally broad spectrum of action. They target the transpeptidase domain (TP) of penicillin-binding proteins (PBPs), which are membrane-bound D,D-peptidases related to serine proteases [3]. PBPs are transpeptidases and transglycosylases that cross-link the bacterial peptidoglycan cell wall in firmicutes and thus may maintain its integrity [4].

Staphylococcus aureus has extensive genomic variability and easily acquires tools for resisting against antimicrobials, in particular against BLAs. Therefore, staphylococci were among the first to be identified as troublesome antibiotic-resistant pathogens [5,6]. The methicillin-resistant variant of S. aureus (MRSA) is a major hospital-borne pathogen that causes high morbidity and mortality. It appeared in 1961, less than one
year after methicillin was introduced [7], and its prevalence in US hospitals is now approaching 50% [8].

2. MecA is the main molecular determinant for methicillin resistance

In MRSA, intrinsic resistance appears as reduced sensitivity to methicillin and several other BLAs. This is mainly accomplished by the production of an exogenous PBP termed MecA (*alias* PBP2*₀* or PBP2a). MecA is encoded by *mecA* on a genetic element, SCC mec, acquired by parasexual horizontal transfer from an extraspecies source [9]. The four housekeeping PBPs in *S. aureus* are BLA-sensitive, while MecA shows much lower affinity for BLAs. Therefore, it can take over part of their function when they have been inactivated, thus contributing to the production of clinical isolates which are impervious or less sensitive to BLAs.

MecA synthesis is inducible and modulated by a signal-transduction system encoded by genes from the MRSA *mec* divergon on SCC mec [10]. This system comprises an integral-membrane zinc-dependent sensor/signal transducer, MecR1, and a constitutive transcriptional repressor, methicillin repressor MecI [11]. Both the sensor/transducer and the repressor are located on the same operon, *mecR1–mecI*, immediately upstream from the *mecA* promoter, and they are counter-transcribed [7,12,13]. An orthologous system to MecR1–MecI–MecA has been described in methicillin-sensitive *S. aureus* (MSSA) and *Bacillus licheniformis*, with a *bla* divergon encoding the BlaR1–BlaI–BlaZ and BlaR–BlaI–BlaP (*alias* PenJ–PenI–PenP) protein triads, respectively [12,14,15]. In contrast to the PBP MecA, the effectors BlaZ and BlaP are β-lactamases, which hydrolyse BLAs instead of participating in cell-wall turnover.

MecR1 comprises an N-terminal integral-membrane metalloprotease domain and a C-terminal extracellular PBP-like domain. The latter is very sensitive to environmental BLAs, which do not penetrate the cytoplasmic membrane. MecI is a dimeric 123-amino acid protein with potential homologues in several bacterial genomes [16,17]. It represses transcription of *mecA*, but also of *mecI* and *mecR1*. Thus, constitutive resistance to methicillin may arise through mutations inactivating MecI or down-regulating *mecI* transcription.

3. MecA synthesis regulation

Biochemical and genetic studies on proteins encoded by the *bla* and *mec* divergons have led to a working model for the MecR1–MecI–MecA signal transduction system [10,15,18–26]. Accordingly, dimeric methicillin repressor MecI constitutively represses its own transcription and that of MecR1 and MecA through binding to an extended region containing two palindromes within the *mecI–mecR1* and *mecA* promoter regions (Fig. 1). The repressor is similar to both *S. aureus* and
B. licheniformis BlaI and they are functionally interchangeable [19,27]. MecR1, present in a low copy number, is inserted in the cytoplasmic membrane, probably via four transmembrane helices [22,26]. It is a metalloendopeptidase zymogen that senses the presence of BLAs via its extracellular PBP-domain. Upon BLA-mediated acylation of the PBP moiety, domain rearrangement transmits a conformational change across the membrane. An interaction between the PBP domain and an extracellular region between two of the transmembrane helices of the metalloendopeptidase domain has been shown for B. licheniformis BlaR only in the absence of BLAs [26]. The transmission of this conformational change across the membrane induces activation of the intracellular metalloprotease moiety. Cleavage occurs on the cytosolic side immediately before the last (putative) transmembrane helix that connects the integral-membrane signal transducer with the C-terminal extracellular signal sensor [10,22]. This limited proteolysis step is understood to be autolytic [10] and liberates the metalloprotease activity which, by migrating across the membrane, may reach the site where MecI blocks the membrane, may reach the site where MecI blocks BLAs [26]. The transmission of this conformational change across the membrane induces activation of the intracellular metalloprotease moiety. Cleavage occurs on the cytosolic side immediately before the last (putative) transmembrane helix that connects the integral-membrane signal transducer with the C-terminal extracellular signal sensor [10,22]. This limited proteolysis step is understood to be autolytic [10] and liberates the metalloprotease activity which, by migrating across the membrane, may reach the site where MecI blocks DNA transcription. There, the inhibitor is inactivated at a single bond in a second limited-proteolysis event. Accordingly, both MecR1 and MecI are turned over during signal transduction and require synthesis inducive. Genes murF, fmtA-C, sigB, hmrA and hmrB, dlt, pbp2, and ctaA, as well as the auxiliary genes (aux) alias factors essential for methicillin resistance (genes or mutants femA-F, femR, and femX) have been described [32,34]. Staphylococcal murein hydrolases, like the lytH gene product, also play a pivotal role as lytic enzymes in peptidoglycan growth and turnover. Some factors only have a minute effect on methicillin resistance, like those encoded by the global regulators sar and agr, which control production of virulence factors. Functions for several other required genes and gene products, however, remain elusive, like lfm and the auxI6-encoded protein [4,36,37].

4. Other factors governing staphylococcal methicillin resistance

MecA is the primary determinant of intrinsic methicillin resistance but additional genes are required for a high-level resistance phenotype, besides other environmental factors [4,32–35]. These genes are native constituents of the S. aureus genome and participate mostly but not exclusively in cell-wall biosynthesis and turnover. Genes murF, fmtA-C, sigB, hmrA and hmrB, dlt, pbp2, and ctaA, as well as the auxiliary genes (aux) alias factors essential for methicillin resistance (genes or mutants femA-F, femR, and femX) have been described [32,34]. Staphylococcal murein hydrolases, like the lytH gene product, also play a pivotal role as lytic enzymes in peptidoglycan growth and turnover. Some factors only have a minute effect on methicillin resistance, like those encoded by the global regulators sar and agr, which control production of virulence factors. Functions for several other required genes and gene products, however, remain elusive, like lfm and the auxI6-encoded protein [4,36,37].

5. Three-dimensional structures of proteins engaged in methicillin resistance and related proteins

5.1. MecA/PBP2a/PBP2′

The central actor of methicillin resistance belongs to the high-molecular mass PBPs of class B1, a family containing a TP with very low affinity for BLAs [3,38]. The 78-kDa transmembrane MecA enzyme is a rather inefficient transpeptidase, producing muropeptide dimers only when other PBPs have been switched off. Recent studies suggest that it does not fully substitute these PBPs but contributes its TP to the transglycosylase function of native PBPs during cell-wall synthesis when their TPs have been inactivated under BLA stress [39].

The structure of a soluble variant of MecA lacking the transmembrane part reveals that the proteins consist of a unique N-terminal extension, a central non-penicillin binding domain (NPBD), and a C-terminal TP (Fig. 2(a); [40]). The first domain is novel and features a curved three-stranded antiparallel β-sheet that places a helical bundle made of five α-helices on its concave side. This domain occupies the space of a segment protruding from the main molecular body in the other high-molecular mass PBP class B structural archetype, Streptococcus pneumoniae PBP2x [41]. Together with the
adjacent NPBD, it is shaped like a sugar tong in the streptococcal protein. An extended three-stranded anti-parallel $\beta$-sheet connects the first domain with the intermediate NPBD in MecA. It is constituted by another curved three-stranded $\beta$-sheet, with three $\alpha$-helices attached to its convex side. This domain is reminiscent of the equivalent in PBP2x [41]. Finally, the C-terminal TP folds in the typical manner of serine PBPs/$\beta$-lactamases, with a central core constituted by a five-stranded anti-parallel $\beta$-sheet flanked on either side by $\alpha$-helices. This core shows an insertion of a 125-residue subdomain (Ser400–Leu525) of mainly $\alpha$-helical nature, which occupies the space beyond the outermost strand of the $\beta$-sheet and harbours the nucleophilic serine Ser403. This catalytic residue is acylated in PBPs during peptide cross-linking, but also in an extremely slow and reversible manner by BLAs. In contrast to PBP2x, MecA lacks the C-terminal domain after the TP. Significant flexibility was observed in the NPBD and the region around the active site within the TP. This active site is distorted and must undergo conformational changes for nucleophile acylation to occur. This results in a reduction of affinity for BLAs and a poor acylation rate, the ultimate factors responsible for resistance against BLAs [40].

5.2. MecI and BlaI

Methicillin repressor is a homodimer, both unbound and in complex with double-stranded DNA encompassing the cognate operator sequence [16,17]. Each monomer consists of a compact globular N-terminal DNA-binding domain (DBD), specifically recognising the nucleotide sequence 5'-GTAXT-3' (X for any base), and a C-terminal dimerisation domain (DD). The chain topology of the DBD is consistent with a winged-helix architecture which encompasses a helix-turn-helix motif penetrating the DNA major groove via a recognition helix. This motif is followed by a $\beta$-hairpin wing, engaged in minor groove interactions. This protein/DNA interaction causes unusual convex bending of the DNA
helix in the DNA/MecI complex. The DBDs do not participate in dimer stabilisation as dimerisation is accomplished through the C-terminal DDs, which feature right-handed superhelical ribbons comprising three consecutive helices. These DD helices are closely juxtaposed in both protomers and intimately intertwine, giving rise to a novel fold in the shape of a spiral staircase. In BlaI and MecI proteins, a specific cleavage between Asn101 and Phe102 within the DD is responsible for regulation, rendering an inactive repressor form or a variant with strongly reduced affinity for double-stranded DNA. The scissile bond flanking residues are strongly conserved within the MecI/BlaI family [17] and scission reduces the interaction surface by more than two thirds. This may eventually cause the dimer to fall apart, restituting mecA, mecRI and mecI transcription.

The structure of the MecI DBD correlates well with that of the isolated DBD of B. licheniformis BlaI (Fig. 2(c)), encompassing the first 82 residues of the full-length 128-residue protein, except for the segment connecting the first two α-helices, which includes a three-residue β-strand. In MecI this feature is reduced to one residue [16,17,42]. In addition, the structure of unbound full-length S. aureus BlaI is in agreement with the foregoing (Fig. 2(d)), except for the distance between the DBDs, which is 9 Å shorter due to a rigid-body translation of one of them than in MecI (Safo, M.K., Zhao, Q., Musayev, F.N., Robinson, H., Scarsdale, N., Archer, G.L., personal communication). Upon DNA binding, a rearrangement of these domains could be conceived to fit the major groove in a similar manner as in MecI. These differences account for the flexibility within this family of repressors and provide an explanation for their interchangeability.

5.3. BlaR C-terminal PBP domain

BlaR/BlaR1/MecR1 are high-molecular weight class C PBPs [3,8], consisting of a ~38-kDa N-terminal (putative) integral-membrane metalloprotease and a ~27-kDa C-terminal extracellular BLA sensor connected by a linker. The isolated sensor domain belongs to the serine PBPs and can be acylated by a variety of PBPs [32]. A 256-residue form from B. licheniformis has been structurally characterised [25]. Sequence alignments indicate that it is a valid model for the equivalent part of MecR1. The structure reported is in its non-acylated form, which is similar to class D β-lactamases (Fig. 2(e)), with two subdomains and the catalytic pocket located at their interface. One subdomain (at the right in Fig. 2(e)) has an α/β structure consisting of a central mixed parallel/antiparallel six-stranded β-sheet flanked by two helical segments on either side. The adjacent subdomain is helical and shows four helices surrounding a central hydrophobic helix. This helix contributes to the active site through the nucleophilic serine, Ser402, and the catalytic lysine, Lys405. A more polar environment of the latter residue is believed to impede β-lactamic activity, conferring its PBP-instead of β-lactamase-character on the protein. This is accomplished through increased stability of the acyl-enzyme intermediate once formed, which prevents subsequent hydrolysis [25].

5.4. FemA and FemX

Optimal MecA-mediated methicillin resistance requires a specific structure for the peptidoglycan: a muropeptidic pentaglycerine bridge must be built on the amino group of a pentapeptide lysine side chain [44]. The synthesis of this peptide is regulated by at least three constitutive proteins in S. aureus FemA, FemB, and FemX [45]. The latter catalyses the transfer of the first glycine, while the first two enzymes, encoded by the same operon, are responsible for attachment of the two subsequent glycerylglycine dipeptide to the stem peptide lysine. These enzymes are non-ribosomal peptidyl transferases which use aminoacyl-tRNAs as amino acid donors. Reduction of the length of the peptide chain by femAB inactivation leads to anomalies in cell growth, cell-wall turnover, and peptidoglycan cross-linking, as well as hypersensitivity towards all BLAs [32].

S. aureus FemA contains 433 residues in two globular subdomains and an inserted coiled coil (Fig. 2(f); [46]). The two subdomains are topologically similar to each other and consist of a mixed α/β structure centred on a strongly twisted β-sheet. This fold is reminiscent of GCN5-related N-acetyltransferases, which catalyse the transfer of an acetyl group from acetyl coenzyme A to a primary amine [45,46]. The N-terminal subdomain (cyan in Fig. 2(f)) has a strongly bent seven-stranded mixed parallel/antiparallel sheet, flanked on its concave side by two helices and on the opposite side by three helices. The subsequent subdomain (orange) is inserted into the previous subdomain and harbours seven strands arranged in a strongly twisted mixed parallel/antiparallel sheet. Two additional strands are provided by a β-hairpin from the preceding subdomain to the left edge of the sheet. Two helices are found on one side of the sheet and three on the other. An L-shaped channel separates the two subdomains, providing a putative binding site for the large disaccharide hexapeptide lipid substrate. FemA is reminiscent of a left hand, with the fingers made up by strands of the first subdomain β-sheet with the connecting loops. These fingers pave the floor of the active-site cleft, together with the palm. A two-helix segment of the second subdomain (partially disordered in the connecting loop; see Fig. 2(f), right) grasps the substrate like a thumb. A 65-residue insertion on the edge of the second sheet, between the penultimate and the antepenultimate strand, makes up the antiparallel two-helix coiled-coil moiety, protruding almost
The targets remain to be identified.

6. Conclusion

It is difficult to quantify the impact of microbial resistance on health, though published data indicate that morbidity and mortality are increased by delays in effective treatment for infections caused by resistant pathogens. Additionally, the prolonged illness and hospitalisation of patients with resistant infections, and the additional procedures and drugs required, have financial implications. Accordingly, there is a need to develop new magic bullets against microbes, but a complementary approach implies to defeat the defences build up by them to circumvent the activity of already available efficient drugs like BLAs. To this end, it is of pivotal importance to obtain a detailed picture of the development of the MRSA phenotype. The analysis of intervening molecules is a rapidly evolving field—all the structures discussed have been published in the last two years. This information may pave the way to a better understanding of the molecular mechanisms underlying methicillin resistance that may in turn lead to the development of new therapeutic approaches to overcome this health problem.

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References


