Bacteriocins to Thwart Bacterial Resistance in Gram Negative Bacteria

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An overuse of antibiotics both in human and animal health and as growth promoters in farming practices has increased the prevalence of antibiotic resistance in bacteria. Antibiotic resistant and multi-resistant bacteria are now considered a major and increasing threat by national health agencies, making the need for novel strategies to fight bugs and super bugs a first priority. In particular, Gram-negative bacteria are responsible for a high proportion of nosocomial infections attributable for a large part to \textit{Enterobacteriaceae}, such as pathogenic \textit{Escherichia coli}, \textit{Klebsiella pneumoniae}, and \textit{Pseudomonas aeruginosa}. To cope with their highly competitive environments, bacteria have evolved various adaptive strategies, among which the production of narrow spectrum antimicrobial peptides called bacteriocins and specifically microcins in Gram-negative bacteria. They are produced as precursor peptides that further undergo proteolytic cleavage and in many cases more or less complex posttranslational modifications, which contribute to improve their stability and efficiency. Many have a high stability in the gastrointestinal tract where they can target a single pathogen whilst only slightly perturbing the gut microbiota. Several microcins and antibiotics can bind to similar bacterial receptors and use similar pathways to cross the double-membrane of Gram-negative bacteria and reach their intracellular targets, which they also can share. Consequently, bacteria may use common mechanisms of resistance against microcins and antibiotics. This review describes both unmodified and modified microcins [lasso peptides, siderophore peptides, nucleotide peptides, linear azole(in)re-containing peptides], highlighting their potential as weapons to thwart bacterial resistance in Gram-negative pathogens and discusses the possibility of cross-resistance and co-resistance occurrence between antibiotics and microcins in Gram-negative bacteria.

Keywords: bacteriocins, microcins, antibiotics, resistance, Gram-negative bacteria, enterobacteria

INTRODUCTION

Since their discovery antibiotics have been routinely used in human medicine and in livestock production as therapeutic agents or growth promoters. Use of antibiotics for livestock greatly exceeds that of uses for humans, with approximately 70–80 percent of total consumption (Van Boeckel et al., 2017). Furthermore, the global use of antibiotics would rise by 67% by 2030 in...
high-income countries and nearly double in Brazil, the Russian Federation, India, China and South Africa (Van Boeckel et al., 2015). According to the World Health Organization (World Health Organization [WHO], 2017) the overuse and misuse of antibiotics in human and animal, as well as the intrinsic capacity of antibiotics to induce broad spectrum killing (Wester et al., 2002) has led to the emergence of multidrug-resistant bacteria (MDR) that are rapidly increasing worldwide and have now become a serious public health problem. In 2016, the United Nations General Assembly recognized the use of antibiotics in the livestock sector as one of the primary causes of antimicrobial resistance (AMR) (Van Boeckel et al., 2017). Moreover, it has been shown that farm animal and human microbiota are reservoirs for AMR (Gibson et al., 2016; Pärnänen et al., 2018; Brown et al., 2019; Sun et al., 2020). Currently, AMR is already killing 700,000 people a year, and it is predicted to cause 10 million deaths per year by 2050 with a cumulative cost of US$ 100 trillion (de Kraker et al., 2016). According to the Centers for Disease Control and Prevention (CDC) AMR challenge, Enterobacteriaceae, including Escherichia coli, Shigella, Salmonella, and Klebsiella spp. amongst others, present a serious and/or urgent threat to world health. Indeed, as Gram-negative bacteria, Enterobacteriaceae are notorious for their capacity to resist antimicrobial therapy (Hawkey, 2015; Li et al., 2015; Zowawi et al., 2015). Furthermore, even though Enterobacteriaceae represent only a small percentage of the host microbiota and are not all pathogens, they are still responsible for important morbidity (Doi et al., 2017; MacVane, 2017), making them an important target for new drug development.

The AMR crisis is exacerbated by the fact that resistances are emerging and disseminating faster than the development of new drugs. Indeed, over the past three decades the number of developed and approved antibiotics has more than halved (Ventola, 2015), leading to an increasing demand for new antimicrobial agents or strategies. Genetically modified phages, antibacterial modified oligonucleotides, inhibitors of bacterial virulence and CRISPR-Cas9 strategy are also discussed for extrapolating them to the field of antimicrobial therapeutics (Dickey et al., 2017; Ghosh et al., 2019). Meanwhile, other promising strategies, such as probiotics, lysins and antimicrobial peptides are in various stages of development (Ghosh et al., 2019). Globally, although several alternatives exist in nature, the challenge still remains to demonstrate their efficacy and their use in human and animal.

Bacteriocins form a large family of antimicrobial peptides (AMP) produced by bacteria (Klaenhammer, 1988). Their biological characteristics and activities have been deeply described in a new web-accessible database named BACTIBASE, which is freely available at the http://bactibase.pba-lab.org web-based platform. Bacteriocins can be either unmodified or posttranslationally modified peptides, the latter thus belonging to the large family of ribosomally synthesized and posttranslationally modified peptides (RiPPs) (Arnison et al., 2013; Montalbán-López et al., 2020). Known as inhibitors of pathogens in vitro, many bacteriocins have a high specific activity against clinical strains including antibiotic-resistant ones (Cotter et al., 2013). Their effectiveness as inhibitors of pathogenic and spoilage microorganisms has been largely explored (Davies et al., 1997; Deegan et al., 2006). It is thus widely believed that some could be usable for therapeutic purposes and as an alternative to conventional antibiotics (Snyder and Worobo, 2014; Egan et al., 2017).

Bacteriocins produced by enterobacteria are called microcins (Baquero and Moreno, 1984). They form a restricted and underexplored group of bacteriocins compared to the hundreds of members of those from lactic acid bacteria, with only some twenty members identified so far, among which only around fifteen have been more deeply characterized (Table 1 and Supplementary Figure S1). Microcins are less than 10 kDa modified or unmodified peptides (Rebuffat, 2012) having key ecological functions, and particularly a role in microbial competitions (Baquero et al., 2019; Li and Rebuffat, 2020). They have potent activity with minimum inhibitory concentrations (MIC) ranging in the nanomolar to micromolar range and narrow spectra of antimicrobial activity directed essentially against Gram-negative bacterial congeners (Rebuffat, 2012; Baquero et al., 2019). To exert their crucial roles in competition, microcins share a common strategy to penetrate into their bacterial targets. They piratize nutrient uptake pathways of phylogenetically close bacteria vying for the same resources. The iron import pathways is the most frequently attacked (Rebuffat, 2012). When inside bacteria, microcins interfere and perturb a variety of bacterial mechanisms, such as transcription (Adelman et al., 2004), translation (Meltlitskaya et al., 2006), DNA structure (Vizán et al., 1991), mannose transport (Bieler et al., 2006), energy production (Trujillo et al., 2001; Zhao et al., 2015), or the cell envelope function (Destoumieux-Garzón et al., 2003; Gerard et al., 2005; Zhao et al., 2015). Due to their specific characteristics and complex mechanisms of action, microcins are viewed as a possible alternative to conventional antibiotics, helping with the immediate AMR problem (Cotter et al., 2013; Mills et al., 2017; Lu et al., 2019; Palmer et al., 2020). Because of their narrow spectrum of inhibition, they would potentially have less side effects than antibiotics, allowing preservation of the microbiota diversity and minimizing the risk of resistance dissemination.

However, since there is a finite number of entry points and potential targets within a bacterium, microcins and antibiotics can share similar bacterial receptors and pathways to reach their intracellular targets. Moreover, as for antibiotics, the application of specific microcins might be curtailed by the development of resistance (Cotter et al., 2013). Thus, bacteria might evolve common mechanisms of resistance against microcins and antibiotics. This review will highlight the potential of microcins as an alternative to antibiotics to fight against bacterial resistance in Gram-negative pathogens and discuss the possibilities of cross-resistance and co-resistance occurrence in Gram-negative bacteria.

CHARACTERISTICS OF MICROCINS

Bacteriocins that are produced by both Gram-positive and Gram-negative bacteria have been defined by James et al. (2013) as ribosomally synthesized peptides capable of mediating inhibitory effects against bacteria. In Enterobacteriaceae and
TABLE 1 | Structural characterization of microcins assembled into posttranslationally modified microcins (classes I and IIb) and unmodified microcins (class IIa) that contain or not disulfide bridges.

| Class          | Microcin | MM (Da) | PTMs/disulfide bonds | Structure                  | Producing organism | References |
|----------------|----------|---------|----------------------|----------------------------|-------------------|------------|
| Class I (modified) | McC  | 1177    | Peptidyladenylate with the C-terminal Asp linked to AMP via a phosphoramide linkage and bearing an aminopropyl on the phosphate | Nucleotide peptide | E. coli | Guijarro et al., 1995 |
|                | MccJ25 | 2107    | Macrolactam ring between Gly1 and Gly2 threaded by the TyrCysTyr side chains (lasso topology) | Lasso peptide | E. coli | Rosengren et al., 2003 |
|                | MccB17 | 3093    | Gly90Ser92Cys51 and Gly64Cys57Ser56 motifs modified to oxazole-thiazole and thiazole-oxazole heterocycles | Linear azol(ine)-containing peptide (LAP) | E. coli | Li et al., 1996 |
| Class IIa (unmodified) | MccV  | 8734    | 1 disulfide bond (Cys76 – Cys87) | Unmodified peptide | E. coli | Fath et al., 1994 |
|                | MccL   | 8884    | 2 disulfide bonds (Cys29 – Cys35; Cys78 – Cys80) | Unmodified peptide | E. coli LR05 | Pons et al., 2004 |
|                | MccS(2) | 9746    | 2 putative disulfide bonds (Cys57, Cys90; Cys109, Cys118) | Unmodified peptide | E. coli G3/10 | Zschüttig et al., 2012 |
|                | MccPDI(2) | 9953    | 2 putative disulfide bonds (Cys57, Cys90; Cys109, Cys118) with Cys57-Cys90 bond required for activity | Unmodified peptide | E. coli 25 | Eberhart et al., 2012 |
|                | MccN/24 | 7222    | No disulfide bond (no Cys residue) | Unmodified peptide | Uropathogenic E. coli | Kaur et al., 2016 |
| Class IIb      | MccE492 | 7887(3) | Linear trimer of N-2,3-(dihydroxybenzoyl)-l-serine (DHBS) anchored at the C-terminal Ser64 | Siderophore peptide | K. pneumoniae | Thomas et al., 2004 |
|                |        | 8178(3) | | | | |
|                | MccM   | 7284(5) | Linear trimer of N-2,3-(dihydroxybenzoyl)-l-serine (DHBS) anchored at the C-terminal Ser77 | Siderophore peptide | E. coli Nissle 1917 | Vassiliadis et al., 2010 |
|                |        | 8115(5) | | | | |
|                | MccH47 | 4865(5) | Linear trimer of N-2,3-(dihydroxybenzoyl)-l-serine (DHBS) anchored at the C-terminal Ser60 | Siderophore peptide | E. coli Nissle 1917 | Vassiliadis et al., 2010 |
|                |        | 5696(5) | | | | |

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(a) Average masses with the cysteines involved in disulfide bonds when relevant.
(b) Also termed thiazole-oxazole modified microcin (TOMM).
(c) Putative structure.
(d) Molecular mass without PTM.
(e) Molecular mass including the DHBS trimer PTM.

more specifically in E. coli, microcins (for extensive reviews see Baquero and Moreno, 1984; Duquesne et al., 2007a; Baquero et al., 2019) have been shown to be produced along with colicins, which are large antibacterial proteins (Cascales et al., 2007). To distinguish them from colicins, the name “microcin” was coined since their first discovery (Asensio and Perez-Diaz, 1976), based on their smaller size of less than 10 kDa. Such as most bacteriocins, microcins are active against phylogenetically related bacteria including enteropathogenic Klebsiella, Shigella, Salmonella and E. coli, notorious for their capacity to develop antibiotic resistances, and considered serious and urgent threats by the CDC. These Gram-negative bacteriocins are ubiquitously distributed in Nature and their production is consistently observed in multiple genera. Those include Escherichia, Salmonella, Shigella, Klebsiella, Enterobacter, and Citrobacter (Gordon and O’Brien, 2006; Gordon et al., 2007; Budic et al., 2011; Drissi et al., 2015; Wang et al., 2016; Cheung-Lee et al., 2019). The development of DNA sequencing methods and the availability of an increasing number of genomes revealed that clusters of genes orthologous to microcin biosynthesis and self-immunity genes are widespread in bacteria. Indeed, analogs of historically described microcins produced by Enterobacteriaceae, essentially in the RiPP family, have been predicted and most often deeply characterized in other Gram-negative bacteria including human pathogens, Helicobacter (Bantysh et al., 2014), Burkholderia (Knappe et al., 2008), Pseudomonas (Metelev et al., 2013), Klebsiella (Metelev et al., 2017a,b; Travin et al., 2020), Acinetobacter (Metelev et al., 2017a), Citrobacter (Cheung-Lee et al., 2019), or in the symbiotic nitrogen-fixing bacterium Rhizobium (Travin et al., 2019) (Supplementary Figure S1A). They were even predicted inGram-positive bacteria and cyanobacteria (Bantysh et al., 2014). This points that a sharp distinction between bacteriocins from Gram-positive and Gram-negative bacteria is artificial and that
The chemical diversity of microcin-like peptides is intended to expand rapidly.

The Two Classes of Microcins

Compared to the huge number of Gram-positive bacteriocins, microcins are distinguished by a high structural heterogeneity inside a restricted number of identified and well-characterized representatives. A widely accepted classification was proposed by Duquesne et al. (2007a) based on both the peptide size and degree of posttranslational modification (PTM). The known microcins are grouped into two classes, class I with molecular masses below 5 kDa and the presence of extensive PTM and class II with molecular masses between 5 and 10 kDa that can be modified or not (Table 1). A brief description of the microcins from the two classes is provided below to help following the next sections. For more detailed overview of the microcins, see two recent reviews (Raquero et al., 2019; Li and Rebuffat, 2020).

Class I assembles three plasmid-encoded microcins that have been well structurally characterized as RiPPs (Supplementary Figure S1A): microcin C (McC) a nucleotide peptide, microcin B17 (MccB17) a linear azol(in)e-containing peptide, and microcin J25 (MccJ25), a lasso peptide. McC is presently the only nucleotide member of the family. However, similar biosynthetic gene clusters are distributed within bacterial genomes (Bantysh et al., 2014), which suggests an unexplored diversity for such gene clusters (Figure S1A). mature MccB17 contains 43 amino acids that are structured into thiazole and oxazole heterocycles (4 thiazoles and 4 oxazoles rings either isolated or fused into oxazole/thiazole- and thiazole/oxazole-bis-heterocycles) by the PTM enzymes (Li et al., 1996; Ghilarov et al., 2019) (Supplementary Figure S1A). Such heterocycles are also found in hybrid non-ribosomal peptide-polyketide natural products such as the anti-tumor drug bleomycin, as well as in RiPPs such as cyanobactin (McIntosh and Schmidt, 2010) or streptolysin (Mitchell et al., 2009), forming the LAP [also termed thiazole/oxazole-modified microcin (TOMM)] peptide family (Melby et al., 2011). Microcin B-like bacteriocins produced by Pseudomonas, Klebsiella and Rhizobium have been reported (Metelov et al., 2013, 2017b; Travin et al., 2019). MccJ25 was isolated first from the E. coli strain AT25 isolated from an infant feces bearing the mjcABCD gene cluster (Salomón and Farias, 1992) (Figure 1). Its maturation from a 58 amino acid precursor into a 21 amino acid lasso peptide is ensured by two enzymes, McjB and McjC, encoded in the microcin gene cluster (Duquesne et al., 2007b; Yan et al., 2012). This unique lasso topology, which is characterized by threading of the C-terminal tail through a seven to nine lactam ring close by an isopeptide bond, is locked in

![Figure 1](image-url)
place with the two bulky side chains of Phe and Tyr aromatic amino acids for MccJ25 (Rosengren et al., 2003) (Supplementary Figure S1A). It is responsible for the sturdiness of MccJ25 and is required for its antibacterial activity (Rebuffat et al., 2004; Wang and Zhang, 2018). Genome mining approaches have revealed a wide distribution of lasso peptides in Gram-positive and Gram-negative bacteria (Maksimov et al., 2012; Hegemann et al., 2013; Tietz et al., 2017; Cheung-Lee and Link, 2019). Many lasso peptides produced by proteobacteria do not show antibacterial activity (Hegemann et al., 2013). This questions their ecological role or can be due to difficulty to decipher the reasons for their narrow activity spectrum.

Class II microcins form a more homogeneous group than their class I cousins (Table 1 and Supplementary Figures S1B,C), although they are subdivided into class IIA, encompassing MccL (Pons et al., 2004), MccN/24 (Kaur et al., 2016), MccPDI (Eberhart et al., 2012), MccS (Zschüttig et al., 2012) and MccV (Gratia, 1925), and class IIB (MccE492, MccH47, MccM, Vassiliadis et al., 2010). MccN was formerly termed Mcc24 (O’Brien and Mahanty, 1994) and is termed MccN/24 in this review. What distinguishes class IIA from class IIB is the presence or not of a siderophore moiety derived from enterobactin anchored at the peptide C-terminal serine carboxylate (Supplementary Figures S1B,C). This catechol-type siderophore PTM sparked coining the name “siderophore microcins” to class IIB microcins (Rebuffat, 2012). Class II microcins result from a proteolytic processing of a precursor with a leader peptide extension, which occurs at a conserved double-glycine (or Gly-Ala) cleavage site, concomitantly with secretion. They have molecular masses between 5 and 10 kDa and exhibit high amino acid sequence similarities, even between class IIA and IIB (Supplementary Figures S1B,C). For examples, the class IIA unmodified MccV and MccN/24 possess high sequence similarities with the class IIB MccH47 and MccE492, respectively, although they do not carry a C-terminal PTM (O’Brien, 1996; Corsini et al., 2010). It was suggested that the conserved C-terminal sequence of these microcins can direct the presence or not of the siderophore PTM and that the C-terminal regions of MccV and MccH47 can be interchanged (Azpiroz and Laviña, 2007). It was further proposed that both class IIA and IIB microcins possess a modular structure (Azpiroz and Laviña, 2007; Morin et al., 2011).

Class IIA microcins have been characterized from E. coli strains from various origins. The MccN/24 producer is an uropathogenic E. coli (Kaur et al., 2016) and the MccL producer comes from poultry intestine (Sablé et al., 2003), while MccS is produced by a probiotic strain, E. coli G3/10 (Symbioflor®; DSM17252) (Zschüttig et al., 2012). The producing strains are in some cases multi-microcin producers, such as E. coli LR05 that secretes MccB17, MccJ25 and the uncharacterized MccD93 in addition to MccL (Sablé et al., 2003). Their gene cluster organization includes the four basic genes only, one structural gene encoding the precursor peptide, two export genes and one immunity gene (Zschüttig et al., 2012) (Figure 1). If the five class IIA microcins are all devoid of PTMs, they are also all except MccN/24, stabilized by one (MccV) or two (MccL, MccPDI, MccS) disulfide bonds (Yang and Konisky, 1984; Sablé et al., 2003; Gerard et al., 2005; Morin et al., 2011; Zschüttig et al., 2012) (Table 1 and Supplementary Figure S1B).

Contrasting with class IIA and class I, class IIB microcins (Supplementary Figure S1C) are chromosome-encoded (Poey et al., 2006). MccE492 is secreted by Klebsiella pneumoniae human fecal strain RYC492 (de Lorenzo, 1984) bearing the mceABCDEFGHIJ gene cluster (Destoumieux-Garzón et al., 2006; Vassiliadis et al., 2007; Nolan and Walsh, 2008) (Figure 1). It is the first siderophore microcin to be characterized (Thomas et al., 2004), although it was primarily described as an unmodified peptide (Wilken et al., 1997). Actually, it was shown further to be secreted under both modified and less active unmodified forms, due to its PTM process (Vassiliadis et al., 2007). The MccE492 PTM was identified as a glucosylated linear trimer of N-(2,3 dihydroxybenzoyl)-L-serine (DHBS) linked to the C-terminal serine carboxylate (Supplementary Figure S1C). The functions of the enzymes involved in establishment of the MccE492 PTM, MccC, MccD, MccE/MceJ, were identified (Vassiliadis et al., 2007; Nolan and Walsh, 2008). MccH47, initially isolated from the human fecal E. coli strain H47 (Laviña et al., 1990) and MccM were both characterized as siderophore microcins produced by several E. coli strains, including the probiotic strain Nissle, 1917 (Mutaflo®) (Vassiliadis et al., 2010). MccH47 and MccM carry the same PTM as MccE492 (Vassiliadis et al., 2010). Siderophore microcins possess a modular structure, where the N-terminal region is responsible for their cytotoxicity and the C-terminal region, which carries the siderophore moiety, is involved in recognition and uptake. For an overview on siderophore microcins, see Massip and Oswald (2020).

Biosynthesis of Microcins

Microcin production takes place in the stationary phase (Baquero and Moreno, 1984) of bacterial growth, with the exceptions of MccE492 (de Lorenzo, 1984) and MccPDI (Eberhart et al., 2012). They are encoded by gene clusters, which exhibit a conserved organization, but contain a variable number of genes ranging from four to ten, according to the presence or not of PTMs on the mature microcin (Figure 1). These gene clusters are generally plasmid-born, except the chromosomally encoded class IIB microcins. The general biosynthetic pathway of microcins (which also applies to other bacteriocins) starts with the ribosomal synthesis of a precursor peptide that is typically composed of two regions, an N-terminal leader part and a core region. The core peptide of modified microcins, which belong to the wide RiPP family, is the region where the PTMs take place (Montalbán-López et al., 2020). In some cases, such as the siderophore microcins, the modifications may result from the non-ribosomal pathway, making these microcins a rare bridge spanning ribosomal and non-ribosomal biosynthesis pathways (McIntosh et al., 2009). The leader is involved in binding to or activation of many of the PTM enzymes, but also maintains the maturing peptide inactive during the process (Arnison et al., 2013), thus contributing to the protection of producing cells as regard their own toxic microcin. For many modified microcins (MccJ25, MccC), this binding involves a peptide binding domain (RiPP precursor peptide recognition element, RRE), also present in a wide proportion of RiPP PTM enzymes and
similar to a small protein involved in the biosynthesis of the RiPP pyrroloquinoline quinone (PQQ) (Burkhart et al., 2015; Sikandar and Koehnke, 2019). Recently, the crystal structure of the MccB1D synthetase ensuring the extensive modifications in MccB17 was solved, deciphering the organization and functioning of such a multimeric heterocyclase-dehydrogenase catalytic complex at the molecular level and affording the spatial relationships between the two distinct enzymatic activities and the leader peptide binding site (Ghilarov et al., 2019).

In all but a few cases, and irrespective of if the microcin is modified or not, maturation requires removal of the leader region to give the active bacteriocin (Drider and Rebuffat, 2011). This proteolytic cleavage is performed either before and independently of (class I), or concomitantly with (class II) export of the mature microcin (Beis and Rebuffat, 2019). It can be ensured either (i)- concomitantly with the PTM establishment by one of the dedicated enzymes (MccJ25 leader is cleaved off by the McjB leader peptidase encoded in the microcin gene cluster (Yan et al., 2012), or (ii)- by a protease from the producer, which is not encoded in the microcin gene cluster (MccB17 leader is cleaved off before export by the conserved proteins TldD/TldE which assemble as a heterodimeric metalloprotease to ensure this function) (Ghilarov et al., 2017), or (iii)- by a bifunctional ATP binding cassette (ABC) transporter of the peptidase-containing ATP-binding transporters (PCAT) family, which is encoded in the microcin gene cluster (cleavage of the class II microcin leader peptides is performed simultaneously with export of the maturated microcins by an ABC exporter endowed with an N-terminal protease extension) (Hävarstein et al., 1995; Massip and Oswald, 2020).

Self-Immunity of Microcin Producers

Microcin gene clusters vary in the number of genes contained and the presence of genes encoding PTM enzymes, and they all carry genes ensuring self-immunity (Figure 1). Each microcinogenic strain is protected against its arsenal of microcins and the self-immunity mechanisms differ from one microcin to another. For instance the self-immunity mechanism to MccL depends on a single gene mccL that encodes an immunity protein (Sablé et al., 2003). Overall, self-immunity of the producers relies either on specific immunity proteins encoded in the gene clusters that bind to the toxic entities making them inefficient, or on efflux systems, mainly ABC transporters, which ensure export of the microcins to the external medium and simultaneously self-immunity of the producing bacteria. As examples, self-immunity to MccJ25 is provided exclusively by McjD, a highly specific ABC exporter which ensures simultaneously export of the microcin (Beis and Rebuffat, 2019), while full self-immunity to MccB17 requires both an immunity protein McbG and an ABC exporter McbEF (Collin and Maxwell, 2019).

MECHANISMS OF ACTION

Comparison of the mechanisms used by antibiotics and microcins to kill sensitive bacteria shows that they may share different bacterial receptors, translocators and final targets (Table 2 and Figure 2). Thus, it is obvious that these two groups of antimicrobials may cross in several mechanisms of action. However, it is also expected that several mechanisms of action of microcins are very specific and are not involved in the inhibition activity of antibiotics. This characteristic is particularly relevant to address in terms of the risk of cross-resistance between microcins and antibiotics. These similarities and differences are highlighted below.

The Uptake Systems

The first obstacle to be overcome by an antimicrobial compound to reach its final target is the bacterial cell envelope (Collet et al., 2020). The extent of this barrier varies according to the target to be reached, the chemical structure of the antimicrobial compound and the bacterial species. For Gram-negative bacteria, antimicrobials have to pass first the outer membrane. Then, they can access the cytoplasmic membrane bilayer (inner membrane) and either insert inside or cross it for those antimicrobials having intracellular targets. Many antibiotics are hydrophilic compounds of low molecular mass and uptake across the outer membrane is ensured by passive diffusion using pores formed by specific β-barrel membrane proteins called porins. Porins are the most abundant proteins of the outer membrane in Gram-negative bacteria. They are classified as non-specific (general porins) and specific (selective porins), according to their threshold size and amino acids lining the aqueous channel (Choi and Lee, 2019). The transport varies according to the size, charge and hydrophilicity of the molecule. Recently, the dual function of the porin OmpF both as receptor and translocator for the pore-forming colicin N, has been elegantly demonstrated (Jansen et al., 2020). However, more hydrophobic or higher molecular mass compounds above the porin threshold require other strategies, among which hijacking receptors or transporters required for vital functions is a major one. Indeed, Gram-negative bacteriocins, colicins and microcins, widely parasite such receptors to enter the periplasmic space, and particularly those involved in iron import. This receptor hijacking qualifies many microcins as “Trojan horse” compounds, as they mimic vital compounds that require being imported in cells, to penetrate sensitive bacteria (Duquesne et al., 2007a; Nolan and Walsh, 2008; Severinov and Nair, 2012).

Iron acquisition is an essential factor for microbial life. However, under aerobic conditions, free iron availability is limited by the very low solubility of ferric iron, and especially within a host, where iron is competed for by both the microbial community and the host (Wilson et al., 2016). To secure iron, bacteria have evolved to develop efficient Fe(III)-chelating agents (K₅ ranging from 10⁻³⁵ to 10⁻⁵⁷), termed siderophores, to scavenge iron from their surrounding environment and import it. A study by Lewis et al. (2010) showed that siderophores are sufficient for allowing the culture of bacteria previously unculturable in laboratory conditions. Siderophores are non-ribosomally synthesized (Grosa and Walsh, 2002) and are important for...
| Antibiotic/Microcin | Mechanisms of action | Uptake system (OM/IM) | Process/Target | Mechanism of resistance |
|---------------------|----------------------|-----------------------|----------------|-------------------------|
| Penicillins, Cephalosporins, Beta-lactams | – Bacterial cell wall disruption/Peptidoglycan breaking | – Inactivation/β-lactam ring | – β-lactam ring cleavage by β-lactamases |
| | | – Mutations/TonB Porins | – Decrease of uptake of the antibiotic due to modifications of TonB sequence |
| | | – Efflux pumps overexpression | – Decrease of uptake of the antibiotic |
| | | – Pumping out of the antibiotic | |
| Fosfomycin | – Bacterial cell wall/Peptidoglycan biosynthesis: UDP-β-N-acetylglucosamine enolpyruvyl transferase, MurA | – GlpT, UhpT sugar transporters | – Mutations/MurA | – Cys-Arg mutation in MurA active site |
| | – Sugar transport into the cytoplasm | | | – Mutations in GlpT, UhpT transporters |
| Polymyxins, Colistin/polymixin E | – Membrane permeabilization/LPS binding leading to detergent effect | – Porins | – Enzymatic modification/LPS | – Modification of LPS by the MCR1 phosphoethanolamine transferase |
| | – Endotoxin neutralization | | – Efflux pumps overexpression | – Pumping out of the antibiotic |
| Rifamycins Rifampicin | – Protein synthesis-Transcription step/β subunit of RNAP | – Siderophore receptor FhuA – TonB system | Mutations/RNAP β subunit | – Mutations in rpoB gene |
| Streptolydigin | – Protein synthesis-Transcription step/inhibition of RNAP catalytic function by binding β and β′ subunits | – Porins | – Mutations/RNAP β and β′ subunits | – Mutations in rpoB and rpoC |
| Albomycin | – Protein synthesis – Translation step/Aminoaetyl-tRNA synthetase | – Siderophore receptor FhuA – TonB system | – Enzymatic modification/Processed albomycin | – Acetylation of processed albomycin by transacylase RimL |
| Quinolones (nalidixic acid, ciprofloxacin, norfloxacin, ...) | DNA replication/Type II topoisomerases (DNA gyrase, topoisomerase IV) | | | – Mutations in gyrA, gyrB or parC, parE (Ser83 in GyrA) |
| | | | | – Protection of DNA gyrase and topoisomerase IV by the gyrA interacting protein qnr |
| | | | | – Piperazine ring acetylation (AAC(6')-Ib-c) |
| | | | | – Decrease of uptake of the antibiotic |
| Chloramphenicol | – Protein synthesis/Binding to 50S ribosome subunit inhibiting the formation of peptide bonds | – Membrane transporter | – Enzymatic modification/Chloramphenicol | – Acetylation by chloramphenicol acetyltransferases CATs |
| | | | | – Acetylation by acetyltransferases (AACs) |
| | | | | – Phosphorylation by phosphotransferases (APhEs) |
| | | | | – Adenylation by nucleotidytranferases (ANTs) |
| Aminoglycosides | – Protein synthesis – Translation step/Binding to 30S ribosome subunit | | | |
| | | | | |
| Tetracyclines | Protein synthesis -Translation step/Binding to 30S ribosome subunit that blocks aminoacyl-tRNA binding to RNA-ribosome complex | – Porins OmpF, OmpC | – Acquisition of tet, or tet resistance genes leading to production of ribosomal production proteins Tet | – Methylation by rRNA methylase |
| | | | | – Pumping out of the antibiotic |
| MccJ2S | – Protein synthesis-Transcription step/Binding to β′ subunit of RNAP (secondary channel) | – Siderophore receptor FhuA – TonB system | – Mutations/RNAP β′ subunit | – Mutation in rpoC that encodes RNAP β′ subunit (T931I) and additional mutations (Q921P, T934M, H936Y) |
| | | | | – Pumping out of the microcin by ABC exporters (MojB, YojI)/TolC |

(Continued)
| Antibiotic/Microcin | Function impaired/Target | Mechanisms of action | Mechanisms of resistance |
|---------------------|-------------------------|----------------------|--------------------------|
| **MccB17**          | − DNA replication and topology maintenance/Binding to DNA gyrase | − Porin OmpF/ − SbmA | − Mutations/GyrB, GyrA OmpF, OmpR SbmA − Efflux pumps expression | − Mutations in GyrB and GyrA: GyrB (W<sup>751</sup>R): full resistance; GyrB (K<sup>447</sup>E), GyrA (S<sup>83</sup>W): partial resistance − Mutations in ompF and ompR − Pumping out of the antibiotic by ABC exporter (McbEF) − Acetylation of processed McC by transacetylases [either encoded in McC gene cluster (MccE) or chromosome-encoded (RimL)] − Cleavage of the heptapeptide-nucleotide amide bond by carboxypeptidases [serine carboxypeptidase encoded in McC gene cluster (MccF)] − Cleavage of the phosphoramidate bond in aspartamide adenosine by histidine triad hydrolases − Pumping out of the antibiotic by ABC exporter MccC |
| **MccC**            | − Protein synthesis − Translation step/Aspartyl-tRNA synthetase (Asp-RS) | − Porin OmpF/YejABEF | − Inactivation of the antibiotic Processed McC − Efflux pumps expression | |
| **MccE492**         | − Inner membrane bilayer permeability/Formation of channels − Sugar transport/Binding to inner membrane components of mannose phosphotransferase system permease (ManPTS) | − Siderophore receptors FepA-, Cir-, Flu-TonB system | − Mutations in uptake system at the inner membrane/Catechol siderophore receptors − Mutations in mannose uptake system/ManXYZ | − Mutations/deletions in FepA, Cir, Flu, TonB − Deletion of inner membrane complex ManXYZ |
| **MccH47**          | − Energy production (ATP synthesis)/Binding to F<sub>0</sub> subunits of ATP synthase | − Siderophore receptors FepA Cir Flu - TonB system | − Mutations in uptake system/Catechol siderophore receptors | − Mutations/deletions in FepA, Cir, Flu |
| **MccL**            | Membrane potential | − Siderophore receptor Cir -TonB system/SdaC | − Mutations in uptake systems/Catechol siderophore receptor | − Mutations in Cir, TonB |
| **MccV**            | Inner membrane | − Siderophore receptor Cir -TonB system/ − SdaC | − Mutations in uptake systems/Catechol siderophore receptor, SdaC | − Mutations in Cir, TonB, SdaC |
| **MccPDI**          | − Energy production/ATP synthase − Inner membrane permeability | − Porin OmpF | − Mutations/Thiol-redox enzymes, OmpF | − Mutations in dsbA, dsbB encoding thiol-redox enzymes making S-S bonds |
FIGURE 2 | Mechanisms of action of antibiotics (A) and microcins (B) against Gram-negative bacteria showing the membrane proteins involved in uptake into sensitive bacteria and the final targets. β-LAC, β-lactams; QNL, quinolones; TET, tetracycline; AMG, aminoglycosides; FOS, fosfomycin; CHL, chloramphenicol; CS, colistin; RIF, rifampicin; ALB, albomycin; P, pore; LPS, lipopolysaccharide. A letter and a number are assigned to each antibiotic and each microcin respectively, which are used in the scheme to identify the path they follow for their killing activity.
enteropathogen survival (Hantke, 2003). Concomitantly, iron availability has been observed to regulate MccE492 gene expression (Marcoleta et al., 2013). The resulting Fe(III) siderophore complex is then internalized by the producing strains via high affinity siderophore receptors anchored at the outer membrane, which are specifically involved in this function, but also ensure other strategic roles in microbial communities (Kramer et al., 2019). Siderophore receptors consist of a 22-stranded antiparallel β-barrel with external loops serving as ligand binding sites and an N-terminal globular domain forming a plug that occludes the barrel (Krewulak and Vogel, 2008). They are specific to the different siderophore chemical types, such as FhuA for ferrichrome or Cir, Fiu, and FepA for catechol siderophores in enterobacteria. These receptors are coupled to the TonB-ExbB-ExbD three-component machinery anchored at the inner membrane (TonB system), which transfers the energy source from the proton motive force of the cytoplasmic membrane to the outer membrane (Krewulak and Vogel, 2008), thus permitting active transport.

All microcins, whatever they are of class I or II, use either the siderophore receptor or the porin path to reach their final target (Figure 2B). Siderophore microcins uptake requires the FepA-, Cir-, Fiu-TonB systems, with FepA having the most important role (Destoumieux-Garzón et al., 2006; Azpiroz and Laviña, 2007; Vassiliadis et al., 2010). Unmodified microcins use the Cir-TonB system (MccV, MccL) (Chehade and Braun, 1988; Morin et al., 2011), or the porin OmpF which screens incoming products in a non-specific manner (Sato et al., 2000; Kaeriyama et al., 2006) (MccPDI) (Zhao et al., 2015), while class I microcins either use FhuA (MccJ25) (Pugsley et al., 1986; Salomón and Farias, 1993; Mathavan et al., 2014), or OmpF (MccB17, MccC) (Laviña et al., 1986; Novikova et al., 2007) to reach the periplasmic space (Figure 2B). In the case of loss of function of the TonB system, MccE492, MccH47, and MccM retain antimicrobial activity, suggesting the involvement of another translocator, such as the TolA-TolQ-TolC system known to mediate the import of certain colicins (Lazdunski et al., 1998). Similar observations were made for MccL and MccV (Gerard et al., 2005; Morin et al., 2011), suggesting that the function of the ExbB protein could be replaced by its homolog TolQ in TonB-dependent microcin activity. However, although the presence of the siderophore PTM enhances its efficiency, the non-modified form of MccE492 (without the C-terminal siderophore) is also able to kill sensitive bacteria, but at a significantly lower level. On their side, antibiotics, which are essentially low molecular mass hydrophobic compounds, are most often transported inside target bacteria via porin or iron siderophore receptor pathways (Table 2).

Mechanisms of Action Common to Antibiotics and Microcins

Disruption of the Cytoplasmic Membrane

Permeabilization and/or disruption of the bacterial cytoplasmic membrane of Gram-negative bacteria is the main mechanism of action of the non-ribosomal peptide antibiotics polymyxins B and E (Table 2 and Figure 2A), which share a high degree of structural similarity (Schindler and Teuber, 1975). Polymyxin E (also called colistin) binds to the lipopolysaccharide (LPS) both in the bacterial outer membrane and in the cytoplasmic membrane and this interaction is essential for cytoplasmic membrane permeabilization, cell lysis and the bactericidal activity of this antibiotic (Sabinis et al., 2019). It should be noted that all polymyxins are inactive against Gram-positive bacteria, except a few species such as Streptococcus pyogenes (Trimble et al., 2016).

Several class II microcins target the inner membrane, by perturbing either its integrity using different mechanisms of peptide membrane interaction, or the proteins which are embedded. This constitutes at least the primary part of their mechanism of action (Table 2 and Figure 2B). Indeed, the final killing trajectory of MccE492 appears to stop at the inner membrane. MccE492 induces a rapid depolarization and permeabilization of E. coli cytoplasmic membrane, without provoking cell lysis (Lagos et al., 1993; Destoumieux-Garzón et al., 2006). It forms well-defined ion channels in planar phospholipid bilayers that are constituted of supramolecular peptide assemblies (Lagos et al., 1993; Destoumieux-Garzón et al., 2006). It also interacts with the mannose phosphotransferase system permease ManXYZ (Bieler et al., 2010), associating specifically with its inner membrane components ManYZ. Therefore, MccE492 both perturbs the inner membrane permeability and interferes with the transport of mannose to kill sensitive congeners. Besides, MccE492 is known to form amyloid fibrils (Bieler et al., 2005; Arranz et al., 2012; Aguilera et al., 2016) that play a role in modulating its antimicrobial activity. These aggregates have been observed more significantly with the unmodified form of MccE492, suggesting their formation is not only an additional mechanism of protection of the producer strain, but also may act as a toxin reservoir. MccV destabilizes the membrane potential (Yang and Konisky, 1984) and further interacts with an inner membrane transporter, the serine permease SdaC (Gerard et al., 2005), which is involved in serine transport and acts as a specific receptor for MccV. It can be suggested that a perturbation of serine transport in sensitive bacteria could result, or that SdaC could drive MccV to form channels in the inner membrane. MccE492 and MccV thus illustrate the combined use of two different mechanisms involving the inner membrane or its components to kill sensitive bacteria. MccL primary target is also the cytoplasmic membrane. It provokes disruption of membrane potential of E. coli cells, but without inducing permeabilization of the inner membrane (Morin et al., 2011). A potential inner membrane target for MccL has not been identified. Finally, it has to be mentioned that at higher concentrations than the MIC, MccJ25 induces perturbations of the cytoplasmic membrane permeability and disruption of the cytoplasmic membrane gradient in Salmonella enterica (Rintoul et al., 2001; Ben Said et al., 2020), and perturbation of the respiratory chain enzymes in E. coli, accompanied with stimulation of the production of reactive oxygen species (Bellomio et al., 2007).

Inhibition of Protein Biosynthesis

The bacterial 70S ribosome is composed of two ribonucleoprotein subunits forming the 30S and 50S subunits...
(Yoneyama and Katsumata, 2006). Aminoglycosides (AGs), such as streptomycin or gentamicin, and tetracyclines bind to the 16S ribosomal RNA of the 30S subunit (Chopra and Roberts, 2001; Krause et al., 2016). AGs bind to the A-site of the ribosome, causing inhibition of translation of mRNA by codon misreading on delivery of the aminoacyl-tRNA (Table 2 and Figure 2A). For their part, tetracyclines prevent incoming aminoacyl-tRNA from binding to the A site of the mRNA translation complex. As well, chloramphenicol inhibits protein synthesis by preventing the binding of t-RNAs to the A site of the ribosome (Kapoor et al., 2017). The bacterial ribosome is also the target for other antibiotic classes, such as the macrolides and ketolides or the streptogramins.

Contrasting with MccB17 and its Pseudomonas congeners which exert their antimicrobial activity by perturbing DNA topology setting up (see section below Inhibition of Nucleic Acid Biosynthesis), other MccB17-like bacteriocins perturb protein synthesis. Klebsazolin from K. pneumoniae, which exhibits moderate antimicrobial activity against certain E. coli, Klebsiella and Yersinia strains (Metelev et al., 2013) targets the 70S ribosome and interferes with translation elongation. Moreover, it binds to the peptide exit tunnel, overlapping with the binding sites of macrolides or streptogramin-B. Similar to klebsazolin, the MccB17-like phazolin produced by Rhizobium sp., which exhibits narrow-spectrum antibacterial activity against some symbiotic bacteria of leguminous plants (Travin et al., 2019), also targets the 70S ribosome by obstructing the peptide exit tunnel, but through different binding mechanisms.

Albomycin, which consists of an antibiotic part linked to a siderophore moiety, inhibits aminoacyl t-RNA synthetases (aaRSs) that are essential for protein synthesis (Severinov and Nair, 2012) (Table 2 and Figure 2A). Similar, Mcc targets the aspartyl-tRNA synthetase (Metelev et al., 2006), making it a translation inhibitor (Table 2 and Figure 2B). After having crossed the outer membrane thanks to the porin OmpF, Mcc requires the inner membrane ABC transporter YejABEF (Novikova et al., 2007) for its translocation within the cytoplasm. A comprehensive analysis by Vondenhoff et al. (2011) has shown that to mediate binding and translocation of substrates, the YejABEF transporter requires an N-terminal formyl-methionine and an arginine. These requirements are achieved with the formylated f-MRTGNAD heptapeptide part of the Mcc precursor. However, unlike other microcins, which are fully processed within the producing cells before export, further Mcc maturation is necessary within the target bacteria to attain its cytotoxic form. Mcc undergoes a double-step processing. First of which is the deformylation of the formylated heptapeptide precursor, essentially nullifying the detoxification process of its immunity protein mceE. This deformylation allows the second maturation step, which is ensured by broadly specific endoproteases PepA, PepB, and PepN, which remove the peptide moiety of the microcin. This last processing step releases the toxic entity, which is a non-hydrolyzable analog of aspartyl-adenylate (Asp-RS) that blocks aspartyl-tRNA synthetase and thus transcription (Kazakov et al., 2008). This subtle cheating mechanism nicely exemplifies the Trojan horse strategy used by microcins. Moreover, Ran et al. (2017) observed that when increasing the concentration until the mM level, Mcc was able to inhibit the activity of β-galactosidase, respiration chain dehydrogenases, and 6-phosphogluconate dehydrogenase without damaging the inner membrane, showing that Mcc develops a second mechanism of action that operates at higher concentrations.

Inhibition of Nucleic Acid Biosynthesis

Quinolone antibiotics (nalidixic acid, ciprofloxacin, . . .) inhibit DNA synthesis by targeting two essential type II topoisomerases, DNA gyrase and topoisomerase IV, and converting them into toxic enzymes that fragment the bacterial chromosome (Table 2 and Figure 2A). These interactions result in erroneous unwinding of DNA, introduction of double strand breaks and cell death (Fabrega et al., 2009). Besides, rifampycin inhibits DNA-dependent RNA polymerase (RNAP) activity by forming a stable complex with the enzyme. It binds in a pocket of the RNAP β subunit, deep within the DNA/RNA channel, while away from the active site. The inhibitor directly blocks the path of the elongating RNA when the transcript becomes two to three nucleotides in length. It thus suppresses the initiation of RNA synthesis (Campbell et al., 2001).

The target of MccB17 is also a topoisomerase (Table 2 and Figure 2B). MccB17 enters sensitive bacteria using the OmpF porin, diffuses through the periplasmic space and binds to the inner membrane transporter SbmA to be delivered into the cytoplasm (Laviña et al., 1986). It induces gyrase-dependent formation of a stable cleavage complex instead of the transient break that normally happens during the catalytic cycle. It causes covalent links between DNA gyrase and double stranded DNA, hence blocking DNA replication and maintenance. Similar to fluoroquinolones, MccB17 targets the cleavage of both DNA strands, which is a critical step in the DNA gyrase supercoiling cycle, but the MccB17-induced cleavage pattern is different from that of quinolones (for a review on MccB17 activity see Collin and Maxwell, 2019). The stringent role of the heterocycles in MccB17 activity has been evidenced (Roy et al., 1999). Introduction of an extra oxazole ring at position Serβ (Roy et al., 1999). Bis-heterocycles play a particularly essential role, with the central MccB17 region that contains two thiazoles and a thiazole/oxazole forming the critical core for DNA cleavage (Collin and Maxwell, 2019). Moreover, the C-terminal part of MccB17 is crucial for both uptake by sensitive cells and DNA gyrase inhibition, while the N-terminal region is only moderately important for uptake (Shkundina et al., 2014). Interestingly, MccB17 congeners that belong to the LAP family of RiPPs do not share all similar mechanisms, targeting either DNA gyrase or the 70S ribosome. Indeed, MccB17-like compounds from P. syringae are active against E. coli and essentially Pseudomonas species including P. aeruginosa, through DNA gyrase inhibition (Metelev et al., 2013), while the other analogs do not (see section above “Inhibition of Protein Biosynthesis”).

Such as rifampicin, the lasso peptide MccJ25 targets the RNAP (Table 2 and Figure 2B). To reach its intracellular target, MccJ25 hijacks the ferriochrome receptor FhuA to cross the outer membrane (Mathavan et al., 2014) and is internalized
into the cytoplasm by the inner membrane protein SbmA. Finally, MccJ25 binds to the RNAP secondary channel, which connects the enzyme surface with the RNAP catalytic center, and through which nucleotide triphosphate substrates (NTP) migrate to the catalytic center (Adelman et al., 2004; Mukhopadhyay et al., 2004), whereby inactivating transcription in a partial competitive manner. The loop is involved in recognition and uptake of MccJ25 by the iron-siderophore transporter FhuA, while the macro lactam ring and C-terminal tail are responsible for binding to the RNA polymerase target (Destoumieux-Garzón et al., 2005; Semenova et al., 2005). The crystal structure of MccJ25 bound to E. coli RNAP was determined and the residues critical for the interaction were identified (Braffman et al., 2019). MccJ25 binds deep within the secondary channel, such as to clash with NTP binding and explaining the partial competitive mechanism of inhibition with respect to NTPs previously proposed (Mukhopadhyay et al., 2004). Besides, it was shown that at higher concentrations, MccJ25 induces perturbations of the cytoplasmic membrane permeability and disruption of the cytoplasmic membrane gradient of S. enterica Newport (Rintoul et al., 2001). At much higher concentrations, it can also stimulate the production of reactive oxygen species (Bellomio et al., 2007). This shows once again the multiple mechanisms brought into play by a given microcin, which both explains their high efficiency and suggests lower risks of resistance acquisition. Several antibacterial lasso peptides, have been shown to also target RNAP through binding to the secondary channel, although their different antibacterial activity spectrum. This is the case for capistruin produced by Burkholderia thailandensis and active against Burkholderia and Pseudomonas species (Knappe et al., 2008; Kuznedelov et al., 2011; Braffman et al., 2019), ubonodin from B. ubonensis and active against pathogenic members of the B. cepacia complex (Cheung-Lee et al., 2020), citrocin from Citrobacter sp., active against E. coli and Citrobacter sp. (Cheung-Lee et al., 2019). By contrast, acinetocid and klebsidin from human-associated strains of Acinetobacter and Klebsiella display no activity or low activity against K. pneumoniae, while they bind RNAP (Metelev et al., 2017b), showing that the spectrum of activity of lasso peptide microcins appears to be driven by the uptake in target bacteria rather than the intracellular target. This is in agreement with the spectrum of activity of MccJ25 against a collection of Salmonella strains, which is associated mainly with differences in the FhuA sequences (Ben Saïd et al., 2020).

Mechanisms of Action Specific to Microcins
MccH47 is bactericidal and targets the membrane bound F0 proton channel subunits of ATP synthase (Trujillo et al., 2001; Rodriguez and Laviña, 2003; Palmer et al., 2020), causing an unregulated influx of protons. It uses FepA-, Cir-, Fiu-TonB dependent receptors to reach its inner membrane target (Patzer et al., 2003). The mechanism of action of the class Ila MccPDI is poorly identified. It was told to require close bacterial proximity to be cytotoxic, hence the name PDI (Proximity Dependent Inhibition) (Eberhart et al., 2012), since co-cultures of producing and sensitive strains separated by a semi-permeable film inhibit its activity. Why proximity is required for activity is unknown, but it could be only a consequence of a concentration-dependence effect (Lu et al., 2019). MccPDI that uses the porin OmpF to cross the outer membrane (Zhao et al., 2015; Lu et al., 2019) was shown (Zhao et al., 2015) to require a functional ATP synthase for exerting its cytotoxic activity, while (Lu et al., 2019) proposed it would induce membrane damage.

Mechanisms of Action Specific to Antibiotics
Inhibition of Cell Wall Formation
The cell envelope of Gram-negative bacteria consists of a phospholipid bilayer inner membrane that wraps the cytoplasm, and an asymmetric outer membrane essentially composed of phospholipids at the inner leaflet and LPS at the outer leaflet, which protects the cell from the environment. In between is the periplasm that shelters a thin peptidoglycan layer (Collet et al., 2020). This double-membrane complex system and in particular the peptidoglycan, often called the cell wall, is a main target for antibiotics and antimicrobials. β-lactam antibiotics, which include in particular penicillins, cephalosporins and carbapenems, harbor the β-lactam ring in their structure that mimics the D-alanyl D-alanine terminal amino acid residues of the precursor subunits of the peptidoglycan layer, and so far interacts with penicillin binding proteins (PBPs). This induces a disruption of the peptidoglycan layer leading to the lysis of the bacterium (Kapoor et al., 2017). Besides, fosfomycin inhibits bacterial cell wall biosynthesis in an early stage; it integrates the cell and inactivates an essential enzyme in peptidoglycan synthesis (Dijkmans et al., 2017). β-lactams, mainly carabpenems and second, third and fourth generation of cephalosporins as well as fosfomycin have a broad spectrum antibacterial activity.

Inhibition of Folic Acid Metabolism
Trimethoprim and sulfonamides act at distinct steps in folic acid metabolism. Sulfonamides inhibit dihydropteroate synthase, which acts at an early step in folic acid biosynthesis in a competitive manner with higher affinity for the enzyme than the natural substrate, p-aminobenzoic acid (PABA). For its part, trimethoprim inhibits dihydrofolate reductase, thus operating at a later stage of folic acid synthesis (Yoneyama and Katsumata, 2006).

Mechanisms of Resistance and Potential Cross- and Co-Resistance Between Antibiotics and Microcins
Various mechanisms of resistance to antibiotics and/or to microcins are reported including essentially modifications of the cellular target by mutations or protein interactions, changes in the structure of the antimicrobial molecule, perturbations of binding or penetration of the antibiotic into sensitive cells and specific cell wall modifications. Several mechanisms are specific, but bacteria may use common mechanisms of resistance against
microcins and antibiotics that could induce cross-resistance, which occurs when a single mechanism provides resistance to several antimicrobial molecules differing in their structures, simultaneously. In contrast, co-resistance occurs when two or more different resistance genes encoding several unrelated resistance mechanisms are located on the same genetic element (plasmid, transposon) (Chapman, 2003). In the following section, we describe different mechanisms of resistance and the possible occurrence of cross- and co-resistance between antibiotics and microcins (Table 2 and Figure 3).

Prevention of Intracellular Accumulation of the Toxic Entity: Efflux Pumps and Decreased Uptake

On one side, outer membrane porins and inner membrane transporters, which are involved in the uptake of antibiotics and microcins into sensitive cells, and on the other side efflux pumps, which pump the toxic compounds out of the bacteria, both constitute a first line resistance strategy (Ghai and Ghai, 2018). Porins, which ensure passive uptake of substrates across the outer membrane (see section mechanisms of action above), serve as the first gate for many antibiotics and several class I and II microcins. Furthermore, efflux pumps can be specific for a single substrate or can confer resistance to multiple antimicrobials by facilitating their extrusion before they can reach their intended targets (Anes et al., 2015). In Gram-negative bacteria, overexpression of efflux pumps is one of the mechanisms of resistance to β-lactams (Amaral et al., 2014) and to quinolones encoded by qepA and oqxAB genes (Fabrega et al., 2009). Likewise, reduced porin levels, which induce decrease of antibiotic concentration inside sensitive cells, is another mechanism of resistance to β-lactams in Gram-negative bacteria (Pfeifer et al., 2010), including K. pneumoniae (Jacoby et al., 2004) and P. aeruginosa (Li et al., 1994). Besides, mutations and deletions of genes encoding porins induce resistance to antibiotics. Indeed, ompF mutant was resistant to several β-lactam antibiotics in some Gram-negative pathogens, including E. coli and the deletion of OmpA resulted in increased susceptibility to several antibiotics including β-lactams in A. baumannii (Smani et al., 2014).

For microcins, the E. coli ABC exporter of unknown function Yojl, mediates resistance to MccJ25 by pumping the microcin out of the cells with the help of TolC, maintaining its concentration below the toxic concentration (Delgado et al., 2005). Yojl is located at the inner membrane and is coupled to the TolC protein at the outer membrane which ensures the last
export step, similar to the MccJ25 gene cluster-encoded ABC exporter McjD, which warrants both microrin export and self-immunity for the producing cells (Bountra et al., 2017; Beis and Rebuffat, 2019). Similarly, McC is expelled from producing cells through a major facilitator superfamily (MFS) efflux pump (Severinov and Nair, 2012). Thus, the activation of several efflux pumps simultaneously could induce a co-resistance to antibiotics and microcins.

The iron-siderophore receptor FhuA is not only required for iron import, but it is also a target for bacteriocins (colicin M, MccJ25) and antibiotics (albomycin, rifamycin). Indeed, FhuA external loops L3, L4, L7, L8, and L11 are involved in the sensitivity to colicin M and the antibiotics albomycin and rifamycin. So far, a further mutation, insertion or deletion in the sequence encoding these loops may induce a cross-resistance between colicin M and these two antibiotics (Wang et al., 2018). Concomitantly, MccJ25 was also shown to require a primary interaction with the FhuA external loops L5, L7, L8 and L11 for its recognition and further internalization via this receptor (Destoumieux-Garzón et al., 2005). The level of sensitivity to MccJ25 also varies depending on the acquisition of specific FhuA, with a maximal sensitivity obtained with *E. coli* FhuA, while several *Salmonella* serovars are resistant due to a lack of efficiency of their FhuA receptor for MccJ25 uptake (Vincent et al., 2004; Ben Said et al., 2020). Similarly, various mutations in FhuA, especially in the cork domain, were reported to reduce the uptake and consequently the sensitivity to albomycin (Endriss et al., 2003). It could thus be hypothesized too that cross-resistance can occur between MccJ25 and albomycin. Besides, membrane permeabilization induced by a synthetic cationic peptide (KFF)_2K was shown to induce the sensitivity of MccJ25 resistant clinical isolates, thus making the microrin entry independent of FhuA and SbmA proteins (Pomares et al., 2010), and thus confirming that microrin uptake is the first source of resistance to MccJ25. Therefore, both uptake decrease of the toxic entity and pumping it out of the sensitive cells are efficient mechanisms to confer resistance to MccJ25.

Resistance to siderophore microcins which carry a catechol siderophore PTM is also primarily induced by uptake impairment (Thomas et al., 2004; Massip and Oswald, 2020). As seen before, MccE492, MccM and MccH47 are recognized and internalized in sensitive bacteria via the TonB-dependent FepA, Fiu and Cir iron-catecholate receptors. According to Thomas et al. (2004), a *fepA*, *fiu* double mutation, the triple *cir, fhu, fepA* mutation and the *tonB* mutation induce complete resistance to MccE492, MccM, and MccH47, while deletion of *exbB* and *exbD* does not affect the sensitivity to all three siderophore microcins (Vassiliadis et al., 2010). Although it does not carry a siderophore PTM, MccL requires the TonB dependent catecholate receptor Cir for uptake. Mutations/deletions in Cir and TonB, or suppression of the proton motive force, which is required for the TonB function, afford MccL resistance in *E. coli* and *Salmonella*, while the proteins involved in serine or sugar transport are not involved (Morin et al., 2011). On the other hand, a mutation in the energy transducer TonB was shown to reduce uptake and confer resistance to ceftazidime. Moreover, ceftazidime-resistant TonB mutants were shown to be cross-resistant to fluoroquinolones and lacticvin, a siderophore-conjugated non-β-lactam antibiotic (Calvopina et al., 2020). Thus, a high probability exists for a possible cross-resistance between these antibiotics and microcins.

Resistance to MccN/24 is afforded by mutations in genes encoding the outer membrane porin OmpF (Jeanneau et al., 1994), or the inner membrane transporter SdaC involved in serine uptake and used for MccV activity (Gerard et al., 2005). Resistance to MccPDI also involves OmpF and more precisely the K<sup>F</sup>G<sup>48</sup> N<sup>49</sup> amino acid motif found in the predicted outer loop L1 of the porin (Zhao et al., 2015; Lu et al., 2019). In addition, mutations in DsbA and DsbB proteins, presumably involved in the formation of disulfide bonds in OmpF, induce resistance to MccPDI (Zhao et al., 2015). Mutations in *ompF* and *ompR* genes encoding OmpF induce a reduced sensitivity to MccB17. Moreover, a mutation in the *sbmA* gene encoding the inner membrane transporter SbmA, which translocates MccB17 from the inner membrane to the cytoplasm, induces high resistance to MccB17 (Laviña et al., 1986).

As regard the efflux systems involved in resistance to microcins, resistance to MccN/24 is controlled by the multiple antibiotic resistance (*mar*) operon (Carlson et al., 2001), which modulates efflux pump and porin expression via two encoded transcription factors, MarR and MarA (Sharma et al., 2017). MarA plays an important role in antibiotic resistance by activating the expression of the *acrAB-tolC* encoded efflux pump (Zhang et al., 2008) and also regulates biofilm formation (Kettles et al., 2019). Resistance to MccN/24 in *Salmonella* cells appears concomitantly with a multiple antibiotic resistance phenotype to ciprofloxacin, tetracycline, chloramphenicol and rifampicin (Carlson et al., 2001). So far, cross-resistance between MccN/24 and antibiotics raised above is quite possible.

Additional mechanisms involve specific cell wall modifications. Those include upexpression of capsule polysaccharides that can increase resistance to various antimicrobials including both antibiotics, in particular polymixins, and antimicrobial peptides (Campos et al., 2004). Interestingly, capsule polysaccharides are not involved in MccJ25 resistance of the YojI deficient strain (Delgado et al., 2005). Alterations of the LPS resulting in truncated LPS structures promote, among other pleiotropic effects, resistance to antimicrobial peptides and hydrophobic antibiotics (Pagnout et al., 2019).

**Changes in Target Sites**

To allow DNA supercoiling, bacteria use two type II topoisomerases, DNA gyrase and topoisomerase IV, which are both the targets of quinolones. They form a ternary cleavage complex gyrase/DNA/quinolone, thus blocking DNA replication. Mutations in genes encoding DNA gyrase (*gyrA, gyrB*) and topoisomerase IV (*parC, parE*) lead to quinolone resistance. Besides, a plasmid-mediated protection of DNA gyrase and topoisomerase IV from the action of quinolones is ensured in a non-specific manner by the gyrase interacting protein Qnr. Qnr is a 218 amino acid pentapeptide repeat protein (PRP) encoded by *qnr* genes, which blocks the action of quinolones on the DNA gyrase and topoisomerase IV in a lesser
extent (Fabrega et al., 2009; Jacoby et al., 2015). Indeed, one of these mutations is the well-known GyrB W751R mutation which induces resistance to quinolones and is also linked to resistance to MccB17 (Vizán et al., 1991). GyrB Trp751 is strongly implicated in the interaction of DNA gyrase with MccB17 (Hedde et al., 2001) and gyrB point mutation changing Trp751 for Arg leads to a protein variant resistant to MccB17 (del Castillo et al., 2001). Additionally, partial resistance to MccB17 is provided by mutations at position 83 in GyrA or 447 in GyrB (Jacoby et al., 2015). Consequently, cross-resistance to MccB17 and quinolones could occur. Otherwise, it is well known that immunity genes are responsible for protecting the producing bacteria from their own bacteriocin. Indeed, three genes mcbE, mcbF, and mcbG are involved in cell protection from endogenous and exogenous MccB17. Interestingly, strains harboring these genes are shown to be highly resistant to fluoroquinolones (Tran and Jacoby, 2002). These mechanisms seem to be responsible for co-resistance to MccB17 and quinolones.

Mutation of the gene rpoB encoding the β′ subunit of RNAP (see section mechanisms of action above) induces resistance to rifampicin (Campbell et al., 2001; Goldstein, 2014). Likewise, alterations in the 30S or 50S subunit of the ribosome lead to resistance to antibiotics that act on these proteins, mainly tetracycline, chloramphenicol, streptomycin and aminoglycosides (Kapoor et al., 2017). Similarly, first studies performed to understand the mechanism of action of MccJ25 have shown that a point mutation causing a substitution of Thr931 for Ile in the conserved segment of the rpoC gene coding for the largest RNAP subunit β′ conferred resistance to MccJ25, suggesting a mechanism involving occlusion of the RNAP secondary channel (Delgado et al., 2001; Yuzenkova et al., 2002). It was shown further from the crystal structure of the MccJ25-RNAP complex that MccJ25 binds within the RNAP secondary channel and interferes with the traffic of NTPs to the catalytic center (Braffman et al., 2019). Furthermore, additional rpoC mutations affecting amino acids in the conserved segments G, G′ and F and exposed into the RNAP secondary channel, also led to MccJ25 resistance in vivo and in vitro. While MccJ25 acts on the β′ subunit, and rifampicin on the β subunit, streptomycin acts on both subunits. So far, a cross-resistance between MccJ25 and the above cited antibiotics mainly streptomycin and rifampicin appears to be highly expected (Yang and Price, 1995; Temiakov et al., 2005).

For other antibiotics and microcins, no specific cross-resistance appears to be predictable. Chromosomally mediated colistin resistance occurs mainly via the addition of cationic moieties onto the negatively charged lipid A, while the plasmid mediated colistin resistance (MCR) is acquired via a plasmid-borne copy of an mcr gene. MCR-1 is the most prevalent MCR enzyme reported for the first time in 2015 followed by nine homologs described to date (Carroll et al., 2019). MCR-1-mediated colistin resistance confers protection against this last resort antibiotic via the presence of modified LPS within the cytoplasmic membrane, rather than the outer membrane (Sabnis et al., 2019). More precisely, the phosphoethanolamine transferase activity of MCR-1 adds a cationic phosphoethanolamine moiety to the anionic lipid domain A of LPS, which results in a net negative charge decrease and thus a lower affinity for the polymyxins.

Fosfomycin inhibits the bacterial cell wall synthesis at the early initiating step of the peptidoglycan synthesis. More specifically, it inhibits UDP-N-acetylglucosamine enolpyruvyl transferase (or MurA), the enzyme involved in transfer of the enolpyruvyl part of phosphoenolpyruvate to the 3′-hydroxy group of UDP-N-acetylglucosamine, which is the first step in the biosynthesis pathway of peptidoglycan. Mutations in the murA gene confer resistance to fosfomycin due to the replacement of cysteine with aspartate in the active site of MurA, which prevents fosfomycin binding (Falagas et al., 2019). Moreover, resistance to fosfomycin can occur from chromosomal mutations in the structural genes that encode the GlpT and UhpT membrane transporters. GlpT and UhpT transport glycerol-3-phosphate and glycerol-6-phosphate sugars in bacteria, respectively and are used by fosfomycin to facilitate its entry in bacteria. These mutations block fosfomycin cell penetration (Falagas et al., 2019).

On the microcin side, the F1-F9-ATP synthase has been shown to be the target of MccH47 (Rodriguez and Laviña, 2003) and MccPDI (Zhao et al., 2015). E. coli ATP synthase consists of a membrane-bound F0 sector, which ensures proton translocation, connected to a cytoplasmic F1 sector. They form a complex made up of eight different subunits, which are encoded by the atp operon, atpBEFHAGDC. Three subunits form the F0 proton channel and five subunits the catalytic F1 domain. Mutations on genes atpB, atpE, atpF encoding the three subunits F0a, F0c, F0b respectively, which constitute the F0 proton channel, result in resistance to MccH47 (Rodriguez and Laviña, 2003). Furthermore, deletion of genes encoding subunits in the F1 and F0 domains of ATP synthase (atpA and atpF encoding F1α and F0b subunits, or atpE and atpH encoding F0c and F1δ subunits), result in a loss of susceptibility to MccPDI simultaneously to the loss of ATP synthase function (Zhao et al., 2015). None of these mechanisms appears to be shared between antibiotics and microcins.

**Inactivation of the Toxic Entity**

Several Gram-negative bacteria produce different enzymes that are able to modify antibiotics and thus induce resistance, such as the very well-known β-lactamases, which disrupt the specific structure of β-lactams (Sawa et al., 2020). β-lactamases are classified into four classes including group 1 (class C) cephalosporinases, group 2 (classes A and D) broad-spectrum, inhibitor-resistant, and extended-spectrum β-lactamases as well as serine carbapenemases, and group 3 (class B) metallo-β-lactamases (Bush and Jacoby, 2010). Other enzymes including aminoglycosides modifying enzymes, such as phosphotransferases (APFs), nucleotideidytranferases (ANTs) and acetyltransferases (AACs), which phosphorylate, admenate and acetylate these compounds, respectively could also be involved in development of resistance (Ramirez and Tolmasky, 2010).

Acetylation is a widespread and efficient mechanism of resistance against different antibiotics. Modification of the piperazine ring of the fluoroquinolones is induced by an acetylase AAC(6′)-Ib-cr, which provides one of the mechanisms of resistance of bacteria to quinolones (Fabrega et al., 2009).
Chloramphenicol is also inactivated by acetylation which is performed by chloramphenicol acetyltransferases (CATs) (Smale, 2010). Acetylation is also a major mechanism of resistance to McC, then suggesting a high risk of cross-resistance between chloramphenicol and McC. Before its ultimate processing by non-specific aminopeptidases, which happens in sensitive cells to release the toxic non-hydrolyzable analog of aspartyl-adenylate, McC is exported outside the producer by the Mcc pump and uptaken by sensitive cells using the porin OmpF and the inner membrane transporter YejABEF (see section Mechanisms of action). However, although most of produced McC is efficiently exported, intracellular processing also occurs inside the producing cells that ineluctably leads to the accumulation of the toxic entity that cannot be exported by the Mcc pump and results in self-poisoning. Therefore, E. coli mcc gene clusters include genes (mccE and mccF) that encode proteins ensuring the self-immunity of the producer. The MccE acetyltransferase acetylates the α-amino group of processed McC, making it unable to bind to AspRS (Agarwal et al., 2011). So far, MccE makes E. coli simultaneously resistant to albomycin and McC (Novikova et al., 2010). MccE belongs to the general control non-repressible 5-related N-acetyltransferases (GNAT) superfamily, and shows high similarity with chromosomally encoded acetyltransferases RimL, RimJ, and RimL, which acetylate the N-termini of ribosomal proteins S18, S5, and L12 (Salah Ud-Din et al., 2016). Indeed, E. coli RimL induces resistance to McC by acetylating the amino group of the processed McC aspartate by the same mechanism as MccE (Kazakov et al., 2014). Similarly, when overproduced, RimL makes cells resistant to albomycin by acetylating processed albomycin, which contains a pyrimidine nucleotide instead of adenosine. Subsequently, a potential cross-resistance between McC and albomycin is quite possible (Kazakov et al., 2014). The MccF serine protease hydrolyses the carboxamide bond between the C-terminal aspartamide and AMP of both intact and processed McC, thus inactivating the aspartyl-adenylate (Agarwal et al., 2012). Moreover, McC inactivation is also ensured by phosphoramidases belonging to the histidine-triad (HIT) superfamily hydrolases that can either inactivate is also ensured by phosphoramidases belonging to the aspartyl-adenylate (Agarwal et al., 2012). Moreover, McC is exported outside the producer by the MccC pump and results in self-poisoning. Therefore, E. coli mcc gene clusters include genes (mccE and mccF) that encode proteins ensuring the self-immunity of the producer. The MccE acetyltransferase acetylates the α-amino group of processed McC, making it unable to bind to AspRS (Agarwal et al., 2011). 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The MccF serine protease hydrolyses the carboxamide bond between the C-terminal aspartamide and AMP of both intact and processed McC, thus inactivating the aspartyl-adenylate (Agarwal et al., 2012). Moreover, McC inactivation is also ensured by phosphoramidases belonging to the histidine-triad (HIT) superfamily hydrolases that can either be encoded in certain mcc-like biosynthetic clusters or by genes located elsewhere in bacterial genomes (Yagmurov et al., 2020). Resistance to McC-like compounds produced by S. enterica, Nocardiopsis kunsanensis, P. fluorescens or Hyalangium minutum is conferred by hydrolysis of the phosphoramidase bond in the toxic aspartamide-adenylate (Yagmurov et al., 2020). Therefore, it appears that resistance to Mcc and McC-like microcins by toxin inactivation can occur via both enzymes encoded in the microcin biosynthesis clusters and more generalist and non-specific enzymes sharing structural similarities. Finally, impairment of the final three-dimensional structure of the antibacterial peptide, such as by preventing the formation of disulfide bridges, could be a last mechanism resulting in resistance to microcins. This has been poorly explored until now, but is however illustrated by MccPDI for which mutations in dsbA, dsbB genes induce resistance to MccPDI (Zhao et al., 2015). Genes dsbA, dsbB encode DsbA and DsbB thiol-redox enzymes that usually catalyze disulfide bond formation for proteins that are transported into the periplasm, and which would be possibly involved in formation of the disulfide bond that stabilizes this microcin.

INHIBITORY EFFECT OF MICROCINS AGAINST ANTIBIOTIC RESISTANT GRAM-NEGATIVE BACTERIA

The spectrum of inhibitory activity of microcins includes a wide number of bacteria which are phylogenetically related to the producing strain including Salmonella, Shigella and E. coli. The inhibition activity of the different microcins against non-multidrug-resistant strains has been reported in the literature. However, the potency of these microcins specifically against MDR bacteria has not been systematically described and only few studies have addressed this special issue.

MccJ25 was shown to exhibit a high antimicrobial activity against MDR Salmonella and E. coli (Martin-Gómez et al., 2019; Yu et al., 2019). The antimicrobial activity of MccJ25 was also extensively studied against a collection of MDR strains of S. enterica spp. enterica (Ben Said et al., 2020). Interestingly, this study has shown that Salmonella strains exhibit various sensitivity profiles to MccJ25 and that MIC values vary from 0.06 to 400 µg/mL (0.028–189 nM), independently of the resistance profiles to antibiotics or the serovars. Other studies have shown that MccJ25 displays a great inhibitory potential against Salmonella and E. coli (Sablé et al., 2000; Delgado et al., 2001; Rintoul et al., 2001; Soudy et al., 2012). MccPDI is known to inhibit foodborne pathogenic enterohemorrhagic E. coli serotypes O157:H7 and O26 (Eberhart et al., 2012) as well as Shigella strains and E. coli isolates that are MDR strains (Lu et al., 2019). Likewise, MccH47 has demonstrated a potent effect against Enterobacteriaceae MDR strains including Salmonella and E. coli carbapenemase, extended spectrum β-lactamase and metallo-β-lactamase producers. MccH47 has MIC values less than 75 µg/mL (13 µM) for all tested strains (Palmer et al., 2020).

The remaining microcins revealed similar narrow spectra of activity against non-MDR Enterobacteriaceae, mainly Salmonella and E. coli. Indeed, MccE492 was shown to have inhibitory activity in vitro against a wide range of Enterobacteriaceae including Klebsiella, Enterobacter, E. coli and Salmonella while MccM was shown to inhibit Salmonella and E. coli (Vassiliadis et al., 2010). MccN/24 is active against E. coli and S. enterica Typhimurium, but not against L. monocytogenes or Campylobacter jejuni (Wooley et al., 1999). It was also reported by Kaur et al. (2016) that MccN/24 exhibits a potent activity against Salmonella strains. Furthermore, MccV is active against some pathogenic E. coli with MIC values ranging from 7.7 × 10⁻³ to 13.25 µg/mL (0.89–1517.94 nM) (Boubezari et al., 2018). MccS is lethal to virulent enterohemorrhagic and enteropathogenic E. coli through inhibiting the adherence of EPEC E. coli to intestinal epithelial cells in an in vitro adherence assay (Zschüttig et al., 2012). MccL exhibits a strong antibacterial activity against Enterobacteriaceae, including the S. enterica serovars Typhimurium and Enteritidis (Morin et al., 2011). Only a few studies have systematically assessed the efficiency of microcins (and, more generally, of bacteriocins), for the
inhibition of MDR bacteria, and/or the microcin/bacteriocin and antibiotic cross-resistance (Ben Said et al., 2020; Kuznetsova et al., 2020). Although the activities reported so far are encouraging, more systematic studies on the inhibitory potential of microcins against MDR strains remain necessary to confirm the potential of microcins as alternatives to antibiotics against MDR and are thus of high research priority. Future directions of research should relate to both qualitative and quantitative in vitro characterization of the inhibitory activity of different microcins against a large panel of clinical isolates of MDR pathogenic bacteria of medical and veterinary interest, coming from well characterized reference collections. The development of resistance of these strains against the various microcins deserves being investigated as well as studying the possible synergistic effects between microcins and certain antibiotics or biocides, as already started with Gram-positive bacteriocins (Mathur et al., 2017). Indeed, the identification of compounds with synergistic or additive effects could represent an effective strategy to limit the development of bacteria resistant to both microcins and antibiotics. Such an approach, and more widely combination treatment therapeutic strategies, could be facilitated by the development of optimized methods to quantify synergy effects more rapidly and efficiently (Fatsis-Kavalopoulos et al., 2020).

**MICROCINS AND THE IMMUNE SYSTEM**

Inflammation is one of the key processes allowing the immune system being alerted of risks for the host, such as pathogen attacks. But its dysregulation results in chronic inflammation and subsequent diseases, pointing that inflammation results in both beneficial and adverse effects. In general, interactions of bacteriocins or microcins with the immune system have not been investigated deeply, which hampers evaluating previsible risks and benefits for all characterized microcins. MccE492 was reported to induce apoptosis against human cell lines without inducing an inflammatory response (Hetz et al., 2002; Lagos et al., 2009). But most of all, two microcins, MccB17 and especially MccJ25, have been chiefly studied in this regard.

A pioneer study showed that polyclonal antibodies were raised in rabbits against mature MccB17, indicating that it could induce immune reaction once introduced in host body (Yorgey et al., 1993). In an in-depth study on the effects of oxazole compounds on intestinal inflammation (Iyer et al., 2018) have shown that, similar to environmental or synthetic ones, short-size oxazole compounds derived from MccB17 degradation were able to induce inflammation in mouse intestinal epithelial cells, while full-length MccB17 was not (Iyer et al., 2018; Collin and Maxwell, 2019). This effect was attributed to a cascade response where oxazole compounds activate IDO1, the rate-limiting enzyme in tryptophan catabolism, and in turn tryptophan-derived metabolites activate the aryl hydrocarbon receptor Ahr, which limits CD1d-restricted production of the anti-inflammatory cytokine IL-10 and results in natural killer T-cell mediated intestinal inflammation (Iyer et al., 2018). It was pointed that this oxazole-induced intestinal inflammation is independent of the antimicrobial activity of the compounds. Moreover, it was proposed that the CD1d-dependent immunomodulatory effect is limited by the size of the compounds, explaining the absence of effect of native MccB17, although its content in oxazole rings.

An in vitro study showed that MccJ25 protects IPEC-J2 cells against enterotoxigenic E. coli (ETEC) without raising cytotoxicity and alleviates the inflammatory responses through modulation of the levels of pro-inflammatory cytokines, interleukins 6 (IL-6), IL-8 and tumor necrosis factor-α (TNF-α) (Yu et al., 2018a). An anti-inflammatory effect of MccJ25 associated with killing of the pathogen was shown in an ETEC-infected mouse model (Ding et al., 2020; Yu et al., 2020). Similar to gentamicin treated mice, the levels of pro-inflammatory cytokines were significantly decreased in jejunum, ileum and colon tissues of mice administered MccJ25, compared to the control group, while the anti-inflammatory IL-10 level increased. Inhibition of ETEC-induced expression of inflammatory cytokines in the jejunum was proposed to be due to down-regulation by MccJ25 of the NF-κB and mitogen-activated protein kinase (MAPK) pathways (Ding et al., 2020). Moreover, absence of immunomodulatory effect and toxicity of MccJ25 was observed at the therapeutic dose (9 mg/kg), much higher doses only (18 mg/kg) being able to cause a low toxicity (Yu et al., 2018b). Furthermore, MccJ25 also decreases the serum concentration levels of the pro-inflammatory cytokines IL-6, IL-1β, and TNF-α, together with an increase in anti-inflammatory IL-10 in weaned pigs (Wang et al., 2020) and in broiler chicken (Yu et al., 2017) fed with MccJ25-supplemented diet. Taken together, these in vivo studies conducted in different animal models indicate that MccJ25 diet supplementation can lower inflammation together with affording protection against pathogens, providing interesting perspectives in inflammatory intestinal diseases. Therefore, it appears that none of the studied microcins appears to induce adverse inflammation imbalance and have a detrimental effect on the host.

**POTENTIAL APPLICATIONS OF MICROCINS AND FUTURE PROSPECT**

Microcins exhibit a number of advantages for potential applications, among which their absence of toxicity to eukaryotic cells and their chemical stability. Indeed, the three-dimensional structures or PTMs of most microcins increases their stability to harsh conditions, such as those that are encountered in the gut (Naimi et al., 2020). This favors their delivery to the gut without the help of specific formulations, if not for avoiding immunity response. However, unfortunately, the spectrum of inhibitory activity of the different microcins has not been deeply investigated, hampering significant development in veterinary or human medical domains. The antimicrobial activity of most microcins (MccB17, MccC and a few others) was determined in order to decipher their mechanism of action and the most tested bacterium was E. coli (Heddle et al., 2001; Metlitskaya et al., 2006; Severinov and Nair, 2012). Thus, while for a few microcins the spectrum of inhibition is well known, for the remaining this information is still missing. A more systematic study involving a significant number of clinical and veterinary
pathogens, including MDR strains, remains necessary to establish the exact spectrum of inhibition of each microcin.

An important characteristic making microcins good candidates as alternatives to antibiotics is that they are prominent actors of competitions in microbiota and particularly in the gut microbiota, which is the most studied. Microcins play a significant role in niche competition (for a review see Li and Rebuffat, 2020), essentially in interference competition, which involves the secretion of harmful molecules such as the microcins, for direct attack of competitors. But also in a lesser extent, they are involved in the indirect process of exploitative competition, as exemplified by siderophore microcins which are able to capture iron and thus deplete the surroundings of this essential element. Thereby, the siderophore microcins MccH47 and MccM, both produced by the probiotic *E. coli* strain Nissle 1917, have been shown to mediate competition among Enterobacteriaceae in mouse model and to impair the growth of the pathogen *S. enterica* serovar Typhimurium in the inflamed gut, where iron is scarce, without perturbing significantly the microbiota equilibrium (Sassone-Corsi et al., 2016). Thanks to their natural role in their niche, which involves both high potency and narrow spectrum of activity, the molecules from microbiota, such as the microcins in the gut microbiota (Donia and Fischbach, 2015; Garcia-Gutierrez et al., 2019), or other bacteriocins in the rumen (Oyama et al., 2017), are thus of high potential. However, exploration of the capacity of microorganisms belonging to various microbiota still remains underdeveloped so far. Its development in combination with genome mining approaches and innovative computational technologies should allow finding novel microcins, and possibly novel mechanisms of action.

To explore the potential applications of microcins in animal and human health, *in vivo* studies have been conducted, although they are still few and only concern a few microcins, essentially MccJ25. For instance, a significant decrease of *S. Typhimurium* was recorded in chicken, using an *E. coli* transformant strain producing MccN/24, although continuous administration of the transformant was needed to ensure colonization within the *in vivo* model (Wooley et al., 1999). MccJ25 has been shown to decrease *S. enterica* counts in the liver and spleen in mice (Lopez et al., 2007) and in the gastrointestinal tract of turkeys (Forkus et al., 2017), and to relieve diarrhea and systemic inflammation in weaned pigs (Yu et al., 2017). Furthermore, MccJ25 was shown to improve performance, fecal microbiota composition and systematic inflammation of broilers (Wang et al., 2020). Further studies are needed however to validate the potential of microcins as therapeutic agents in human or veterinary medicine.

Finally, developing safe probiotics engineered to produce potent microcins is a complementary and efficient approach. It relies on previous studies of commercially available probiotics, *E. coli* Nissle 1917 (Mutaflor®) and *E. coli* G3/10 (Symbioflor2®), producers of microcins MccH47 and MccM (Sassone-Corsi et al., 2016; Massip and Oswald, 2020) and MccS (Zschüttig et al., 2012), respectively, which were shown to act in bacterial competition and kill pathogens in inflamed gut (Sassone-Corsi et al., 2016), or suppress adherence of enteropathogenic *E. coli* (Zschüttig et al., 2012). Thus, *S. enterica* carriage was significantly reduced in turkey gastrointestinal tract using *E. coli* Nissle engineered to produce MccJ25 (Forkus et al., 2017). Furthermore, *E. coli* Nissle was engineered to produce MccH47 in response to tetrathionate, which is produced in gut inflammation conditions and is favorable to *Salmonella* growth (Palmer et al., 2018). In this system, MccH47 was produced in response to the tetrathionate environmental signal serving as an inducing molecule, and inhibited the pathogen *S. Typhimurium*, both in static inhibition assays and in ecological competition experiments.

**CONCLUSION**

As it can be seen through this review, microcins offer an attractive track for designing novel antimicrobial strategies and envisage alternatives to conventional antibiotics, despite the potential risks of resistance, cross-resistance and co-resistance that have been pointed. The microcin attractiveness relies first on their two-step mechanisms of action. The first step ensures uptake of the microcin and involves most often a Trojan horse strategy. It is exemplarily illustrated by MccC, for which the last processing step of the uptaken harmless nucleotide peptide is ensured in the targeted bacteria by common proteases. It is also exemplified by siderophore microcins (MccE492, MccM, MccH47) or the lasso microcin MccJ25 that mimic the natural ligands of siderophore receptors to hijack them. The second step implies either membrane perturbations or inhibition of critical enzymes, and therefore vital functions in bacteria. Indeed, in certain cases such strategies are shared by antibiotics, which can result in cross-resistance, as pointed in this review. These two steps can also constitute a drawback toward resistance development as inhibiting one of them could potentially confer resistance to microcin. However, a few microcins, such as MccC and MccJ25, bring into play a second and independent mechanism that intervenes at higher concentrations. Such a secondary mechanism has not been brought to light for other microcins, but it must be said that it has not been thoroughly investigated. Such a succession of different mechanisms limits the emergence of bacterial resistance, as the energetic costs induced by setting up distinct resistance mechanisms simultaneously is hard to assume by the bacteria.

Other characteristics, which have been underlined in the review, support their interesting potential: (i) a potent activity in the GI tract, (ii) a narrow spectrum of activity, which makes them active against pathogens while preserving host microbiota, (iii) an important role in microbial competitions, which makes them actors in maintaining microbiota equilibrium, (iv) an efficient activity *in vivo* in different animal models. Developing strategies based on Nature-derived mechanisms and molecules that are able to minimize both niche perturbations and resistance thus appears as a promising direction in the light of recent analysis of the frequency and mechanisms of resistance of antimicrobial peptides and antibiotics (Kintses et al., 2019). Finally, as the production costs of antimicrobial peptides and in particular of RIPPs remain high, a possible strategy to use microcins and simultaneously increase their potency could be to associate them to conventional antibiotics. This would take...
full advantage of the lower costs of production of antibiotics, of an increased potency when synergistic effects are obtained, and of the possibility of combining distinct mechanisms of action. Therefore, relying on the current knowledge on the topology of microcins and their targets, the microcin biosynthesis pathways, and their mechanisms of action and of resistance, directions of research involving a more dynamic exploration of diverse microbiota associated with the development of microcin bioengineering would presumably accelerate the diversification of anti-AMR strategies.

**AUTHOR CONTRIBUTIONS**

All authors listed have made a substantial, direct and intellectual contribution to the work, approved the final manuscript for publication. ST and LBS drafted the manuscript and contributed equally to its preparation. SR, IF, and SZ revised the manuscript.

**REFERENCES**

Adelman, K., Yuzenkova, J., La Porta, A., Zenkin, N., Lee, J., Lis, J. T., et al. (2004). Molecular mechanism of transcription inhibition by peptide antibiotic Microcin J25. Mol. Cell. 14, 753–762. doi: 10.1016/j.molcel.2004.05.017

Agarwal, V., Metlitskaya, A., Severinov, K., and Nair, S. K. (2011). Structural basis for microcin C7 inactivation by the McE acetyltransferase. J. Biol. Chem. 286, 21295–21303. doi: 10.1074/jbc.M111.226282

Agarwal, V., Tikhonov, A., Metlitskaya, A., Severinov, K., and Nair, S. K. (2012). Structure and function of a serine carboxypeptidase adapted for degradation of the protein synthesis antibiotic microcin C7. Proc. Natl. Acad. Sci. U.S.A. 109, 4425–4430. doi: 10.1073/pnas.111424109

Aguileria, P., Marcoleta, A., Lobos-Ruiz, P., Arranz, R., Valpuesta, J. M., Monasterio, O., et al. (2016). Identification of key amino acid residues modulating intracellular and in vitro microcin E492 amyloid formation. Front. Microbiol. 7:35. doi: 10.3389/fmicb.2016.00035

Amaral, L., Martins, A., Spengler, G., and Molnar, J. (2014). Efflux pumps of Gram-negative bacteria, what they do, how they do it, with what and how to deal with them. Front. Pharmacol. 4:168. doi: 10.3389/fphar.2013.00168

Anes, J., McCusker, M. P., Fanning, S., and Martins, M. (2015). The ins and outs of RND efflux pumps in Escherichia coli. Front. Microbiol. 6:587. doi: 10.3389/fmicb.2015.00587

Arnison, P. G., Bibb, M. J., Bierbaum, G., Bowers, A. A., Bugni, T. S., Bulaj, G., et al. (2013). Ribosomally synthesized and post-translationally modified peptide natural products, overview and recommendations for a universal nomenclature. Nat. Prod. Rep. 30, 108–160. doi: 10.1039/c2np20085f

Arranz, R., Mercado, G., Martin-Benito, J., Giraldo, R., Monasterio, O., Lagos, R., et al. (2012). Structural characterization of microcin E492 amyloid formation. Identification of the precursors. J. Struct. Biol. 178, 54–60. doi: 10.1016/j.jsb.2012.02.015

Asensio, C., and Perez-Diaz, J. C. (1976). A new family of low molecular weight antibiotics from enterobacteria. Biochem. Biophys. Res. Commun. 69, 7–14. doi: 10.1016/s0006-291x(76)80264-1

Azpiroz, M. F., and Laviña, M. (2007). Modular structure of microcin H47 and colicin V. Antimicrob. Agents Chemother. 51, 2412–2419. doi: 10.1128/AAC.01606-06

Bantysh, O., Serebryakova, M., Makarova, K. S., Dubiley, S., Datsenko, K. A., and Severinov, K. (2014). Enzymatic synthesis of bioinformatically predicted microcin C-like compounds encoded by diverse bacteria. mBio 5:e01059-14. doi: 10.1128/mBio.01059-14

Baquero, F., Lanza, V. F., Baquero, M. R., Del Campo, R., and Bravo-Vazquez, D. A. (2019). Microcins in Enterobacteriaceae. Peptide antimicrobials in the eco-active intestinal chemosphere. Front. Microbiol. 10:2261. doi: 10.3389/fmicb.2019.02261

Baquero, F., and Moreno, F. (1984). The microcins. FEMS Microbiol. Lett. 23, 117–124. doi: 10.1016/0014-0106(84)90005-4

Beis, K., and Rebufat, S. (2019). Multifaceted ABC transporters associated to microcin and bacteriocin export. Res. Microbiol. 170, 399–406. doi: 10.1016/j.resmic.2019.07.002

Bellomo, A., Vincent, P. A., de Arcuri, B. F., Farias, R. N., and Moreiro, R. D. (2007). Microcin J25 has dual and independent mechanisms of action in Escherichia coli. RNA polymerase inhibition and increased superoxide production. J. Bacteriol. 189, 4180–4186. doi: 10.1128/JB.00206-07

Ben Said, L., Emond-Rheault, J. G., Soltani, S., Telhig, S., Zirah, S., Rebufat, S., et al. (2020). Phenomic and genomic approaches to studying the inhibition of multiresistant Salmonella enterica by microcin J25. Environ. Microbiol. 22, 2907–2920. doi: 10.1111/1462-2920.15045

Bieler, S., Estrada, L., Lagos, R., Baeza, M., Castilla, J., and Soto, C. (2005). Amyloid formation modulates the biological activity of a bacterial protein. J. Biol. Chem. 280, 26880–26885. doi: 10.1074/jbc.M502031200

Bieler, S., Silva, F., and Belin, D. (2010). The polypeptide core of microcin E492 stably associates with the mannose permease and interferes with mannose metabolism. Res. Microbiol. 161, 706–710. doi: 10.1016/j.resmic.2010.07.003

Bieler, S., Silva, F., Soto, C., and Belin, D. (2006). Bactericidal activity of both secreted and nonsecreted microcin E492 requires the mannose permease. J. Bacteriol. 188, 7049–7061. doi: 10.1128/jb.00688-06

Boubezari, M. T., Idoui, T., Hammami, R., Fernandez, B., Gomaa, A., and Fliss, I. (2018). Bacteriocinogenic properties of Escherichia coli P2C isolated from pig gastrointestinal tract, purification and characterization of microcin V. Arch. Microbiol. 200, 771–782. doi: 10.1007/s00203-018-1482-6

Bountra, K., Hagelsueken, G., Choudhury, H. G., Corradi, V., El Omari, K., Wagner, A., et al. (2017). Structural basis for bacterial peptide self-immunity by the bacterial ABC transporter McjD. Embo. J. 36, 3062–3079. doi: 10.15252/embj.201797278

Braffman, N. R., Piscotta, F. J., Hauver, J., Campbell, E. A., Link, A. J., and Darst, S. A. (2019). Structural mechanism of transcription inhibition by lasso peptides of microcin J25 and capistruin. Proc. Natl. Acad. Sci. U.S.A. 116, 1273–1278. doi: 10.1073/pnas.1817352116

Brown, E. F., Cooper, A., Carrillo, C., and Blais, B. (2019). Selection of multidrug-resistant bacteria in medicated animal feeds. Front. Microbiol. 10:456. doi: 10.3389/fmicb.2019.00456

Budic, M., Rajic, M., Petkovsek, Z., and Zgur-Bertok, D. (2011). Escherichia coli bacteriocins, antimicrobial efficacy and prevalence among isolates from patients with bacteremia. PLoS One 6:e28769. doi: 10.1371/journal.pone.0028769

**SUPPLEMENTARY MATERIAL**

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LBS and SR wrote the final version of the manuscript. ST, LBS, and SZ designed and prepared the figures.

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Burkhart, B. J., Hudson, G. A., Dunbar, K. L., and Mitchell, D. A. (2015). A prevalent peptide-binding domain guides ribosomal natural product biosynthesis. *Nat. Chem. Biol.* 11, 564–570. doi: 10.1038/nchembio.1856

Bush, K., and Jacoby, G. A. (2010). Updated functional classification of beta-lactamases. *Antimicrob. Agents Chemother.* 54, 969–976. doi: 10.1128/aac.01089-09

Calvopina, K., Dulyangkul, P., Heesom, K. J., and Avison, M. B. (2020). TonB-dependent uptake of beta-lactam antibiotics in the opportunistic human pathogen *Stenotrophomonas maltophilia*. *Mol. Microbiol.* 113, 492–503. doi: 10.1111/mmi.14434

Campbell, E. A., Korzheva, N., Mustaev, A., Murakami, K., Nair, S., Goldfarb, A., et al. (2001). Structural mechanism for rifampicin inhibition of bacterial RNA polymerase. *Cell* 104, 901–912. doi: 10.1016/s0092-8674(01)00286-0

Campos, M. A., Vargas, M. A., Requeiro, V., Lompart, C. M., Alberti, S., and Bengoechea, J. A. (2004). Capsule polysaccharide mediates bacterial resistance to antimicrobial peptides. *Infect. Immun.* 72, 7107–7114. doi: 10.1128/IAI.72.11.7107-7114.2004

Carlson, S. A., Frana, T. S., and Griffith, R. W. (2001). Antibiotic resistance in *Salmonella enterica* serovar Typhimurium exposed to microcin-producing *Escherichia coli*. *Appl. Environ. Microbiol.* 67, 3763–3766. doi: 10.1128/aem.67.8.3763-3766.2001

Carroll, L. M., Gaballa, A., Guldimann, C., Sullivan, G., Henderson, L. O., and Wiedmann, M. (2019). Identification of novel mobilized colistin resistance gene mcr-9 in a multidrug-resistant, colistin-susceptible *Salmonella enterica* serotype Typhimurium isolate. *mBio* 10:e0853-19. doi: 10.1128/mBio.0853-19

Cascales, E., Buchanan, S. K., Duché, D., Kleanthous, C., Lloubès, C., Llobrèu, R., Postle, K., et al. (2007). Colicin biology. *Microbiol. Mol. Biol. Rev.* 71, 158–229. doi: 10.1128/mmbr.00036-06

Chapman, J. S. (2003). Disinfectant resistance mechanisms, cross-resistance, and co-resistance. *Int. Biodeterior. Biodegrad.* 51, 271–276. doi: 10.1016/S0964-8305(03)00044-1

Chehade, H., and Braun, V. (1988). Iron-regulated synthesis and uptake of colicin V. *FEMS Microbiol. Lett.* 52, 177–181. doi: 10.1111/j.1574-6968.1998.tb02591.x

Cheung-Lee, W. L., and Link, A. J. (2019). Genome mining for lasso peptides, past, present, and future. *J. Ind. Microbiol. Biotechnol.* 46, 1371–1379. doi: 10.1007/s10529-019-02197-z

Cheung-Lee, W. L., Parry, M. E., Cartagena, A. J., Darst, S. A., and Link, A. J. (2019). Discovery and structure of the antimicrobial lasso peptide citricin. *J. Biol. Chem.* 294, 6822–6830. doi: 10.1074/jbc.RA118.006494

Cheung-Lee, W. L., Parry, M. E., Zong, C., Cartagena, A. J., Darst, S. A., Connell, N. D., et al. (2020). Discovery of ubonodin, an antimicrobial lasso peptide active against members of the *Burkholderia cepacia* complex. *Chembiochem.* 21, 1335–1340. doi: 10.1002/cbic.201900707

Choi, U., and Lee, C. R. (2019). Distinct roles of outer membrane porins in antibiotic resistance and membrane integrity in *Escherichia coli* strains. *Front. Microbiol.* 10:953. doi: 10.3389/fmicb.2019.00953

Chopra, I., and Roberts, M. (2001). Tetracycline antibiotics, mode of action, applications, molecular biology, and epidemiology of bacterial resistance. *Microbiol. Mol. Biol. Rev.* 65, 232–260. doi: 10.1128/mmbr.65.2.232-260.2001

Collet, J.-F., Cho, S.-H., Iorga, B. L., and Goemans, C. V. (2020). How the assembly and protection of the bacterial cell envelope depend on cysteine residues. *J. Biol. Chem.* 295, 11984–11994. doi: 10.1074/jbc.REV.212.112101

Collin, F., and Maxwell, A. (2019). The microbial toxin Microcin B17, prospects for the development of new antibacterial agents. *J. Mol. Biol.* 431, 3400–3426. doi: 10.1016/j.jmb.2019.05.050

Corsini, G., Karahanian, E., Tello, M., Fernandez, K., Rivero, D., Saavedra, J. M., et al. (2010). Purification and characterization of the antimicrobial peptide microcin N. *FEMS Microbiol. Lett.* 312, 119–125. doi: 10.1111/j.1574-6966.2010.02106.x

Cotter, P. D., Ross, R. P., and Hill, C. (2013). Bacteriocins - a viable alternative to antibiotics? *Nat. Rev. Microbiol.* 11, 95–105. doi: 10.1038/nrmicro2937

Crosa, J. H., and Walsh, C. T. (2002). Genetics and assembly line enzymology of siderophore biosynthesis in bacteria. *Microbiol. Mol. Biol. Rev.* 66, 223–249. doi: 10.1128/mmbr.66.2.223-249.2002

Davies, E. A., Bevis, H. E., and Delves-Broughton, J. (1997). The use of the bacteriocin, nisin, as a preservative in ricotta-type cheeses to control the food-borne pathogen *Listeria monocytogenes*. *Lett. Appl. Microbiol.* 24, 343–346. doi: 10.1046/j.1472-765x.1997.00145.x
Endriss, F., Braun, M., Killmann, H., and Braun, V. (2003). Mutant analysis of the Escherichia coli FhuA protein reveals sites of FhuA activity. J. Bacteriol. 185, 4683–4692. doi: 10.1128/JB.185.16.4683-4692.2003

Fabrega, A., Madurga, S., Giralt, E., and Vila, J. (2009). Mechanism of action of and resistance to quinolones. Microb. Biotechnol. 2, 40–61. doi: 10.1111/j.1751-7915.2009.00063.x

Falagas, M. E., Athanassiou, F., Voulgaris, G. L., Triandrides, N. A., and Vardakas, K. Z. (2019). resistance to fosfomycin, mechanisms, frequency and clinical consequences. Int. J. Antimicrob. Agents 53, 22–28. doi: 10.1016/j.ijantimicag.2018.09.013

Fath, M. J., Zhang, L. H., Rush, J., and Kolter, R. (1994). Purification and germination of a DNA gyrase poison. Proc. Natl. Acad. Sci. U.S.A. 91, 100–104. doi: 10.1073/pnas.91.1.100

Fatt, M. J., Zhang, L. H., Rush, J., and Kolter, R. (1994). Purification and germination of a DNA gyrase poison. Proc. Natl. Acad. Sci. U.S.A. 91, 100–104. doi: 10.1073/pnas.91.1.100

Fatic-Kavalopoulos, N., Roemhild, R., Tang, P.-C., Kreuger, J., and Andersson, D. I. (2020). CombiANT, antibiotic interaction testing made easy. Front. Microbiol. 11, 586433

Gerard, F., Pradel, N., and Wu, L. F. (2005). Bactericidal activity of colicin V is a DNA gyrase poison. Int. J. Antimicrob. Agents 26, 531–537. doi: 10.1016/j.ijantimicag.2005.08.006

Ghilarov, D., Stevenson, C. E. M., Travin, D. Y., Piskunova, J., Serebryakova, D. I., et al. (2019). Architecture of microcin B17 synthetase. An Lasso peptides from proteobacteria. Genome mining employing heterologous expression and mass spectrometry. Biopolymers 100, 527–542. doi: 10.1002/bip.22326

Hetz, C., Bono, M. R., Barros, L. F., and Lagos, R. (2002). Microcin E492, a channel-forming bacteriocin from Klebsiella pneumoniae, induces apoptosis in some human cell lines. Proc. Natl. Acad. Sci. U.S.A. 99, 2696–2701. doi: 10.1073/pnas.052796999

Iyer, S. S., Gensollen, T., Gandhi, A., Oh, S. F., Neves, J. F., Collin, F., et al. (2018). Dietary and microbial oxazoles induce intestinal inflammation by modulating aryl hydrocarbon receptor responses. Cell 173, 1123–1134.e11. doi: 10.1016/j.cell.2018.04.037

Jacoby, G. A., Corcoran, M. A., and Hooper, D. C. (2015). Protective effect of Qnr on agents other than quinolones that target DNA gyrase. Antimicrob. Agents Chemother. 59, 6689–6695. doi: 10.1128/aac.01292-15

Jacoby, G. A., Mills, D. M., and Chow, N. (2004). Role of beta-lactamases and porins with export. J. Hosp. Infect. 57, 217–227. doi: 10.1016/j.jhin.2004.05.012

Kao, S., Tschowri, N., Lyons, K. J., Sharma, P., Hengge, R., Webber, M. A., et al. (2006). OmpC and OmpF are required for growth under hyperosmotic stress above pH 8 in Escherichia coli. Lett. Appl. Microbiol. 42, 195–201. doi: 10.1111/j.1742-7765.2006.01845.x

Kaper, B. H., Saal, S., and Elfgang, M. A. (2017). Action and resistance mechanisms of antibiotics. A guide for clinicians. J. Anaesthesiol. Clin. Pharmacol. 33, 300–305. doi: 10.4103/joa/joapc_349_15

Kaur, K., Tarassova, O., Dangeti, R. V., Azmi, S., Wishart, D., McMullen, L., et al. (2016). Characterization of a highly potent antimicrobial peptide microcin N from uropathogenic Escherichia coli. Femis. Microbiol. Lett. 363:fnw995. doi: 10.1093/femsle/fnw995

Kazakov, T., Kuznecov, K., Semenova, E., Mukhamedyarov, D., Datsenko, K. A., Metlitskaya, A., et al. (2014). The RimL transacetylase provides resistance to translation inhibitor microcin C. J. Bacteriol. 196, 3377–3385. doi: 10.1128/jb.01984-14

Kloos, D. M., Oliver, E., and Littlefield-Wyer, J. (2007). “The diversity of bacteriocins in Gram-negative bacteria,” in Bacteriocins, Ecology and Evolution, eds M. A. Riley and M. A. Chavan (Berlin: Springer).

Gratia, A. (1925). Sur un remarquable exemple d’antagonisme entre deux souches de colibacille. C. R. Soc. Biol. 93, 1041–1042.

Guijarro, J. I., Gonzalez-Pastor, J. E., Baleux, F., San Millán, J. L., Castilla, M. A., Rico, M., et al. (1995). Chemical structure and translation inhibition studies of the antibiotic microcin C7. J. Biol. Chem. 270, 23520–23523. doi: 10.1074/jbc.270.40.23520

Hävarstein, S. L., Diep, D. B., and Nes, I. F. (1995). A family of bacteriocin ABC transporters carry out proteolytic processing of their substrates concomitant with export. Mol. Microbiol. 16, 229–240. doi: 10.1111/j.1365-2958.1995.tb02295.x

Hawkey, P. M. (2015). Multidrug-resistant Gram-negative bacteria, a product of globalization. J. Hosp. Infect. 89, 241–247. doi: 10.1016/j.jhin.2015.01.008

Heidbrede, J. G., Blanc, S. J., Zamble, D. B., Holfelder, F., Miller, D. A., Wentsell, L. M., et al. (2001). The antibiotic microcin B17 is a DNA gyrase poison, characterisation of the mode of inhibition. J. Mol. Biol. 307, 1223–1234. doi: 10.1006/jmbi.2001.4562

Hegemann, J. D., Zimmermann, M., Zhu, S., Klug, D., and Marahiel, M. A. (2013). Lasso peptides from proteobacteria. Genome mining employing heterologous expression and mass spectrometry. Biopolymers 100, 527–542. doi: 10.1002/bip.22326

Hantke, K. (2003). Is the bacterial ferrous iron transporter FeoB a living fossil? FEMS Microbiol. Lett. 223, 69–74. doi: 10.1111/j.1574-6941.2003.tb02295.x
Krewulak, K. D., and Vogel, H. J. (2008). Structural biology of bacterial iron uptake. Biochim. Biophys. Acta 1778, 1781–1804. doi: 10.1016/j.bbamem.2007.07.026

Kuznetsova, K., Semenova, E., Knappe, T., Mukhamedyarov, D., Srivastava, A., Chatterjee, S., et al. (2011). The antibacterial threadless-lipid peptide caspin inhibits bacterial RNA polymerase. J. Mol. Biol. 412, 842–848. doi: 10.1016/j.jmb.2011.02.060

Kuznetsova, M. G., Gizautilina, J. S., Nesterova, L. Y., and Starčič Erjavec, M. (2020). Escherichia coli isolated from cases of colibacillosis in Russian poultry farms (Perm Krai). Sensitivity to antibiotics and bacteriocins. Microorganisms 8:741. doi: 10.3390/microorganisms8050741

Lagos, R., Tello, M., Mercado, G., Garcia, V., and Monasterio, O. (2009). Antibacterial and antimutorigenic properties of microcin E492, a pore-forming bacteriocin. Curr. Pharm. Biotechnol. 10, 74–85. doi: 10.2174/13892010978048643

Lagos, R., Willens, M., Vergara, C., Cecchi, X., and Monasterio, O. (1993). Microcin E492 forms ion channels in phospholipid bilayer membrane. FEBS Lett. 321, 145–148. doi: 10.1016/0014-5793(93)80096-d

Laviña, M., Gaggero, C., and Moreno, F. (1990). Microcin H47, a chromosome-encoded microcin antibiotic of Escherichia coli. J. Bacteriol. 172, 6585–6588. doi: 10.1128/jb.172.11.6585–6588.1990

Laviña, M., Pugeot, A. P., and Moreno, F. (1986). Identification, mapping, and characterization of a gene (sbmA) required for microcin B17 action on Escherichia coli K12. J. Gen. Microbiol. 132, 1685–1693. doi: 10.1099/00221287-132-6-1685

Lazdunski, C. J., Bouveret, E., Rigal, A., Journet, L., Llobèrs, R., and Bésnédetti, H. (1998). Colicin import into Escherichia coli cells. J. Bacteriol. 180, 4993–5002. doi: 10.1128/ JB.170.12.4993-5002.1998

Lewis, K., Epstein, S., D’Onofrio, A., and Ling, L. L. (2010). Uncultured microorganisms as a source of secondary metabolites. J. Antibiot. 63, 468–476. doi: 10.1038/ia.2010.87

Li, Z. X., Ma, D., Livermore, D. M., and Nikaido, H. (1994). Role of efflux pump(s) in intrinsic resistance of Pseudomonas aeruginosa, active efflux as a contributing factor to beta-lactam resistance. Antimicrob. Agents Chemother. 38, 1742–1752. doi: 10.1128/aac.38.8.1742

Li, Z. Z., Pleisiat, P., and Nikaido, H. (2015). The challenge of efflux-mediated antibiotic resistance in Gram-negative bacteria. Clin. Microbiol. Rev. 28, 337–418. doi: 10.1128/cmr.00117-14

Li, Y., and Rebuffat, S. (2020). The manifold roles of microbial ribosomal peptides-based natural products in physiology and ecology. J. Bacteriol. 295, 34–54. doi: 10.1128/jb.REV119.006545

Li, Y.-M., Milne, J. C., Madison, L. L., Koler, R., and Walsh, C. T. (1996). From peptide precursors to oxazole and thiazole-containing peptide antibiotics, microcin B17 synthase. Science 274, 1188–1193. doi: 10.1126/science.274.5290.1188

Lopez, F. E., Vincent, P. A., Zenoff, A. M., Salomón, R. A., and Farias, R. N. (2007). Efficacy of microcin J25 in biomatrices and in a mouse model of Salmonella infection. J. Antimicrob. Chemother. 59, 676–680. doi: 10.1093/jac/dkm009

Lu, S. Y., Graça, T., Avillan, J. J., Zhao, Z., and Call, D. R. (2019). Microcin S9 inhibits antibiotic-resistant strains of Escherichia coli and Shigella through a mechanism of membrane disruption and protection by homotrimer self-immunity. Appl. Environ. Microbiol. 85:e0371-19. doi: 10.1128/aem.0371-19

MacVane, S. H. (2017). Antimicrobial resistance in the intensive care unit, A focus on Gram-negative bacterial infections. J. Intensive Care Med. 32, 25–37. doi: 10.1177/0739986316651895

Maksimov, M. O., Pelzer, L., and Link, A. J. (2012). Precursor-centric genome-mining approach for lasso peptide discovery. Proc. Natl. Acad. Sci. U.S.A. 109, 15223–15228. doi: 10.1073/pnas.120897109

Marcoleta, A., Marin, M., Mercado, G., Valpuesta, J. M., Monasterio, O., and Lagos, R. (2013). Microcin E492 amyloid formation is retarded by posttranslational modification. J. Bacteriol. 195, 3995–4004. doi: 10.1128/jb.00564-13

Martin-Gómez, H., Jorba, M., Albericci, F., Viñas, M., and Tulla-Puche, J. (2019). Chemical Modification of microcin J25 reveals new insights on the stereospecific requirements for antimicrobial activity. Int. J. Mol. Sci. 20:5152.

Massip, C., and Osowld, E. (2020). Siderophore-microcins in Escherichia coli, determinants of digestive colonization, the first step toward virulence. Front. Cell Infect. Microbiol. 10:381. doi: 10.3389/fcimb.2020.00381

Mathavan, I., Zirah, S., Mehmoond, S., Choudhury, H. G., Goulard, C., Li, Y., et al. (2014). Structural basis for hijacking siderophore receptors by antimicrobial lasso peptides. Nat. Chem. Biol. 10, 340–342. doi: 10.1038/nchembio.1499

Mathur, H., Field, D., Rea, M. C., Cotter, P. D., Hill, C., and Ross, R. P. (2017). Bacteriocin-antimicrobial synergy, a medical and food perspective. Front. Microbiol. 8:5120. doi: 10.3389/fmicb.2017.05120

Mcintosh, J. A., Donia, M. S., and Schmidt, E. W. (2009). Ribosomal peptide-targeting novel research. Bacteriocins Against Gram-Negative Bacterial Resistance

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November 3, 2020 Time: 18:8 # 22
O’Brien, G. J. (1996). Molecular Analysis of Microcin 24, Genetics, Secretion and Mode of Action of a Novel Microcin. Ph. D thesis, University of Canterbury, Christchurch.

O’Brien, G. J., and Mahanty, H. K. (1994). Colicin 24, a new plasmid-borne colicin from a uropathogenic strain of Escherichia coli. Plasmid 31, 288–296. doi: 10.1016/0146-6178(94)90076-3

Oyama, L. B., Girdwood, S. E., Cookson, A. R., Fernandez-Fuentes, N., Privé, F., Vallin, H. E., et al. (2017). The rumen microbiome, an underexplored resource for novel antimicrobial discovery. NPJ Biofilms Microbiol. 3:33. doi: 10.1038/s41522-017-0042-1

Pagnout, C., Sohm, B., Razafitananamaharavo, A., Caillat, C., Offroy, M., Leduc, M., et al. (2019). Pleiotropic effects of rfa-gene mutations on Escherichia coli envelope properties. Sci. Rep. 9:9696. doi: 10.1038/s41598-019-46100-3

Palmer, J. D., Mortfeldt, B. M., Piattelli, E., Silby, M. W., Brigham, C. J., and Dougherty, G. R. (2003). Microcin J25 has a threaded sidechain-to-backbone ring structure of ATP synthase necessary and sufficient for microcin H47 antibiotic action. J. Bacteriol. 185, 7056–7065. doi: 10.1128/jb.185.20.6034-6046.2003

Palmer, J. D., Piattelli, E., McCormick, B. A., Silby, M. W., Brigham, C. J., and Dougherty, G. R. (2003). Microcin J25 has a threaded lasso molecule are responsible for interaction with bacterial RNA polymerase. J. Bacteriol. 185, 3859–3863. doi: 10.1128/jb.185.11.3859-3863.2003

Saffin, A., Klockner, A., Beccе, M., Evans, L. E., Furniss, R. C., David, D. F., et al. (2019). Colistin kills bacteria by targeting lipopolysaccharide in the cytoplasmic membrane. bioRxiv [Preprint], doi: 10.1101/479618

Salah Ud-Din, A. I., Tikhonirovа, A., and Roujeинikова, A. (2016). Structure and functional diversity of GCN5-related N-acetyltransferases (GNAT). Int. J. Mol. Sci. 17:1018. doi: 10.3390/ijms17071018

Salomón, R. A., and Farias, R. N. (1992). Microcin 25, a novel antimicrobial peptide produced by Escherichia coli. J. Bacteriol. 174, 7428–7435. doi: 10.1128/jb.174.22.7428-7435.1992

Salomón, R. A., and Farias, R. N. (1993). The FhuA protein is involved in microcin 25 uptake. J. Bacteriol. 175, 7741–7742. doi: 10.1128/jb.175.23.7741-7742.1993

Sassone-Corsi, M., Nuccio, S. P., Liu, H., Hernandez, D., Vu, C. T., Takahashi, A. A., et al. (2016). Microcins mediate competition among Enterobacteiraceae in the inflamed gut. Nature 540, 280–283. doi: 10.1038/nature20557

Sato, M., Machida, A., Ariкako, E., Saitо, H., Kakegawa, T., and Kobayashi, H. (2000). Expression of outer membrane proteins in Escherichia coli growing at acid pH. Appl. Environ. Microbiol. 66, 943–947. doi: 10.1128/aem.66.4.943-947.2000

Sawa, T., Kooguchi, K., and Moriyama, K. (2020). Molecular diversity of extended-spectrum beta-lactamases and carbapenemases, and antimicrobial resistance. J. Intens. Care 8:13. doi: 10.1186/s40706-020-0249-6

Schindler, P. R., and Teuber, M. (1975). Action of polymyxin B on bacterial membranes, morphological changes in the cytoplasm and in the outer membrane of Salmonella typhi murium and Escherichia coli B. Antimicrob. Agents Chemother. 8, 95–104. doi: 10.1128/ac.8.1.95

Severnin, K., and Nair, S. K. (2012). Microcin C, biosynthesis and mechanisms of bacterial resistance. Future Microbiol. 7, 281–289. doi: 10.2217/fmb.11.148

Sharma, P., Haycоcks, J. R., Middlemiss, A. D., Ketleys, R. A., Sellers, L. E., Ricci, V., et al. (2017). The multiple antibiotic resistance operon of enteric bacteria controls DNA repair and outer membrane integrity. Nat. Commun. 8:9444. doi: 10.1038/ncomms10003

Shkundina, I., Serebryakova, M., and Severinov, K. (2014). The C-terminal part of microcin B is crucial for DNA gyrase inhibition and antibiotic uptake by sensitive cells. J. Bacteriol. 196, 1759–1767. doi: 10.1128/jb.00015-14

Sikandar, A., and Koehnke, J. (2019). The role of protein-protein interactions in the biosynthesis of ribosomally synthesized and post-translationally modified peptides. Nat. Prod. Rep. 36, 1576–1588. doi: 10.1039/c8np00064f

Smale, S. T. (2010). Chloramphenicol acetyltransferase assay. Cold Spring Harb. Protoc. 2010.pdb_prot5422. doi: 10.1101/pdb.prot5422

Snavely, A. B., and Worobo, R. W. (2014). Chemical and genetic characterization of bacteriocins, antimicrobial peptides for food safety. J. Sci. Food Agric. 94, 28–44. doi: 10.1002/jsfa.6293
Soudy, R., Wang, L., and Kaur, K. (2012). Synthetic peptides derived from the sequence of a lasso peptide microcin J25 show antibacterial activity. Bioorg. Med. Chem. 20, 1794–1800. doi: 10.1016/j.bmc.2011.12.061

Sun, J., Liao, X. P., D’Souza, A. W., Boolchandani, M., Li, S. H., Cheng, K., et al. (2020). Environmental remodeling of human gut microbiota and antibiotic resistome in livestock farms. Nat. Commun. 11:427. doi: 10.1038/s41467-020-15222-7

Temiakov, D., Zenkin, N., Vassyleva, M. N., Perederina, A., Tahirov, T. H., Kashkina, E., et al. (2005). Structural basis of transcription inhibition by antibiotic streptolysin. Mol. Cell. 19, 655–666. doi: 10.1016/j.molcel.2005.07.020

Thomas, X., Destoumieux-Garzón, D., Peduzzi, J., Afonso, C., Blond, A., Birilirakis, N., et al. (2004). Siderophore peptide, a new type of post-translationally modified antibacterial peptide with potent activity. J. Biol. Chem. 279, 2823–2824. doi: 10.1074/jbc.M40028200

Tietz, J. I., Schwalen, C. J., Patel, P. M., Blair, P. M., Tai, H.-C., et al. (2017). A new genome-mining tool redefines the lasso peptide biosynthetic pathway of genetic determinants for production of and immunity to microcin E492 from Klebsiella pneumoniae. J. Bacteriol. 197, 4789–4794. doi: 10.1128/jb.197.15.4789-4794. 1997

Wester, C. W., Durairaj, L., Evans, A. T., Schwartz, D. N., Hussain, S., and Martinez, E. (2002). Antibiotic resistance, a survey of physician perceptions. Arch. Intern. Med. 162, 2210–2216. doi: 10.1001/archinte.162.19.2210

Wilkins, M., Villanueva, J. E., Cofre, J., Chnaiderman, J., and Lagos, R. (1997). Cloning and expression in Escherichia coli of determinants for production of and immunity to microcin E492 from Klebsiella pneumoniae. J. Bacteriol. 179, 4789–4794. doi: 10.1128/jb.179.15.4789-4794. 1997

Wilson, B. R., Bogdan, A. R., Miyazawa, M., and Tsuji, Y. (2016). Siderophores in iron metabolism, from mechanism to therapy potential. Trends Mol. Med. 22, 1077–1090. doi: 10.1016/j.molmed.2016.10.005

Woolley, R. E., Gibbs, P. S., and Shotts, E. B. Jr. (1999). Inhibition of Salmonella Typhimurium in the chicken intestinal tract by a transformed avirulent avian Escherichia coli. Avian Dis. 43, 245–250. doi: 10.2307/1592614

World Health Organization [WHO]. (2017). Antibiotic Resistance Fact Sheet. Geneva: WHO.

Yamguérov, E., Tsibulskaya, D., Livenskaya, A., Serebyakova, M., Wolf, Y. I., Burkovsk, S., et al. (2020). Histidine-tripeptide hydrolases provide resistance to peptide-nucleotide antibiotics. mBio 11:e00697-20. doi: 10.1128/mBio.00261-07

Yan, K. P., Li, Y., Zirah, S., Gaulard, C., Knappe, T. A., Marshel, M. A., et al. (2012). Dissecting the maturation steps of the lasso peptide microcin J25 in vitro. ChemBiochem 13, 1046–1052. doi: 10.1002/chem.201200016

Yang, C. C., and Konisky, J. (1984). Colicin V-treated Escherichia coli does not generate membrane potential. J. Bacteriol. 158, 757–759. doi: 10.1128/JB.158.2.757-759.1984

Yang, X., and Price, C. W. (1995). Streptolydigin resistance can be conferred by alterations to either the beta or beta’ subunits of Bacillus subtilis RNA polymerase. J. Biol. Chem. 270, 23930–23933. doi: 10.1074/jbc.270.41.23930

Yoneyama, H., and Katsumata, R. (2006). Antibiotic resistance in bacteria and its future for novel antibiotic development. Biosci. Biotechnol. Biochem. 70, 1060–1075. doi: 10.1271/bbb.70.1060

Yorgey, P., Davagnino, J., and Kolter, R. (1993). The maturation pathway of microcin B17, a peptide inhibitor of DNA gyrase. Mol. Microbiol. 9, 897–905. doi: 10.1111/j.1365-2958.1993.tb01747.x

Yu, H., Ding, X., Shang, L., Zeng, X., Liu, H., Li, N., et al. (2018a). Protective ability of biogenic antimicrobial peptide microcin J25 against enterotoxigenic Escherichia coli-induced intestinal epithelial dysfunction and inflammatory responses IPCJ-J2 cells. Front. Cell Infect. Microbiol. 8:242. doi: 10.3389/fcimb.2018.00242

Yu, H., Shang, L., Zeng, X., Li, N., Liu, H., Cai, S., et al. (2018b). Risks related to high-dosage recombinant antimicrobial peptide microcin J25 in mice model, intestinal microbiota, intestinal barrier function, and immune regulation. J. Agric. Food Chem. 66, 11301–11310. doi: 10.1021/acs.jafc.8b03405

Yu, H., Li, N., Zeng, X., Liu, L., Wang, Y., Wang, G., et al. (2019). A comprehensive antimicrobial activity evaluation of the recombinant microcin J25 against the foodborne pathogens Salmonella and E. coli O157:H7 by using a matrix of conditions. Front. Microbiol. 10:5195.4. doi: 10.3389/fmicb.2019.01954

Yu, H., Wang, Y., Zeng, X., Cai, S., Wang, G., Liu, L., et al. (2020). Therapeutic administration of the recombinant antimicrobial peptide microcin J25 effectively enhances host defenses against gut inflammation and epithelial barrier injury induced by enterotoxigenic Escherichia coli infection. FASEB J. 34, 1018–1037. doi: 10.1096/fj.201901717R

Yu, H. T., Ding, X. L., Li, N., Zhang, X. Y., Zeng, X. F., Wang, S., et al. (2017). Dietary supplemented antimicrobial peptide microcin J25 improves the growth performance, apparent total tract digestibility, fecal microbiota, and intestinal barrier function of weaned pigs. J. Anim. Sci. 95, 5064–5076. doi: 10.2527/jas.2017-1494

Yuzenkov, J., Delgado, M., Nechaev, S., Savalia, D., Epstein, V., Artsimovich, L., et al. (2002). Mutations of bacterial RNA polymerase leading to resistance to microcin J25. J. Biol. Chem. 277, 50867–50875. doi: 10.1074/jbc.M209425200
Zhang, A., Rosner, J. L., and Martin, R. G. (2008). Transcriptional activation by MarA, SoxS and Rob of two tolC promoters using one binding site, a complex promoter configuration for tolC in Escherichia coli. Mol. Microbiol. 69, 1450–1455. doi: 10.1111/j.1365-2958.2008.06371.x
Zhao, Z., Eberhart, L. J., Orfe, L. H., Lu, S. Y., Besser, T. E., and Call, D. R. (2015). Genome-wide screening identifies six genes that are associated with susceptibility to Escherichia coli microcin PDI. Appl. Environ. Microbiol. 81, 6953–6963. doi: 10.1128/aem.01704-15
Zowawi, H. M., Harris, P. N., Roberts, M. J., Tambyah, P. A., Schembri, M. A., Pezzani, M. D., et al. (2015). The emerging threat of multidrug-resistant Gram-negative bacteria in urology. Nat. Rev. Urol. 12, 570–584. doi: 10.1038/nrurol.2015.199
Zschüttig, A., Zimmermann, K., Blom, J., Goessmann, A., Pöhlmann, C., and Gunzer, F. (2012). Identification and characterization of microcin S, a new antibacterial peptide produced by probiotic Escherichia coli G3/10. PLoS One 7:e033351. doi: 10.1371/journal.pone.0033351

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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