

Mechanism of Action and Potential for Use of Tea Catechin as an Anti-infective Agent

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Abstract: "Drinking several cups of green tea a day keeps the doctor away" is clearly an overstatement. However, extensive research has revealed that the predominant catechin from tea (*Camellia sinensis*), epigallocatechin gallate (EGCg), has significant medicinal and health-promoting properties. This review summarizes what is presently known about the antimicrobial properties of EGCg, with a particular focus on the synergistic relationship between EGCg and β -lactams in the inhibition of methicillin-resistant *Staphylococcus aureus* (MRSA). The mechanisms of action and prospects for use of tea catechins such as EGCg as an anti-infective agent are discussed.

Keywords: Tea, catechins, EGCg, polyphenols, flavanols, antimicrobial agents, β -lactams, MRSA.

INTRODUCTION

Clinical reports indicate that antibiotic resistance in bacterial pathogens is an escalating problem. Antimicrobial agents from natural products and traditional medicines could serve as viable supplements to the present range of antibiotics. Tea (*Camellia sinensis*) is one of the most popular beverages worldwide. In traditional Chinese medicine, tea was regarded as a panacea, with antipyretic, antidotal, antidiarrhoeal, and diuretic properties, indicating an anti-infective activity with our modern concept. However, until the end of the 1980s, there were limited reports and experimental data describing the antimicrobial properties of tea [1,2].

The beneficial effects of tea are attributable to catechins and their derivatives, which account for 30% of the dry weight of the water-extractable material from tea [3]. In 1985, Matsuzaki and Hara successfully extracted catechins from green tea, enabling further mechanistic characterization of this class of compounds [4]. Fig. (1) shows the chemical structures of catechins including (+)-catechin (molecular weight, MW 290) and the isomers (-)-epicatechin (EC, MW 290), (-)-epigallocatechin (EGC, MW 306), (-)-epicatechin gallate (ECg, MW 442), and (-)-epigallocatechin gallate (EGCg, MW 458). The polyphenol or flavanol moiety is common to all catechins.

EGCg is the major catechin in green tea, where it accounts for about 50 to 65% of total catechins as reviewed by Zaveri [5] and Nagle *et al.* [6]. Extensive research has revealed that EGCg possesses a range of biological and medicinal properties, including antioxidant [7], anti-carcinogen [8,9], anti-obesity [10], antibacterial, antiviral and anti-enzymatic effects [1,2,5]. This review focuses on recent findings about the antimicrobial activity of EGCg, particularly the synergistic effect of EGCg in combination with β -

lactams against methicillin-resistant *Staphylococcus aureus* (MRSA). The mechanisms of action and prospects for use of tea catechin as an anti-infective agent are discussed.

DIFFERENT SUSCEPTIBILITIES OF STAPHYLOCOCCUS AND GRAM-NEGATIVE RODS TO EGCg AND THE MECHANISM OF ANTI-BACTERIAL ACTIVITY OF CATECHINS

In the 1990s, research focused on determining the antibacterial activity and species specificity of individual catechins. EGCg and ECg are the most potent catechins showing antibacterial activity [1,2]. The activity of these two catechins may be attributable to the galloyl moiety, which is absent from EC and EGC (Fig. 1). The antibacterial activity of catechins was regarded as non-specific with limited species selectivity. Further research revealed that *Staphylococcus* and Gram-negative rods such as *Escherichia coli* (*E. coli*) show different susceptibilities to EGCg [2,11], with the minimum inhibitory concentrations (MICs) of 50-100 $\mu\text{g/ml}$ and $> 800 \mu\text{g/ml}$, respectively. Also, more EGCg binds to *Staphylococcus aureus* (*S. aureus*) than *E. coli*, specifically, 38% of EGCg at 50 $\mu\text{g/ml}$ binds to live *S. aureus*, but only 18% to *E. coli* [11]. EGCg-treated *S. aureus* was more sensitive to high ionic strength and low osmotic pressure [12]. These data indicated that cell wall composition was a determinant for EGCg binding and activity. In order to verify this, peptidoglycan, lipopolysaccharide (LPS) and dextran were added to *S. aureus* cultures grown in the presence of EGCg. As summarized in (Fig. 2), peptidoglycan, LPS or dextran alone showed no effect on bacterial growth, however, peptidoglycan blocked the bactericidal activity of EGCg whereas LPS and dextran did not [11,12].

Peptidoglycan is a cross-linked complex of polysaccharides and peptides. The cell wall of *Staphylococcus* is composed of 30-50 layers of peptidoglycan where it provides osmotic protection, aids in cell division and serves as a primer for further biosynthesis of peptidoglycan [13]. EGCg can directly bind to peptidoglycan and induce its precipita-

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tion (unpublished result). Therefore, the EGCg-induced damage of the cell wall and interference with its biosynthesis through direct binding with peptidoglycan are the major reasons for the susceptibility of *Staphylococcus* to EGCg.

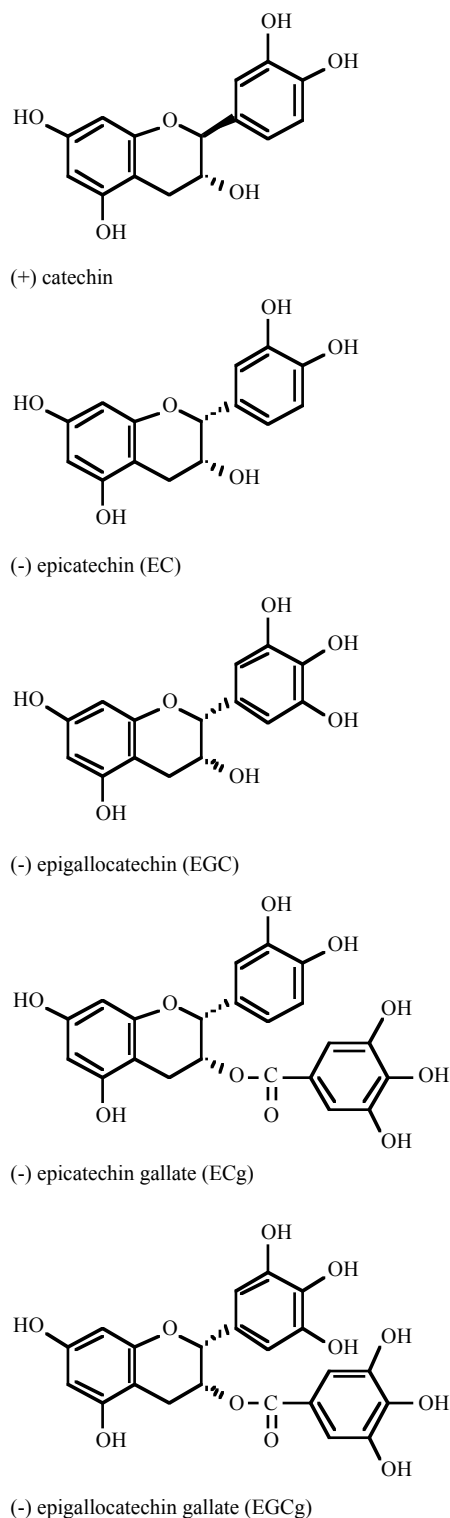


Fig. (1). Chemical structure of catechins.

Although Gram-negative rods have several layers of the peptidoglycan, they are overlaid with an outer membrane composed mainly of LPS. The outer membrane is an impor-

tant permeability barrier which provides protection against various antibacterial materials [14]. Hence, the physiological function of the outer membrane and the low affinity between EGCg and LPS limit the binding of EGCg to peptidoglycan, thereby reducing the susceptibility of Gram-negative rods to EGCg.

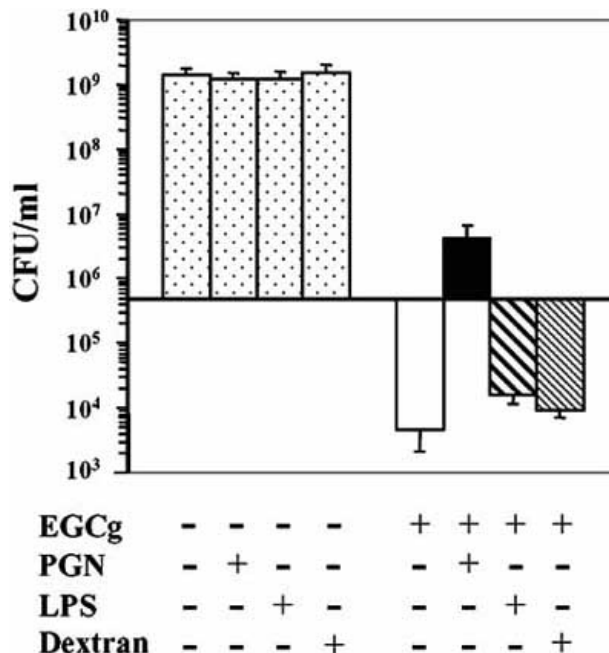


Fig. (2). Antagonism of the bactericidal activity of EGCg by peptidoglycan (PGN). *S. aureus* cells (5×10^5 /ml) were inoculated in Mueller-Hinton broth containing 100 μ g/ml EGCg, 512 μ g/ml PGN, 512 μ g/ml LPS or 2048 μ g/ml dextran and then cultured at 37°C for 24 hours. The bacterial cultures were sampled and serially diluted to spread on Mueller-Hinton agar plates for an additional 24 h cultures. The colony forming units/ml (CFU/ml) of bacterial cultures were calculated.

To elucidate whether peptidoglycan specifically blocks the activity of EGCg, fetal calf serum, peptone or dextran were added to cultures of *S. aureus* containing EGCg. Peptone, fetal calf serum and dextran alone did not affect bacterial growth. However, a combination of fetal calf serum or peptone appeared to interfere with the activity of EGCg. Individual or combined amino acids did not affect the activity of EGCg [11]. These results suggest that components of fetal calf serum and peptone bind to EGCg and block its activity. Catechins are polyphenols and components of condensed tannins [15], which can precipitate proteins by direct binding [15,16]. This property of catechins is mainly responsible for the antibacterial activity but makes EGCg be non-specific and low bioavailable *in vivo*.

Following the observation that EGCg damages liposomes, Ikgai *et al.* hypothesized that EGCg attacks the lipid bilayer of bacterial cell membranes in a similar fashion [17]. However, this activity may be minor since the bacterial cell membrane is protected by an outer cell wall. In phosphate buffered saline (PBS) at neutral pH, EGCg generates

hydrogen peroxide (H_2O_2) which is responsible for bactericidal action of EGCg in PBS [18]. However, this phenomenon can not be confirmed in Mueller-Hinton broth, the standard medium for antibacterial assay (unpublished result). Further research is required to determine the involvement of EGCg-derived H_2O_2 in the bactericidal action.

EFFECTS OF ECGG IN COMBINATION WITH ANTIBIOTICS AGAINST MULTIDRUG-RESISTANT BACTERIA

MRSA is a major nosocomial pathogen which expresses penicillin binding protein 2' (PBP2') with reduced affinity for β -lactam rings. Therapeutic options for treating MRSA infections are limited because most MRSA strains are also resistant to macrolides, aminoglycosides, and fluoroquinolones [19]. Isolates with reduced sensitivity to vancomycin have also been detected [20]. New chemotherapeutic agents are urgently needed to control such multidrug-resistant bacteria. EGCg is an excellent candidate, as extensive research has revealed that β -lactams and EGCg have synergic effects against multidrug-resistant bacteria [12,21-29]. As shown in (Fig. 3), EGCg dose-dependently reverses the high level resistance of clinical isolates of MRSA to β -lactams. EGCg synergizes the activity of β -lactams against MRSA because both EGCg and β -lactams directly or indirectly attack the same target: peptidoglycan synthesis [12]. However, the possibility that EGCg binds with PBP2' and then inhibits its enzymatic activity cannot be excluded.

The most common resistance mechanism to β -lactams is β -lactamase production. Penicillinase occurred in less than 5% of *S. aureus* isolates at the time of penicillin's introduction into clinics in the 1940s, but has dramatically increased to 80-90% of isolates at the present time [30]. The combined effects of EGCg and β -lactams have been examined in various β -lactamase-producing clinical isolates. The combination of EGCg with penicillin shows potent synergy against penicillinase-producing *S. aureus* [31]. Direct binding of EGCg with penicillinase inhibits enzymatic activity and protects the antibacterial activity of penicillin. A synergic effect was not observed when cefotaxime or imipenem were combined with EGCg (100 μ g/ml) to inhibit β -lactamase-producing Gram-negative rods, even though EGCg directly blocked the activity of the β -lactamase extracted from these bacteria [32]. The combined effects on different β -lactamase-producing species appear dependant on the cellular location of β -lactamase [32]. Staphylococcal β -lactamase is extracellular, whereas the β -lactamases of Gram-negative rods are located in the periplasm.

The effects of combining EGCg and non- β -lactam antibiotics have also been studied. Table 1 summarizes the synergic, additive, indifferent and antagonistic effects observed *in vitro* when different antibiotics are combined with EGCg against MRSA [12,26,27].

Additive or indifferent effects on MRSA were observed when EGCg was combined with inhibitors of protein or nucleic acid synthesis [27]. Increased permeability of the cell wall/membrane to antibiotics following EGCg-induced damage of the cell wall may explain the additive effects. It is also reported that EGCg can impair the tetracycline-specific efflux pump, which raises the intracellular drug concentration

and increases the susceptibility of Staphylococcal isolates to tetracycline [33].

EGCg has antagonistic effects on the antibacterial activities of the glycopeptide antibiotics, vancomycin, teicoplanin and polymyxin B. EGCg binds to the peptide backbones of these antibiotics and impairs their activity [27].

MECHANISMS OF ANTIVIRAL AND ANTI-ENZYMATIC ACTIVITIES OF CATECHINS

The effects of tea and tea catechins on viruses, and various enzymes derived from bacteria, viruses and cells have also been studied. Antiviral activities have been observed against tobacco mosaic virus [34], influenza virus [35-42], rotavirus [43], enterovirus [43], HIV [44-47], herpes simplex virus [48,49], adenovirus [50], and Epstein-Barr virus [51,52]. The 50% inhibition concentrations (IC_{50}) of EGCg, ECG, and EGC, for example, against influenza A virus are 10.1-12.8, 9.7-17.7 and 94.6-97.3 μ g/ml, respectively [41]. Anti-enzymatic activities of catechins have been observed against β -lactamases [31,32], reverse transcriptase of HIV [53,54], collagenase [55], fatty acid synthase [56], and various other enzymes [57-62]. We observed that EGCg inhibits penicillinase in a dose-dependent fashion, with an IC_{50} of 10 μ g/ml versus 10 U/ml of the enzyme [31]. EGCg inhibits viruses and enzymes by direct binding to biological molecules [31,32,37,54-62]. EGCg induces agglutination of the influenza virus thus preventing their adsorption to target cells [37]. In addition, the direct binding of EGCg to viral receptors on cell surfaces may also interfere with viral infectivity, following the observation that EGCg binds to CD4 and interferes with binding by the HIV surface protein, gp120 [46].

PROSPECT FOR USE OF TEA CATECHIN AS AN ANTI-INFECTIVE AGENT

The synergistic effects of EGCg and β -lactams against MRSA and penicillinase-producing *S. aureus*, and the potent anti-viral activity of EGCg suggest tea catechins may be valuable therapeutics. However, to date, studies of the antibacterial, antiviral and anti-enzymatic properties of catechins have been restricted to *in vitro* settings. Furthermore, the inhibitory activities are non-specific. EGCg inhibits the reverse transcriptase of HIV only in the absence of serum albumin [54] and the antibacterial activities of EGCg are strongly affected by the presence of proteins in the culture medium [11]. It is likely, therefore, that under *in vivo* conditions, serum proteins and other biological macromolecules may strongly affect the bioavailability of catechins [11,15,16,54] and some of the antimicrobial effects observed *in vitro* may not occur *in vivo*.

Drinking tea or taking EGCg capsules is the only safe way to administer EGCg in humans. It is difficult to estimate the *in vivo* concentration of EGCg following its absorption through the digestive system and distribution to various organs, especially given its propensity to bind with serum or tissue proteins. EGCg of 5.6 μ g/ml was detected in rat blood plasma following administration at 500 mg/kg body weight [63]. In a similar study, EGCg of 2 μ g/ml was detected in human blood plasma 90 minutes after administration of a 525 mg EGCg capsule [64]. These concentrations are not

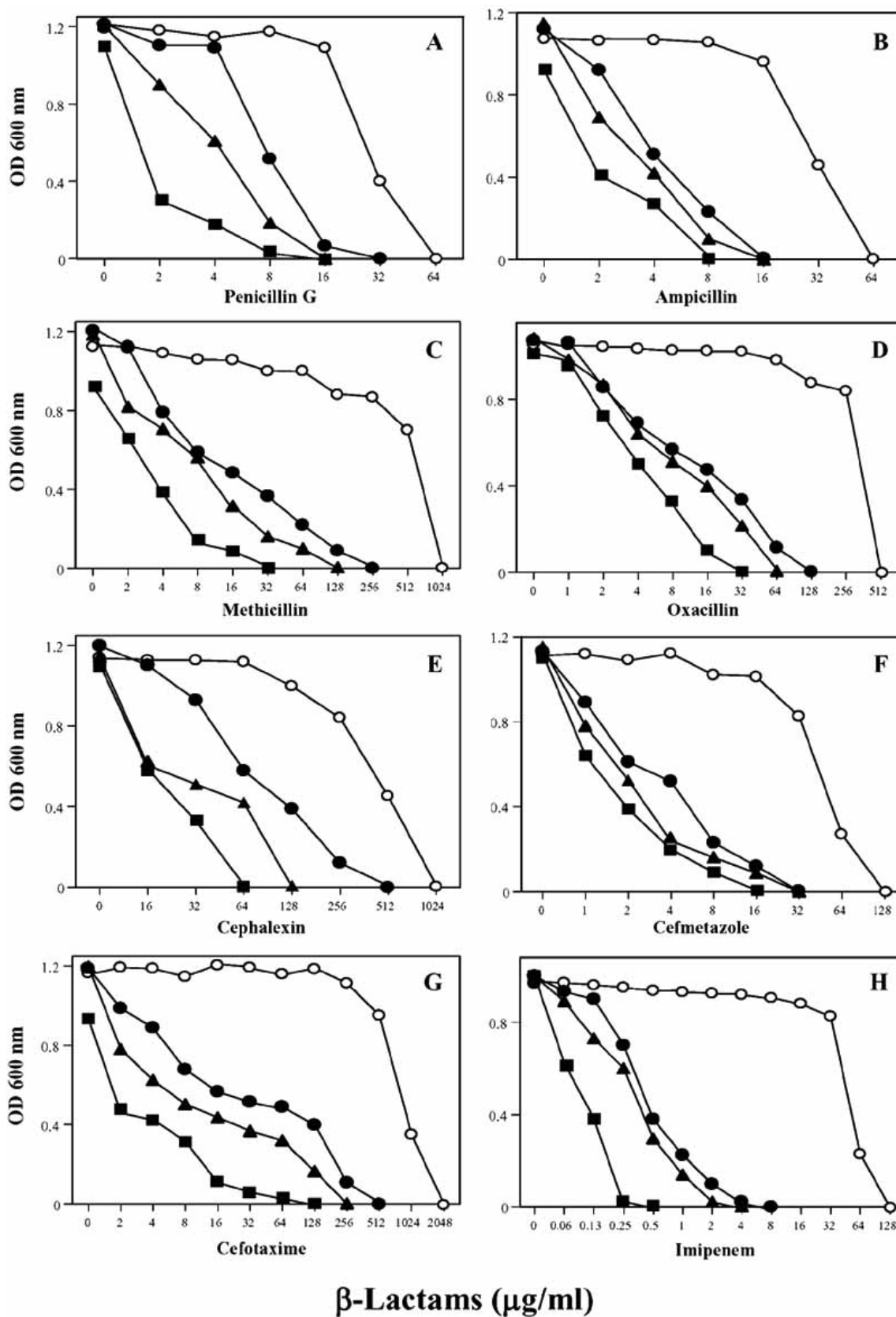


Fig. (3). Synergistic effects between EGCg and β -lactams against MRSA. MRSA cells ($5 \times 10^5/\text{ml}$) were inoculated in Mueller-Hinton broth containing different concentrations of EGCg and various β -lactams, and then cultured at 37°C for 24 hours. Bacterial growth was examined using a spectrophotometer. \circ : β -lactam alone; \bullet : β -lactam plus 6.25 $\mu\text{g/ml}$ EGCg; \blacktriangle : β -lactam plus 12.5 $\mu\text{g/ml}$ EGCg; \blacksquare : β -lactam plus 25 $\mu\text{g/ml}$ EGCg.

Table 1. Effects of EGCg in Combination with Various Antibiotics on MRSA

EGCg + Antibiotic*	Synergy	Addition	Indifference	Antagonism
β -Lactams	+	-	-	-
Inhibitors of protein synthesis	-	\pm	\pm	-
Inhibitors of nucleic acid synthesis	-	\pm	\pm	-
Glycopeptides	-	-	-	+

* Tested antibiotics. β -Lactams: penicillin, ampicillin, methicillin, oxacillin, cephalixin, cefmetazol, cefotaxime, imipenem, panipenem, meropenem; Inhibitors of protein synthesis: tetracycline, minocycline, chloramphenicol, streptomycin, gentamicin, kanamycin, erythromycin; Inhibitors of nucleic acid synthesis: ofloxacin, rifampicin; Glycopeptides: vancomycin, teicoplanin, polymyxin B.

high enough to exert antimicrobial activity. It is plausible that EGCg in combination with antimicrobial agents may be effective against topical or digestive tract infections. The inhalation of catechins was recently tested in order to treat an MRSA infection of the respiratory system [65-67]. Although the inhalation of catechins is safe, the efficacy of this method against MRSA needs to be evaluated further in controlled clinical studies.

In summary, the antimicrobial activity of tea catechins is mainly exerted by direct binding to peptide structure of bacterial components, viruses, and enzymes. The *in vivo* efficacy of EGCg appears hindered by limited absorption, pre-systemic metabolism [68] and non-specific binding with other biological macromolecules [11,15,16,54]. Therefore, considerable research is required to improve the bioavailability of catechins before they can be used as therapeutic compounds.

ABBREVIATIONS

EGCg	=	Epigallocatechin gallate
IC ₅₀	=	50% inhibition concentration
LPS	=	Lipopolysaccharide
MIC	=	Minimum inhibitory concentration
MRSA	=	Methicillin-resistant <i>Staphylococcus aureus</i>
MW	=	Molecular weight

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