Review

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Nosocomial infection and its molecular mechanisms of antibiotic resistance

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Summary **Nosocomial infection is a kind of infection, which is spread in various hospital environments, and leads to many serious diseases (*e.g.* pneumonia, urinary tract infection, gastroenteritis, and puerperal fever), and causes higher mortality than community-acquired infection. Bacteria are predominant among all the nosocomial infection-associated pathogens, thus a large number of antibiotics, such as aminoglycosides, penicillins, cephalosporins, and carbapenems, are adopted in clinical treatment. However, in recent years antibiotic resistance quickly spreads worldwide and causes a critical threat to public health. The predominant bacteria include Methicillin-resistant *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Escherichia coli*, and *Acinetobacter baumannii*. In these bacteria, resistance emerged from antibiotic resistant genes and many of those can be exchanged between bacteria. With technical advances, molecular mechanisms of resistance have been gradually unveiled. In this review, recent advances in knowledge about mechanisms by which (*i*) bacteria hydrolyze antibiotics (*e.g.* extended spectrum β-lactamases, (*ii*) AmpC β-lactamases, carbapenemases), (*iii*) avoid antibiotic targeting (*e.g.* mutated vanA and *mecA* genes), (*iv*) prevent antibiotic permeation (*e.g.* porin deficiency), or (*v*) excrete intracellular antibiotics (*e.g.* active efflux pump) are summarized.**

Keywords: Hospital-acquired infection, mutations, PBP2a, SCC*mec*, OprD, MexEF-OprN.

1. Introduction

Nosocomial infection, also known as hospital-acquired infection, is a kind of infection, which is contracted from the environment or staff of a healthcare facility (*1*). It can be spread in various hospital environments, including nursing homes, wards, operating rooms, or other clinical settings. Infection happens in the clinical setting through a large number of pathways. In addition to contaminated equipment, bedding articles, or aerosols, staff also can spread infection (*2*). An epidemiological investigation implemented by WHO in fifty five hospitals of fourteen countries from four WHO Regions (Europe, Eastern Mediterranean, South-East Asia and Western Pacific) revealed an average of 8.7% of hospital patients had a nosocomial infection. At any time, over 1.4 million people worldwide suffer from infectious complications acquired in hospitals (*3*). The morbidities of nosocomial infection were reported from hospitals in the European, Eastern Mediterranean, South-East Asia and Western Pacific as 7.7, 11.8, 10.0, and 9.0% respectively (*4*). Nosocomial infections could lead to functional disability and mental stress of patients. In addition, nosocomial infections are also one of the leading causes of death (*5*).

In hospitals, patients are exposed to a diversity of microbes. Many different bacteria, viruses, fungi and parasites may lead to nosocomial infections (*6*). Most recently hospital-acquired infections are caused by common bacteria that usually lead to no or milder disease compared to in-patients such as, *Staphylococcus aureus*, *enterococci*, *Pseudomonas spp.* and E*nterobacteriaceae* (*7*). After being infected, patients commonly receive antibiotics. Through selection and

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exchange of genetic resistant elements, antibiotics boost the emergence of multi-drug resistant strains. Bacteria which are sensitive to the antibiotics are suppressed or killed, while resistant strains survive and may become endemic and burst out in the hospital (*8,9*). Based on previous research, the major mechanisms of antibiotic resistance include extended spectrum β-lactamases (ESBLs), AmpC β-lactamases, carbapenemases, staphylococcal cassette chromosome *mec* (SCC*mec*), VanA ligase, porin deficiency, and active efflux pumps (Figure 1). The above mechanisms will be introduced in the following sections.

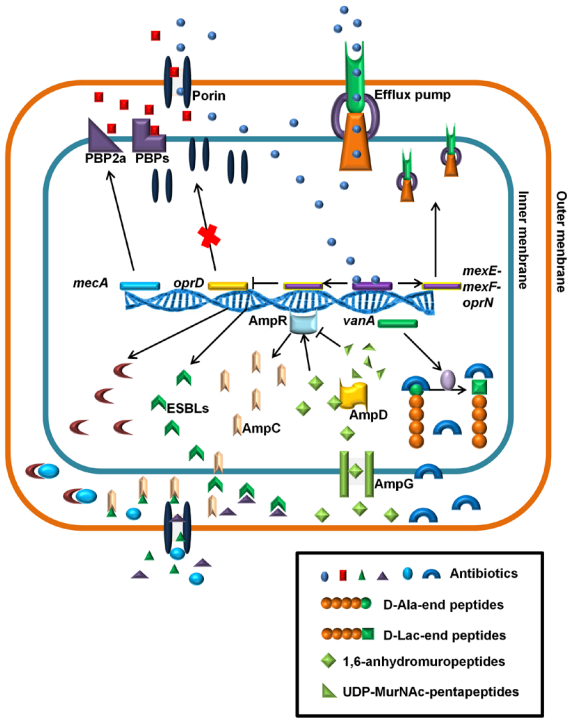

Figure 1. Molecular mechanisms of antibiotic resistance

2. Hydrolyzing antibiotics I: ESBLs

The emergence of third-generation cephalosporins in clinical treatment in the early 1980s was reported as a significant breakthrough to antagonize β-lactamase-mediated antibiotic resistance. Soon after, the first research into plasmid-encoded-lactamases which are able to hydrolyze extended-spectrum cephalosporins was reported in 1983 (*10*). The genes, which encode β-lactamases much similar to SHV-1, TEM-1, and TEM-2, exhibited mutations of single nucleotides and were soon discovered to have the ability to acquire resistance to extended-spectrum cephalosporins (Table 1) (*11,12*). For now,various ESBLs contained in Gram-negative bacteria such as *E. coli*, *K. pneumoniae*, *A. baumannii* and *P. aeruginosa* have proved to be capable of resistance to most of β-lactam antibiotics. Because ESBLs-producing bacteria are able to hydrolyze a large number of β-lactam antibiotics, the utility of those antibiotics for infections caused by such bacteria is reduced. Moreover, the plasmids containing the genes that encode ESBLs usually also contain genes that cause resistance to aminoglycosides and trimethioprim/sulfamethoxazole. There have been more and more reports of plasmid-induced attenuation in susceptibility to aminoglycosides, often being associated with plasmid-

Table 1. Resistance-related β-lactamases

β-lactamases	Families	Targets	Susceptibility (to clavulanic acid)	Classes	*Ref.*
Extended spectrum β-lactamases (ESBLs)	TEM family and SHV family	Targets of broad-spectrumβ-lactamases(Aminopenicillins,benzylpenicillin,carboxypenicillins, narrow-spectrum-cephalosporins), oxyimino-cephalosporins,monobactam	++++	A	(*19-27*)
	CTX-M family	Targets of broad-spectrumβ-lactamases, cefepime	++++	A	(*28-30*)
	XOA family	Same as above	+	D	(*21,31,32*)
	Others (PER-1, VEB family, GES family, IBC-2, BES-1, IBC-1, SFO-1, and TLA-1)	Same as TEM family and SHV family	++++	A	(*33, 34*)
AmpC	FOX family, CMY family, LAT family, DHA family, and MOX family	Targets of broad-spectrumβ-lactamases, cephamycins	0	C	(*35-40*)
Carbapenemase	IMP family and VIM family	Targets of broad-spectrumβ-lactamases,cephamycins,carbapenems	0	B	(*41-44*)
	KPC family	Same as above	+++	A	(*41-44*)
	OXA family	Same as above	+	D	(*41,42,45*)

induced cephalosporin resistance (*13,14*). Even when plasmid-mediated attenuation of susceptibility of quinolone y is absent, there also is an obvious correlation between quinolone resistance and ESBLs production (*15-17*). The reason for such correlation is not yet understood.

2.1. *TEM family*

Mutations of single nucleotides at many sites in genes coding TEM-1β-lactamases can be achieved in the laboratory with complete activity (*18*). Those mutations, which change the ESBLs phenotype transform the configuration of the active site of the enzyme, and allow interaction between active site and oxyimino-β-lactams (*18-20*). Exposing the active site to β-lactam substrates also leads to susceptibility of the ESBLs to β-lactamase inhibitors, such as clavulanic acid. More than one hundred and thirty members of the TEM family are now recognized, and their diversity supplies a useful pathway to trace the transmission of individual resistance genes (*21,22*).

2.2. *SHV family*

SHV-1 coincides in 68% of its amino acids sequence with TEM-1 and shares its molecular structure (23). Like the TEM family, members of the SHV family have certain amino acid mutations at the active site. More than fifty members of the SHV family recently have been identified through unique combinations of amino acid replacements (*24*). The SHV family recently has been shown in surveys of resistant strains in Europe and America (*25,26*). SHV-5 and SHV-12 are prevalent among the members of the SHV family (*27*).

2.3. *CTX-M family*

Another family of ESBLs not a member of the TEM or SHV families was named CTX-M to emphasize its greater activity against cefotaxime compared to ceftazidime. More than forty members of CTX-M are currently known (*28*). Belying their name, some hydrolyze ceftazidime more rapidly than they do cefotaxime. CTX-M-14, CTX-M-3, and CTX-M-2 are the most widespread (*29,30*).

2.4. *OXA family*

Twelve members of the OXA family have recently been discovered *(21*). They were found mainly in *P. aeruginosa* in clinical samples from France and Turkey (*31*). Major members of the OXA family are relatively resistant to clavulanic acid-induced inhibition. Some of them have resistance mainly to ceftazidime, but OXA-17 shows stronger resistance to cefotaxime and cefepime than to ceftazidime (*32*).

2.5. *Other members of ESBLs*

Other members of ESBLs are rare and have been discovered predominantly in *P. aeruginosa* and in some small areas: PER-1 was found in France, Italy, and Turkey; VEB family was found Southeast Asia; and GES family and IBC-2 were found in South Africa, France, and Greece (*33*). A part of these ESBLs were discovered in *Enterobacteriaceae* as well, but other rare ESBLs, such as BES-1, IBC-1, SFO-1, and TLA-1, have been discovered only in *Enterobacteriaceae* (*34*).

3. Hydrolyzing antibiotics II: AmpC β-lactamases

AmpC β-lactamases, which are usually induced by β-lactams, are expressed in many Gram-negative bacteria. Mutations in relevant genes lead to increasing expression levels and promote the emergence of cephalosporin resistance in *Enterobacter cloacae* (*35*). The AmpC β-lactamases in *E. coli* are present at a low expression level and the AmpC-encoded gene is deficient in the chromosome of *klebsiella* and *salmonella* strains. However, plasmid-expressed AmpC β-lactamases can endow those bacteria with similar resistance as *Enterobacter cloacae* mentioned above. Until recently, more than twenty AmpC β-lactamases have been found expressed by plasmids (*36*).

As shown in Figure 1, The ampC-related regulatory pathway includes the following three elements: (*i*) AmpG which is a permease on the inner membrane; (*ii*) AmpD which is an amidase in cytoplasm; and (*iii*) AmpR, a transcription factor, is a member of the LysR family, a group of regulatory proteins (*37*). These three elements are necessary for expression of AmpC β-lactamases in both *Enterobacteriaceae* and *P. aeruginosa* (*38*). In the regular process of cell wall recycling, 1,6-anhydromuropeptides are disassembled from the cell wall and transferred into the cytoplasm by AmpG permease. The 1,6-anhydromuropeptides are cut by AmpD protein to produce tripeptides, which are subsequently transformed into UDP-MurNAc-pentapeptides. UDPMurNAc-pentapeptides couple with AmpR proteins combining in the intergenic region between *ampR* and *ampC*, and generating a structure that inhibits activation of *ampC*. Low expression levels of AmpC are generated, and the β-lactamase localizes to the periplasmic space. When β-lactams, such as cefoxitin and imipenem, permeate the outer membrane of bacteria, they reach the periplasmic space, and combine with target penicillin binding proteins (PBPs). The amount of 1,6-anhydromuropeptides increases, and AmpD is unable to efficiently deal with the high levels of cell wall pieces. The anhydro-MurNAc-peptides substitute UDP-MurNAc-pentapeptides binding to AmpR, leading to a structural change of the enzyme. AmpR is changed into a role of transcriptional promoter, AmpC is produced at higher levels, and concentration of AmpC rises in the

periplasmic space. When the concentration of β-lactam decreases below its "alarm level" the amount of anhydro-MurNAc-peptides in cytoplasm also decreases, and AmpD's ability to efficiently cut these peptides is restored. In another case, mutations of nucleotides in genes leads to deficiency of AmpD or down-regulates expression of ampD damage in the process of wall fragment recycling and leads to increased concentration of anhydro-MurNAc-peptides in the cytoplasm. As a result, the combining of anhydro-MurNAc-peptides to AmpR makes AmpR "locked" in a structure as transcriptional activator of ampC, and produces high levels of AmpC β-lactamases (*37,39,40*).

4. Hydrolyzing antibiotics III: Carbapenemases

Carbapenemases are a kind of β-lactamase with various hydrolytic abilities. They have been identified to have ability to damage penicillins, cephalosporins, and carbapenems. Bacteria generating the carbapenemases, which resist these antibiotics by breaking β-lactams, frequently lead to serious nosocomial infections. Carbapenemases belong to the A, B, and D molecular class of β-lactamases (*41*). Class A and D β-lactamases have a mechanism of serine-based hydrolysis, while class B β-lactamases are metallo-β-lactamases which have zinc in their active site (*42*). The carbapenemases group of class A contains members of the KPC, NMC, IMI, SME, and GES families. Among these families, the KPC carbapenemases are the most predominant, usually existing on plasmids in *Klebsiella pneumoniae* (*43,44*). The carbapenemases group of class D contains OXA β-lactamases usually found in *Acinetobacter baumannii*. The metallo-β-lactamases were first found in *Pseudomonas aeruginosa* strains, but at present, there is an increasing worldwide emergency of this class of β-lactamases in the *Enterobacteriaceae* (*45*).

5. Avoid antibiotics targeting I: *mecA* mutation

SCC*mec* is a mobile genetic element of *Staphylococcus* bacterial strains. This genetic sequence contains the *mecA* gene, which codes for resistant proteins to the antibiotic methicillin, and is the only known way for *Staphylococcus* species to spread the gene in the wild by horizontal gene transfer. *mecA* leads to resistance to methicillin and other β-lactam antibiotics. After *mecA* is absorbed into bacteria, it is inserted into the *S. aureus* chromosome (*46,47*). *mecA* produces penicillin-binding protein 2a (PBP2a), which is much different from former penicillin-binding proteins because mutations have changed its conformation to make it hard to bind methicillin or other β-lactam antibiotics to its active site. Thus, PBP2a can continuously promote the transpeptidation required for peptidoglycan cross-linking to perform cell wall synthesis in the presence of antibiotics. As a result of the incapability of PBP2a to combine with β-lactam moieties, activation of *mecA* promotes resistance to all other β-lactam antibiotics including methicillin (*48*). *mecA* is controlled by regulatory genes *mecI* and *mecR1*. MecI often combines with the *mecA* promoter and plays an inhibitor role (*49*). In the existence of β-lactam antibiotics, MecR1 promotes a signaling transduction pathway that causes activation of *mecA* (*50*). This activation is initiated by MecR1-induced cleavage of MecI, which decreases MecI inhibition. *mecA* is also regulated by two co-repressors BlaI and BlaR1. *blaI* and *blaR1* are homologous to *mecI* and *mecR1*, respectively, and usually play a role as regulators of *blaZ* which leads to penicillin resistance (*51,52*). The nucleotide sequences recognized by MecI and BlaI are the same, thus BlaI can also combine with the *mecA* operator to inhibit activation of *mecA* (*53*).

6. Avoid antibiotics targeting II: *vanA* mutation

Glycopeptides repress cell wall synthesis in Gram-positive bacteria by combining with the C-terminal D-Ala-D-Ala of the pentapeptide precursors of peptidoglycan, further blocking the reactions of transglycosylation and transpeptidation (*54*). Recently, glycopeptide-resistant *enterococci* have spread throughout the whole world. So far, seven types of resistant elements (VanA, -B, -C, -D, -E, -G, and -L) in enterococci have been discovered and they have seven corresponding operons (*vanA*, *-B*, *-C*, *-D*, *-E*, *-G*, and *-L*) which play roles of synthesis of a novel combining site (peptidoglycan precursors terminating in D-Ala-D-lactate in VanA, -B, and -D type or D-Ala-D-serine in VanC, -E, -G, and -L type) leading to a decreased affinity to glycopeptides and substitution of the normal precursors ending in D-Ala-D-Ala (*55-57*).

A two-component regulatory system VanR-VanS controls vancomycin resistance in vancomycin-resistant *enterococci* (VRE) and vancomycin-resistant *Staphylococcus aureus* (VRSA) (*58*). VanS is a membrane-related sensor for vancomycin which regulates the phosphorylation of VanR. VanR is a transcriptional activator of the operon which encodes VanH, VanA and VanX. VanH is a dehydrogenase which converts pyruvate to D-Lac, and VanA is a ligase which combines D-Ala and D-Lac by creating an ester bond between them. Vancomycin can only combine with D-Ala- D-Ala but not to D-Ala-D-Lac, and thus vancomycin resistance appears. VanX is a dipeptidase which cleaves the normal peptidoglycan component D-Ala-D-Ala that prevents it from leading to vancomycin sensitivity. VanY is a D,D-carboxypeptidase that cuts the end D-Ala residue of the peptidoglycan if substitution of D-Ala-D-Ala by VanX is not thorough. Thus, D-Ala-D-Lac substitutes for the normal D-Ala-D-Ala in peptidoglycan synthesis resulting in vancomycin resistance (*59-61*).

7. Prevent antibiotics permeation: *oprD* mutation and porin deficiency

The outer membrane of Gram-negative bacteria has a semi-permeable barricade which decreases the import of antibiotics, and the outer membrane of *P. aeruginosa* is only 8% as permeable as that of *Escherichia coli* (*62*). However, for surviving, *P. aeruginosa* has to allow import of nutrients through the outer membrane, and this is achieved through a system of water-filled protein channels named porins. DNA sequencing of the *P. aeruginosa* genome has recognized one hundred and thirty known or supposed outer membrane proteins, with sixty four of these outer membrane proteins classified into three families of porins (*62*). These porins play a significant physiological role in the transport of sugars, amino acids, and phosphates, and so on (*63,64*). Some hydrophilic antibiotics, such as β-lactams, aminoglycosides, tetracyclines, and some fluoroquinolones, have been shown to pass through the outer membrane porins (*65-68*). Thus, deficiency of porins can diminish susceptibility of *P. aeruginosa* to some antibiotics.

OprD porin-mediated resistance contains mechanisms that down-regulate the transcriptional level of the *oprD* gene and/or mutations which replace the translational production of a normal porin. At the transcriptional level of *oprD*, disturbing mechanisms contain (*i*) breakdown of the *oprD* promoter, (*ii*) terminating the transcription of *oprD* prematurely, (*iii*) co-regulation with trace metal ion resistance, (*iv*) salicylate-induced decrease, and (*v*) down-regulated transcriptional expression by coregulation with the active efflux pump encoded by mexEF-oprN. The oprD promoter breakdown appears as a result of deletions or insertions in the upstream region of oprD. It was reported that a deletion containing the putative promoter and initiation codon blocked transcription of *oprD* (*69-72*). Based on previous research, IS1394 and an ISPa16-like insertion element have been proposed as an upstream region of the *oprD* in imipenem-resistant strains of *P. aeruginosa* showing down-regulated *oprD* expression (*73,74*).

8. Excreted intracellular antibiotics: mexEF-oprN and actived efflux pump

On the one hand, the deficiency of porins such as OprD is an effective obstacle for antibiotic import into the cell, on the other hand, a decrease in antibiotic concentration can also be realized *via* export through membrane-located efflux pumps. Efflux pumps have been classified into five superfamilies (*75,76*). The superfamilies contain (*i*) the ATP-binding cassette (ABC) superfamily, (*ii*) the small multidrug resistance superfamily, (*iii*) the major facilitator superfamily, (*iv*) the resistance-nodulation-division (RND) superfamily, and (*v*) the multidrug and toxic compound extrusion superfamily.

One of the most important regulatory mechianisms is the coincident overproduction of the MexEF-OprN efflux pump and downregulation/upregulation of the OprD porin (*77*). In wild *P. aeruginosa*, MexT is silenced owing to either the existence of repressing mutations or the deficiency of a secondary effecter (*78*). As a result, expression of *mexEF-oprN* stays at a low level, and expression of *oprD* stays at a basal level providing a proper amount of OprD in the outer membrane sufficient for normal cellular intake (*79*). In *nfxC*-type mutants, MexT becomes active *via* a mutation in *mexT*. The activated MexT protein up-regulates transcription of *mexEF-oprN* causing overexpression of the efflux operon and overproduction of the MexEF-OprN efflux pump. At the same time, MexT down-regulates *oprD* at the transcriptional and translational levels, leading to a decreased amount of *OprD* (*80*). On the other hand, loss of MexS, a supposed oxidoreductase/dehydrogenase, has been thought to lead to formation of secondary metabolites which may serve as effecters for MexT (*77*). These effecters could combine with MexT, change the structure of the regulatory protein, and alter MexT into an activating situation. As a result, MexT can up-regulate the expression of *mexEF-oprN* and down-regulate the expression of *oprD*, similar to the mechanism mentioned above. There also is a third mechanism. Loss of the universal regulatory protein MvaT is also associated with the positive regulation of the *mexEF-oprN* operon (*81*). The mechanism of MvaT-related regulation has not been discovered, but it works independent of MexT and MexS. In contrast to the MexT- and MexS-related regulatory mechanisms, loss of MvaT leads to a positive regulation of both *mexEF-oprN* and *oprD* expression.

9. Conclusion

The capacity of bacteria to evolve resistance to antibiotics has long been realized, but our knowledge about the tremendous variety of molecular mechanisms has been enriched enormously most recently. Technology advances in genomics, proteomics, and structural biology have analyzed many of the molecular mechanisms promoting resistance and will continuously provide more and more intensive explanations. Based on these newest discoveries, the development of novel antibiotics, which can resist or grant knowledge of resistance mechanisms will be accelerated. For speeding up development of new antibiotics, academic institutions and pharmaceutical companies should make joint efforts in the future.

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