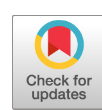
RESEARCH ARTICLE



Evaluating the Potential and Synergetic Effects of Microcins against Multidrug-Resistant *Enterobacteriaceae*

Soufiane Telhig,[a,b] Laila Ben Said,[a] Carmen Torres,[c] Sylvie Rebuffat,[b] Séverine Zirah,[b] Ismail Fliss[a,d]

[a]Food Science Department, Food and Agriculture Faculty, Laval University, Québec City, Québec, Canada

[b]Laboratoire Molécules de Communication et Adaptation des Microorganismes, Muséum National d'Histoire Naturelle, Centre National de la Recherche Scientifique, Paris, France

[c]Department of Food and Agriculture, University of La Rioja, Logrono, Spain

[d]Institute of Nutrition and Functional Foods, Laval University, Québec City, Québec, Canada

ABSTRACT The advent of multidrug-resistant bacteria has hampered the development of new antibiotics, exacerbating their morbidity and mortality. In this context, the gastrointestinal tract reveals a valuable source of novel antimicrobials. Microcins are bacteriocins produced by members of the family *Enterobacteriaceae,* which are endowed with a wide diversity of structures and mechanisms of action, and exert potent antibacterial activity against closely related bacteria. In this study, we investigated the antibacterial activities of four microcins against 54 *Enterobacteriaceae* isolates from three species (*Escherichia coli*, *Klebsiella pneumoniae*, and *Salmonella enterica*). The selected microcins, microcin C (McC, nucleotide peptide), microcin J25 (MccJ25, lasso peptide), microcin B17 (MccB17, linear azol(in)e-containing peptide), and microcin E492 (MccE492, siderophore peptide) carry different post-translational modifications and have distinct mechanisms of action. MICs and minimal bactericidal concentrations (MBC) of the microcins were measured and the efficacy of combinations of the microcins together or with antibiotics was assessed to identify potential synergies. Every isolate showed sensitivity to at least one microcin with MIC values ranging between 0.02 μM and 42.5 μM. Among the microcins tested, McC exhibited the broadest spectrum of inhibition with 46 strains inhibited, closely followed by MccE492 with 38 strains inhibited, while MccJ25 showed the highest activity. In general, microcin activity was observed to be independent of antibiotic resistance profile and strain genus. Of the 42 tested combinations, 20 provided enhanced activity (18 out of 20 being microcin–antibiotic combinations), with two being synergetic.

IMPORTANCE With their wide range of structures and mechanisms of action, microcins are shown to exert antibacterial activities against *Enterobacteriaceae* resistant to antibiotics together with synergies with antibiotics and in particular colistin.

KEYWORDS microcins, bacteriocins, multidrug resistance, antimicrobial activity, RiPPs, synergy

The overuse and misuse of antibiotics in animal and human health generated the emergence of resistance and its spread (1), which are responsible for the antibiotic resistance crisis (2). Recent findings show that human and livestock microbiota have become reservoirs of antimicrobial resistance (AMR) markers (3–5) that can be disseminated to the environment through wastewater treatment or manure (6). This issue is exacerbated by the lack of development of novel antibiotics (7–9). In 2017 the World Health Organization (WHO) warned that "the world is running out of antibiotics" (10). Among the emergent multidrug-resistant (MDR) bacteria, *Enterobacteriaceae* are particularly problematic, with MDR enteropathogens such as *Escherichia coli*, *Klebsiella pneumoniae*, and *Salmonella* strains causing ever frequent outbreaks (11–14). Due to their double membrane and multiple efflux systems (15, 16), *Enterobacteriaceae* infections

Editor Xiaoyu Tang, Shenzhen Bay Laboratory

Copyright © 2022 Telhig et al. This is an open-access article distributed under the terms of the Creative Commons Attribution 4.0 International license.

Address correspondence to Ismail Fliss, ismail.fliss@fsaa.ulaval.ca, or Séverine Zirah, severine.zirah@mnhn.fr.

The authors declare no conflict of interest.

Received 11 January 2022
Accepted 12 April 2022
Published 11 May 2022

TABLE 1 Microcins used in this study

Microcin	Producer	mol wt (Da)	Type of RiPPs	Reference
McC	*E. coli*	1,177	Nucleotide peptide	72
MccJ25	*E. coli*	2,107	Lasso peptide	39
MccB17	*E. coli*	3,093	Linear azol(in)e-containing peptide	41
MccE492	*K. pneumoniae*	8,781	Siderophore peptide	44

are notoriously difficult to treat, highlighting their importance as a target for new drug developments. Faced with these challenges, new treatments with reduced risks of resistance emergence and dissemination are needed.

The gastrointestinal tract, which is the seat for multiple microbial interactions, constitutes a valuable source of novel antimicrobials (17, 18), and in particular of bacteriocins, which are antimicrobial peptides produced by bacteria through the ribosomal pathway. Microcins are low molecular weight bacteriocins produced by members of the family *Enterobacteriaceae* (19–21). More specifically, they are peptides below 10 kDa that exhibit potent antimicrobial activities directed against bacteria closely related to the producing strains. Microcins are particularly diverse, many of them being endowed with complex post-translational modifications, making them representatives of ribosomally synthesized and post-translationally modified peptides (RiPPs) (22, 23). Many bacterial species indigenous to livestock and human microbiota produce microcins (20, 24, 25). Furthermore, commercially available probiotics isolated from animal microbiota exhibit the presence of microcin-producing strains (26, 27). Being indigenous to the gut microbiota and exhibiting a narrow spectrum of antibacterial activity, microcins constitute an attractive alternative to antibiotics. While antibiotics can cause significant changes to microbiota compositions and are most often linked to dysbiosis and related disorders (28–31) and can favor AMR dissemination (32), microcins are expected to have a limited impact on the gut microbiota composition and induce a reduced dissemination of resistance, should it emerge. Indeed, other bacteriocins, such as nisin and pediocin PA-1, which both exhibit a narrow spectrum of activity in comparison with antibiotics, have been shown to induce significantly lower changes to the host microbiota (33–36). Given the narrow spectrum of activity of microcins that can vary within the same genus or species, a comprehensive study of their spectra of activity is required to determine which microcin is more appropriate against a given pathogen. Faced with the AMR problem, drug combinations offer a promising reprieve, whereby combining multiple drugs should increase the energetic costs of resistance development (37). As of yet there are no studies exploring the interactions between microcins or between microcins and antibiotics.

In this study, we explored for the first time the potential of four microcins with specific structures and mechanisms of action, namely, microcins C (McC), J25 (MccJ25), B17 (MccB17), and E492 (MccE492) (Table 1, Fig. 1) (19, 21) to kill, inhibit, or displace a given pathogen using a collection of *Enterobacteriaceae* resistant to antibiotics. *Enterobacteriaceae* from three species, *E. coli*, *K. pneumoniae*, and *S. enterica*, isolated from different origins and exhibiting resistance against different antibiotics were selected for this purpose. The microcins were tested alone or in pairwise microcin-microcin or microcin-antibiotic combinations.

RESULTS

Four microcins, McC, MccJ25, MccB17, and MccE492, belonging to the RiPP family were selected for their potent and narrow spectrum of activity directed against enteropathogens, their stability to harsh conditions, and their diversity of structures and mechanisms of action (Fig. 1). More precisely, McC is a 7-amino acid nucleotide peptide that is cleaved in susceptible cells to release an aspartyl adenylate mimic that targets aspartyl-tRNA synthetase (38). MccJ25 is a 21-amino acid lasso peptide with an

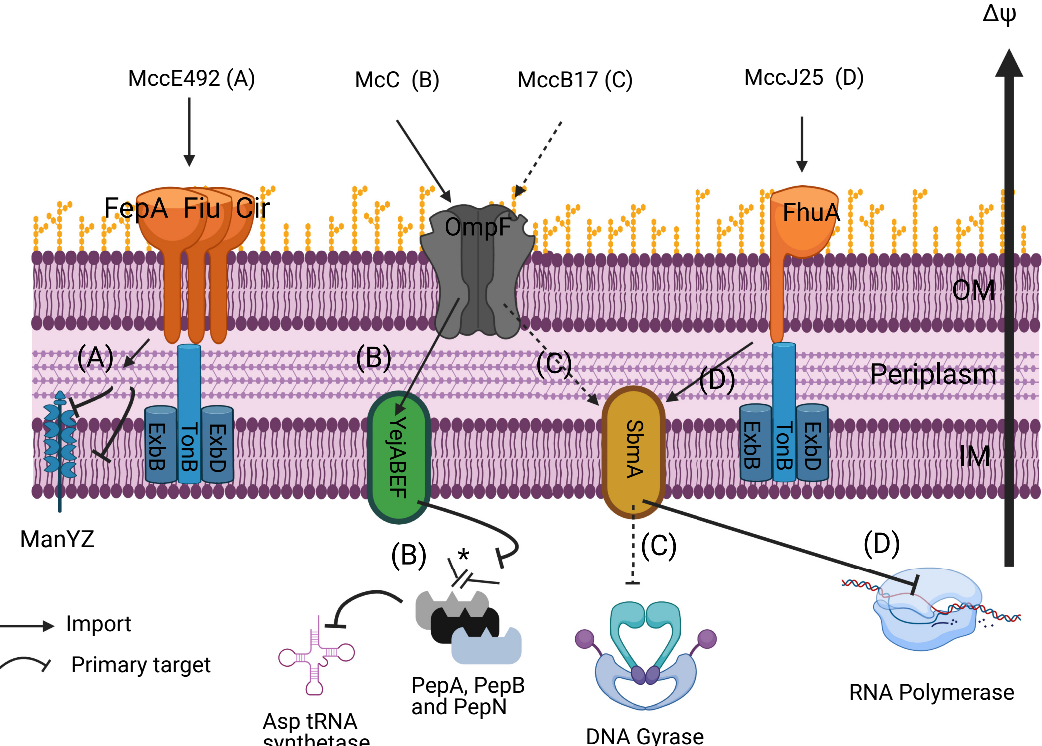


FIG 1 Mechanisms of action of McC, MccJ25, MccB17, and MccE492. MccE492 (A) and MccJ25 (D) gain entry to the periplasm through siderophore receptors FepA/Fiu/Cir (A) or FhuA (D) coupled to the TonB-ExbB-ExbD complex for translocation. McC (B) and MccB17 (C) enter the periplasm through the porin OmpF. McC gains access to the cytoplasm through YejABEF, whereas MccB17 and MccJ25 use SbmA. When in the cytoplasm, MccJ25, MccB17, and McC target RNA polymerase, DNA gyrase, and aspartyl tRNA synthetase, respectively, to kill the cells. For MccE492, it does not enter the cytoplasm but inserts itself into the inner-membrane by stably associating with ManYZ and inducing depolarization of the inner membrane and perturbation of the mannose transport. *, Inside bacteria McC is processed by a deformylase and the peptidases A, B, and N, resulting in the formation of a non-hydrolysable Asp-tRNA mime, thus blocking translation. Figure made with Biorender.

N-terminal macrolactam ring threaded by the C-terminal tail (39), which is imported in susceptible cells through interaction with the membrane proteins FhuA and SbmA and targets RNA polymerase (40). MccB17 belongs to the linear azol(in)e-containing peptide (LAP) family. It contains 43 amino acids, nine of which are converted to thiazole or oxazole rings (41, 42). It is imported in susceptible cells through interaction with the membrane proteins OmpF and SbmA and targets DNA gyrase (43). Finally, MccE492 is a 84-amino acid siderophore-peptide where the C-terminal serine carboxylate is linked to a glycosylated enterobactin derivative (44). It both perturbs the inner membrane permeability and targets the mannose permease (45).

Production and purification of microcins. The four microcins were produced heterologously in *E. coli* and their purification was bio-guided using agar diffusion assays against two reference indicator strains (Table S4). MccJ25, McC, MccB17, and MccE492 were purified at yields of 3.5 mg/L, 9.1 mg/L, 1 mg/L, and 4.0 mg/L of culture, respectively (Table S5).

Spectrum of activity of the microcins. The four purified microcins were tested against 54 pathogenic enterobacteria from three species, *E. coli*, *K. pneumoniae*, and *S. enterica*, isolated from different hosts and/or environments: human and animal, animal food, farm indoor air, and wastewater treatment plant (Tables S1-S3). Most of these strains are MDR and their resistance profiles cover a wide panel of antibiotics differing both in structures and mechanisms of action. Out of all the isolates, only three did not exhibit any antibiotic resistance and the rest showed resistances from one to 11 different antibiotics.

The four microcins exhibited heterogeneous spectra of activities, as illustrated by agar diffusion assays (Fig. 2) and MIC measurements (Fig. 3A, Tables S7 to S9). Every

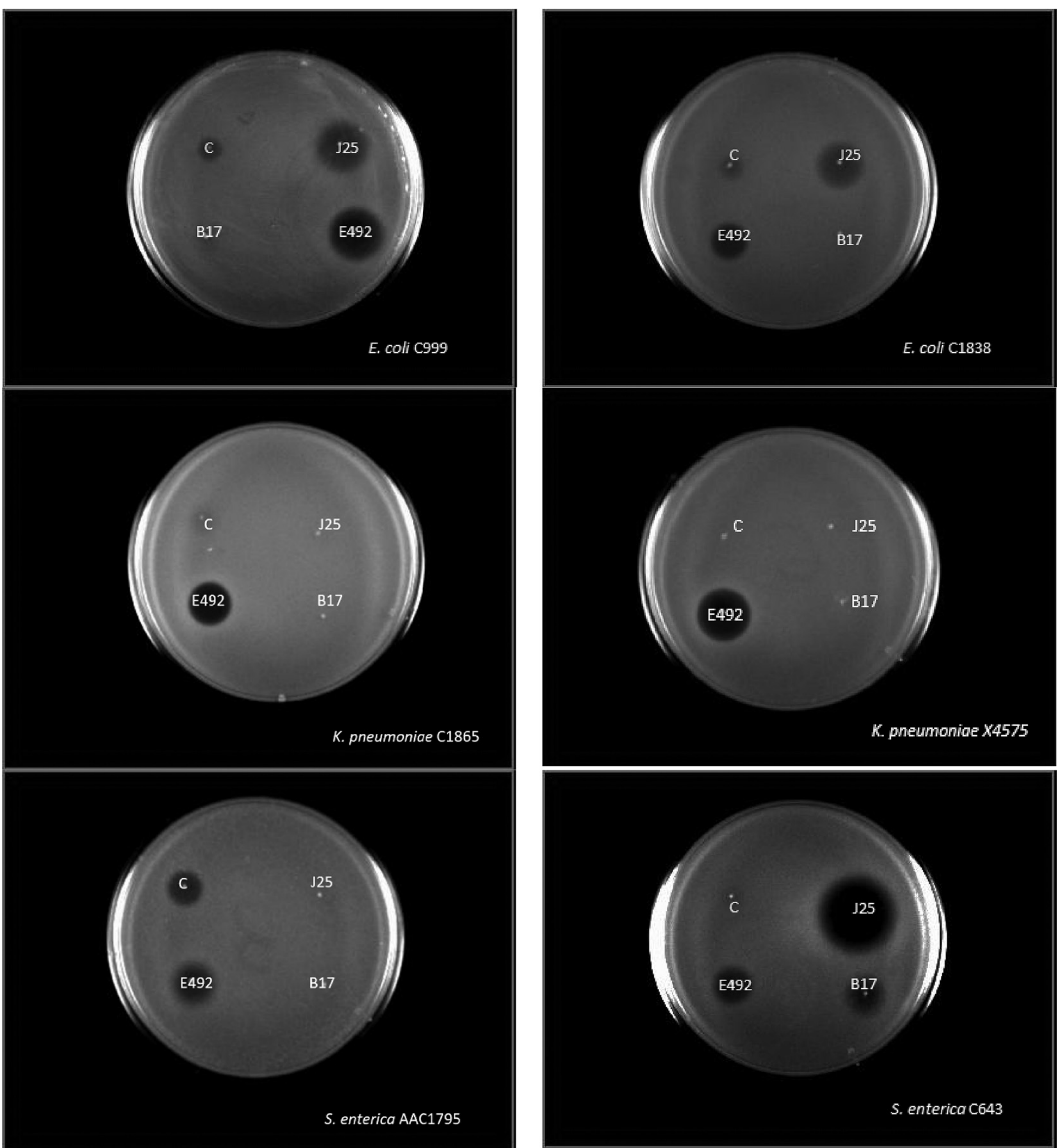


FIG 2 Agar diffusion assays for McC, MccJ25, MccB17, and MccE492 against *E. coli*, *K. pneumoniae*, and *S. enterica* strains. Microcins were deposited at the concentration of 100 μg/mL.

isolate showed susceptibility to at least one microcin with MIC values ranging between 0.02 μM and 42.5 μM. The dendrogram constructed from the susceptibility profiles did not reveal a clear clustering per bacterial species. Nevertheless, general trends were observed. The lowest susceptibility corresponded to inhibition by only one microcin, a trend mostly observed for *K. pneumoniae*, which revealed almost non-susceptible to MccJ25 and MccB17, with the exception of strain *K. pneumoniae* C4750, susceptible to all four microcins (Table S8). By contrast, *E. coli* presented both the lowest number of strains susceptible to only one microcin and the highest number of strains susceptible to all four microcins. The number of strains susceptible to each microcin is shown in Fig. 3B. McC revealed the widest spectrum of activity, with 46 strains (85.2%) inhibited within the range of concentrations tested. It was followed by MccE492, which was active against 38 strains (70.4%), then MccB17 with 23 strains (42.6%) and MccJ25 with 19 strains (35.2%). Despite exhibiting the narrowest spectrum of inhibition, MccJ25 presented the lowest recorded MIC (0.02 μM), and thus, the highest efficacy. We assessed the type of inhibition by calculating the ratio R between the MIC and MBC (Fig. 3C). When $R > 4$, the activity of a given antimicrobial compound is considered

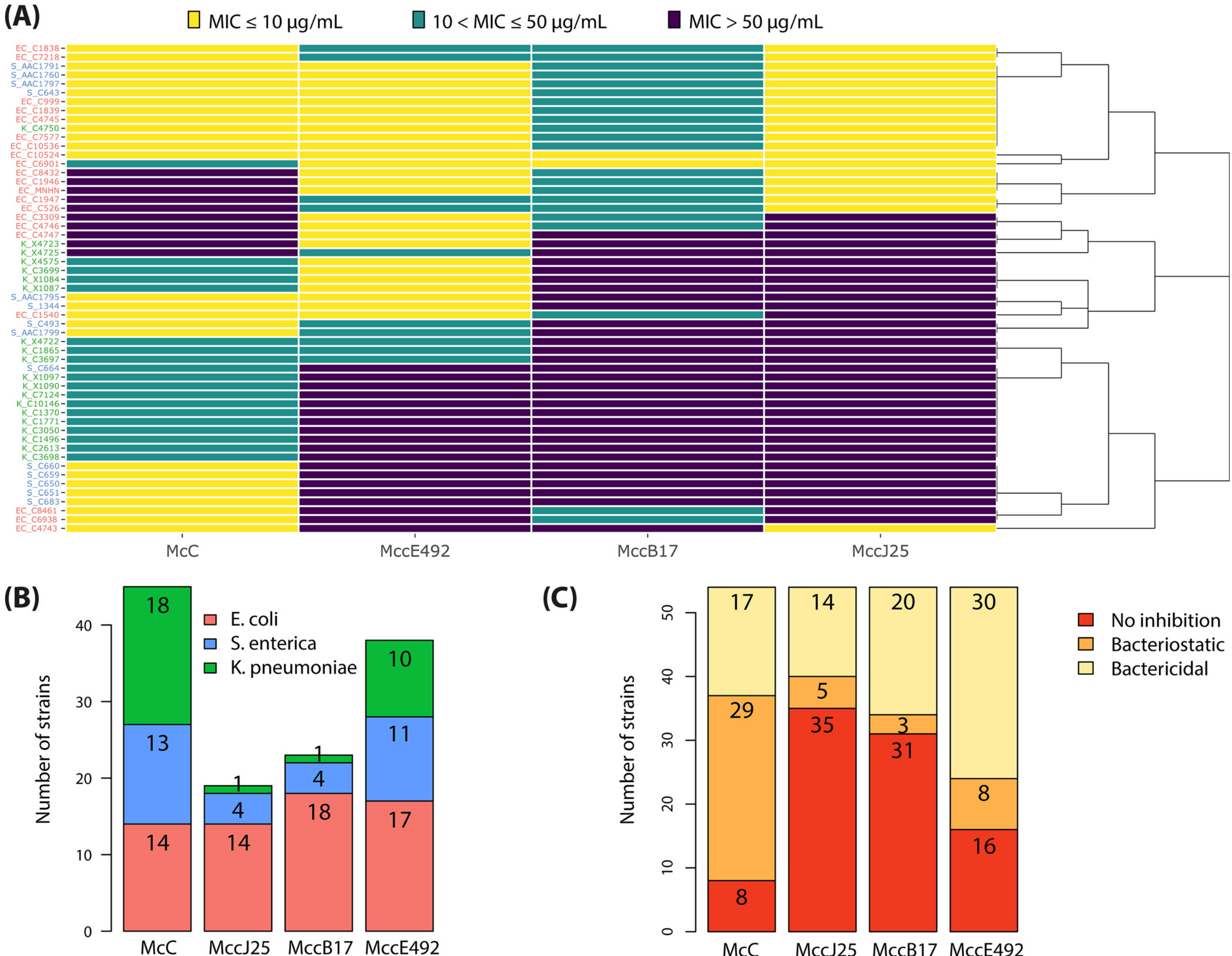


FIG 3 Efficacy of microcins against *Enterobacteriaceae* isolates. McC, MccJ25, MccB17, and MccE492 were tested against a collection of 54 *Enterobacteriaceae* isolates. (A) Heatmap representing the MIC values (in μg/mL). High, medium, and low sensitivity correspond to MIC $\leq$ 10, 10 $<$ MIC $\leq$ 50, and MIC $>$ 50 μg/mL, respectively. The corresponding values in μM are provided in Tables S6 to S8. Strains are noted EC for *E. coli*, K for *K. pneumoniae*, and S for *S. enterica*. (B) Susceptibility to microcins per bacterial species. (C) Inhibition type observed per microcin.

bacteriostatic, while it is considered bactericidal for $R \leq 4$ (46). Microcin inhibition varies between bacteriostatic and bactericidal depending on the tested strains. MccJ25, MccB17, and MccE492 appear mainly bactericidal while McC, which displays the widest spectrum of activity, appears mainly bacteriostatic.

To assess the relationships between microcin susceptibility and antibiotic resistance profiles, we performed multifactorial analysis (MFA) based on categorized susceptibility to microcins and antibiotics (Fig. 4). MFA revealed a clustering for *K. pneumoniae* (except for isolate C4750) and *E. coli*, while *S. enterica* showed a more heterogeneous distribution (Fig. 4A). The strains that showed most susceptible to the microcins were projected on the bottom-right panel, while the strains less susceptible to microcins (weakly susceptible to MccC), and especially all *K. pneumoniae*, clustered on the upper left panel. Representation of the microcin and antibiotic susceptibility categories in the first two components of the multicomponent analysis (MCA) (Fig. 4B) suggested several associations between resistances to a specific microcin and a specific antibiotic (namely, gentamicin/MccJ25, tobramycin/MccB17, and amoxicillin-clavulanic acid/McC). The MFA analysis shows that resistance phenotypes toward MccJ25, gentamicin, and tobramycin pointed in the same direction. However, only the relationship between gentamicin and MccJ25 resistances was confirmed by Chi-square test for independence.

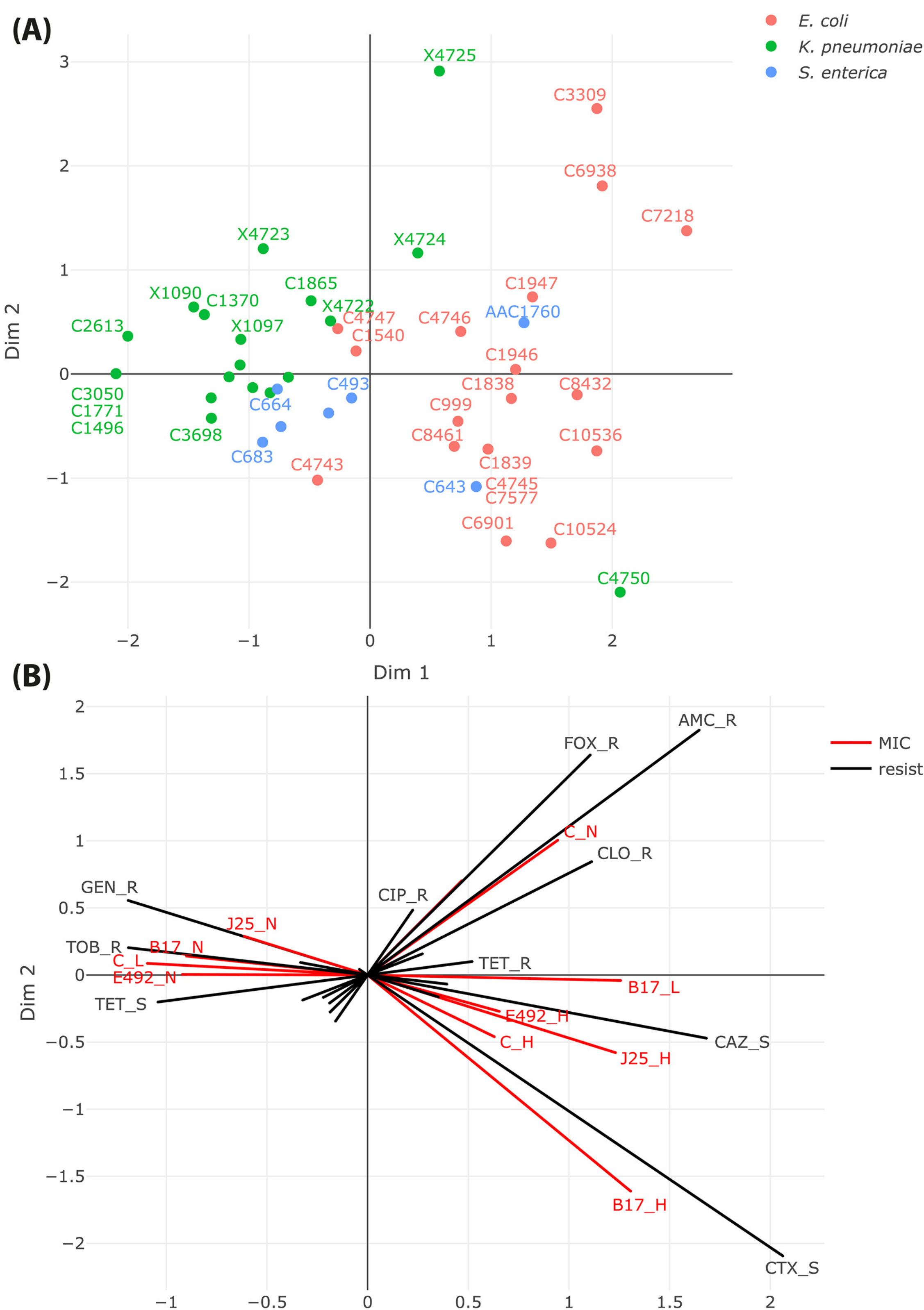


FIG 4 MFA correlations identified between microcin and antibiotic susceptibilities. (A) Representation of the strains in the first two dimensions, colored per species. (B) Representation of the susceptibility categories in the first two dimensions. In red: susceptibility to microcins (H, high, MIC $\leq$ 10 μg/mL, L, low, 10 $<$ MIC $\leq$ 50 μg/mL, N, no activity up to 50 μg/mL); in black: susceptibility to antibiotics (R, resistant, S, susceptible).

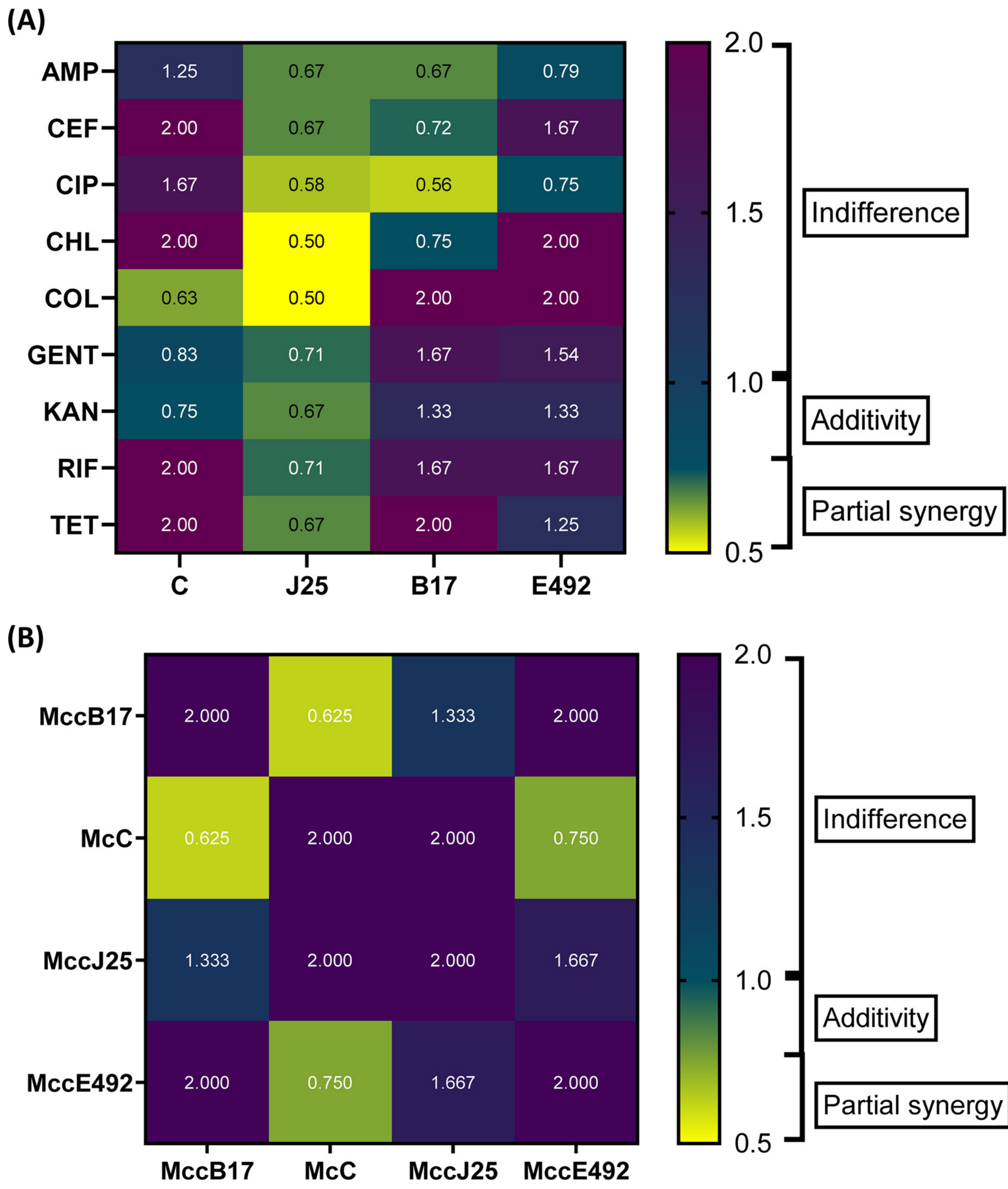


FIG 5 FIC values measured for all consortia tested in the study against *E. coli* and *Salmonella* indicator strains. (A) shows the FIC values for the antibiotic microcin-consortia. (B) shows the FIC values for the microcin-microcin consortia. FIC ≤ 0.5: synergy, 0.5 < FIC ≤ 0.75: partial synergy, 0.75 < FIC < 1: additivity and 1 ≤ FIC ≤ 2 = indifference.

Microcin-microcin and microcin-antibiotic combinations. We further looked at the possibility of combining microcins in different consortia either with each other or with different antibiotics presenting diverse mechanisms of action. The MICs of the antibiotics and microcins alone against the indicator strains were first determined (Table S6). Then, 42 consortia were tested and the fractional inhibitory concentration (FIC) index, indicative of the combination effect (47), was determined (Fig. 5). Out of all the tested combinations, 22 were observed to be indifferent (52.4%), two were additive (4.8%), 16 were partially synergetic (38.1%), and two were synergetic (4.8%) (Fig. 6). Interestingly, no antagonistic effect was detected.

All the combinations between MccJ25 and antibiotics exhibited interactions within the partial synergy range, whereas McC combined with antibiotics produced two interactions within the partial synergy range with colistin (FIC = 0.63) and kanamycin

TABLE 2 Most potent synergetic interactions[a]

Microcin	MICc/MIC	Antibiotic	MICc/MIC	FIC index
MccJ25	1/4	Chloramphenicol	1/4	0.50
MccJ25	1/4	Colistin	1/4	0.50
MccJ25	1/2	Ciprofloxacin	1/16 to 1/32	0.58
MccB17	1/2	Ciprofloxacin	1/16	0.56
McC	1/2	Colistin	1/16	0.63

[a]MICc, MIC of the compound in the combination; MIC, MIC of the compound alone. MICc/MIC is the ratio between the resulting MIC of an antimicrobial agent within the consortium and the MIC of the same antimicrobial by itself.

(FIC = 0.75) (Fig. 5). The interactions between microcins and antibiotics were much more synergetic than pairwise microcin-microcin interactions. Out of the 20 positive interactions (FIC $<$ 1), pairwise microcin-microcin combinations only accounted for two (10%) with MccB17/McC and MccE492/McC. Indeed, the only synergetic effects were observed for MccJ25/colistin and MccJ25/chloramphenicol combinations. MccB17 accounted for five partially synergetic in total. McC exhibited three partially synergetic and one additive effects in total, while MccE492 showed one additive and two partially synergetic interactions (Fig. 5). The most potent beneficial interactions between microcins and antibiotics are listed in Table 2.

DISCUSSION

The objective of this study was to evaluate the potential of microcins as a treatment against *Enterobacteriaceae* notorious for their capacity to develop resistances to conventional antibiotics (15, 16) and or persist within the host (48, 49). Despite reviews discussing the prospects of using microcins as novel therapeutics, due to their high specificity, potent antibacterial activity and reduced collateral damage to the host's microbiome (19, 21, 22, 50), there have been few comprehensive studies on this point.

Hence, we first investigated whether four microcins (McC, MccJ25, MccB17, and MccE492) can exert bacterial inhibition against a variety of *Enterobacteriaceae*, both MDR and non-MDR. In total, 54 natural isolates were tested, belonging to the species *E. coli*, *K. pneumoniae*, and *S. enterica*, all considered urgent and/or serious threats by the Centre for Disease Control and Prevention (CDC). None of the studied *Enterobacteriaceae* were non susceptible to all microcins within the range of tested concentration (up to 50 μg/mL). However, there was a high variability in the effectiveness of the different microcins to inhibit the growth of bacteria. The lowest recorded MIC values were 0.03 μM, 0.1 μM, 1 μM and 2 μM for MccJ25, MccE492, McC, and MccB17, respectively. Concomitantly, McC recorded the widest spectrum out of all studied microcins, with 85.2% of strains inhibited within the range of concentrations used, followed by MccE492 with 70.4% of strains inhibited. According to the data, the most efficient microcins were MccE492 and MccJ25, albeit with the narrowest spectrum for MccJ25 (35.2%). In the case of MccE492, these observations are in line with previous studies, whereby siderophore microcins including MccE492 were found to confer a significant fitness advantage (26). Moreover, D'Onofrio et al. (51) have shown that adding exogenous siderophores to synthetic media can promote the growth of previously uncultured bacteria, via iron acquisition. Their importance is increased in the context of a host, where iron availability is reduced, suggesting MccE492, and siderophore microcins by extension, are promising therapeutics.

Looking at the type of inhibition effectuated by microcins it was observed to be variable throughout the different strains, changing between bacteriostatic and bactericidal. Indeed, the highest variation in the type of inhibition activity is observed for McC and MccJ25. Both microcins were recorded to have two different mechanisms of action, with one acting in the cytoplasm and the second at the level of the inner membrane but at much higher concentrations, close to mM (52–55). Although the two mechanisms are assumed to be independent, in reference to the concept that has been proposed for conventional antibiotics (56), it could also be considered that for both microcins the main mechanism of action could result, at least for a part, in

deleterious changes in the inner membrane, leading consecutively to a bactericidal effect. Both alternatives could explain the variety observed within their activity. Moreover, it cannot be discarded neither that reduction of microcin uptake at the outer membrane, essentially via the involved receptor or porin, reduces the microcin concentration in the cytoplasm, which thus cannot reach the level required for a bactericidal activity. Furthermore, the activities of the tested microcins seemed independent of the species and antibiotic resistance profiles of the pathogens. Given that the receptors and targets of these microcins are well characterized, a comprehensive genomic study is needed to shed more light on any potential association between susceptibility to a given microcin and the antibiotic resistance profile or the species of a given strain.

Comparing microcin susceptibilities and antibiotic resistance profiles of the strains revealed a correlation between MccJ25 and gentamicin resistances. Although the mechanisms of action of these two pairs of antimicrobial molecules are different, this result suggests that cross-resistance may occur between microcins and conventional antibiotics. It must be noted that the resistance to antibiotics of the collection of *Enterobacteriaceae* was measured phenotypically. Furthermore, despite the detection of antibiotic resistance genes coding for acquired resistance enzymes, it remains unclear whether these resistances are due to acquired AMR genes or if mutational events on the targets of the antibiotics could also be implicated. Hence, concerning the statistical relevance of the correlation between gentamicin and MccJ25 resistance, an in-depth study of the mechanisms of aminoglycoside resistance should be performed in these isolates, mostly those related to mutations in the antibiotic target, to detect potential microcin-antibiotic resistance interactions. Moreover, a larger collection of gentamicin-susceptible and gentamicin-resistant isolates could be analyzed in the future.

Secondly, we investigated whether it was possible to enhance microcin activity by combining them between each other or with a collection of antibiotics. This is a standard method to circumvent antibiotic resistance and in keeping with the comprehensive aim of this study, we chose antibiotics covering a wide range of mechanisms (membrane degradation, inhibition of cell wall biosynthesis or of protein synthesis by targeting the 30S and 50S ribosome, etc.). There were no observations of any antagonistic effects, yet no observation of a highly significant synergy (FIC < 0.5). The most promising consortia were observed between microcins and antibiotics. MccJ25 presented the most enhanced interactions, with the best FIC indexes measured for combinations with chloramphenicol and colistin. Colistin interacts with the bacterial cytoplasmic membrane changing its permeability (57), which could explain its ability to enhance MccJ25 activity, presumably by increasing the microcin uptake, which would then rely on both import through FhuA and SbmA and membrane permeabilization. At high concentrations, MccJ25 has also been observed to cause membrane perturbations and disruption of the cytoplasmic membrane gradient (55), which could also contribute to the synergy between colistin and this microcin. MccJ25 and chloramphenicol both inhibit protein synthesis but using different mechanisms, i.e., by blocking transcription through binding to RNA polymerase for MccJ25 (54) and by targeting translation for chloramphenicol, which reversibly binds to the 50S ribosomal L16 protein (58). These combined effects could explain the synergetic effect between MccJ25 and chloramphenicol. Tetracycline, kanamycin, and gentamicin, all 30S inhibitors (59, 60), present a lower FIC index when combined to MccJ25. This suggests that combinations of MccJ25 with 50S inhibitors are more beneficial than with 30S inhibitors.

Given that there is a finite number of entry pathways into a target cell, there was overlap in the mechanisms of the tested compounds. For instance, MccJ25 and rifampicin share the FhuA and SbmA receptors they use for uptake, and RNA polymerase as their cytosolic target, yet no antagonism was observed between them. According to Mathavan et al. (61), MccJ25 occupies a location within FhuA similar to that of ferrichrome, its natural ligand. Moreover, ferrichrome and the antibiotics rifampicin and albomycin were also shown to occupy similar sites within FhuA (62). Furthermore, while MccJ25 targets the β' RNA polymerase sub-unit (63), rifampicin targets the β sub-unit (64). These different mechanisms at the level of the RNA polymerase interaction coupled with the lack of tight

structural specificity of FhuA for its ligands could explain the lack of antagonism between MccJ25 and rifampicin. On the other hand, SbmA also presents the ability to accommodate and transport various substrates including antimicrobial peptides such as MccB17 (65) and proline-rich AMPs (66). Indeed, the use of SbmA is shared by both MccB17 and ciprofloxacin on one hand and MccJ25 and ciprofloxacin on the other. Both consortia recorded similarly partially synergetic effects at 0.56 and 0.59, respectively. The lack of antagonism despite sharing SbmA as a receptor has also been recorded for ciprofloxacin and rifampicin (67, 68), suggesting that both SbmA and FhuA structural specificity is not a limiting factor when designing drug combinations. It could be hypothesized that the use of these consortia, would increase the potential of cross-resistance emergence. Nevertheless, combining ciprofloxacin and rifampicin was shown to reduce the frequency of resistance emergence in comparison with ciprofloxacin alone, which was attributed to rifampicin killing any ciprofloxacin resistant subpopulation (67).

No significant enhancing effects were recorded by combining MccE492 with antibiotics. MccE492 activity relies on simultaneously disrupting mannose transport and inner membrane pore formation through binding to the mannose permease (45). This suggests that microcins with a cytoplasmic target are more prone to exert synergic activities with antibiotics.

Combining two by two, the microcins did not reveal much synergetic effects, with the exception of McC and MccB17. This could be due to the narrow spectrum of activity of microcins. It must be noted that our results show different spectra of activity of the microcins, coupled with the lack of antagonism when combining different microcins. It could thus be beneficial to use microcin consortia, not necessarily for synergetic effects, but to cover a wider spectrum of pathogens. It must be noted, however, that the frequency of natural resistance emergence to microcins is still poorly studied. Yet, both the lack of antagonism and enhancing effects between microcin and antibiotics shown in this study appear promising, especially in the case of colistin, due to the toxicity of the latter.

To summarize, the microcins tested were effective against the collection of *Enterobacteriaceae* isolates within the range of concentrations tested. McC exhibited the widest range of activity, whereas MccJ25 accounted for the lowest MIC values. Furthermore, both McC and MccJ25 activities presented the highest variation in type of inhibition. Finally, all the tested combinations exhibited significantly varying FIC indexes, with microcin-antibiotics combination having the most synergetic effects observed.

MATERIALS AND METHODS

Bacterial strains and plasmids. The *E. coli*, *K. pneumoniae*, and *S. enterica* isolates along with their serotypes and resistance profiles to antibiotics are listed in Tables S1, S2, and S3, respectively. These strains were obtained from the collection of the University of La Rioja (Logroño, Spain) and from Agriculture Canada's pathogen collection and their phenotypes and genotypes of resistance were known from previous studies. The *E. coli* strains used for heterologous production of microcins together with their microcin-encoding plasmids, and the indicator strains used for susceptibility assays are listed in Table S4. Two reference indicator strains were also used, *E. coli* ATCC 25922 and *S. enterica* subsp. *enterica* serovar Newport ATCC 6962 (later termed *S.* Newport ATCC 6962).

Production and purification of microcins. The microcin producing strains were cultured in LB medium or in M63 minimal medium (for 1 L, 3 g KH_2PO_4, 7 g K_2HPO_4, 2 g $(NH_4)_2SO_4$, 1 g Casamino Acids) supplemented with glucose (2 g/L), thiamine (1 mg/L), and $MgSO_4$ (0.2 g/L). Ampicillin (Amp) or chloramphenicol (Chl) were added as selection factor when appropriate, at 50 μg/mL and 34 μg/mL, respectively. For all microcins, an overnight culture of the producing *E. coli* strain was grown at 37°C and 200 rpm and used at 1% to inoculate 500 mL of supplemented M63 medium, for an overnight culture in 2 L erlemneyers at 37°C and 200 rpm. The cultures were centrifuged at 12,000 rpm and 4°C for 20 min. For McC, MccJ25, and MccE492, the culture supernatants were collected. For MccB17, the pellet was collected and suspended in 25 mL acetic acid 100 mM, EDTA 1 mM, and heated at 100°C under shaking at 80 rpm. The resulting suspension was then centrifuged at 4,250 *g* and 4°C for 20 min for collection of the clear supernatant. The culture supernatants of strains producing McC, MccE492, and MccJ25 and pellet extract of strain producing MccB17 were treated by solid phase extraction on a Sep-Pak C18 35 cc (Waters) for MccB17, McC, and MccJ25, or on a Sep-Pak C8 35 cc (Waters) for MccE492. In all cases, the cartridges were conditioned with methanol, acetonitrile (ACN), and 0.1% trifluoroacetic acid (TFA) (A1, for McC, MccB17 and MccJ25) or formic acid (FA) in milliQ water (A2, for MccE492), successively. After loading the supernatants, the cartridges were washed with A1 or A2 and then eluted with A1 or A2 together with increasing the amount of ACN. McC, MccB17, MccJ25, and MccE492 were eluted with 10%, 25%, 30%, and 40% ACN, respectively. The modified form of MccE492, in which the C-terminal Ser

residue is connected to three N-(2,3-dihydroxybenzoyl) units through a β-D-glucose moiety (44), was purified. The SPE fractions were then concentrated and submitted to reverse phase high performance liquid chromatography (RP-HPLC), using mobile phase A1 (for McC, MccB17 and MccJ25) or A2 (for MccE492) together with ACN. McC, MccB17, and MccJ25 were purified from SPE fractions on a Mandel Shimadzu 2D HPLC system, using a C18 Phenomenex column (Luna 10 μm, 250 mm $\times$ 21.10 mm) at 6 mL/min, and a gradient from 0% to 50% ACN in 20 min, and to 100% ACN in 10 min. MccE492 was purified on a biocompatible RSLC HPG-3400RS chromatographic system (Thermo Fisher Scientific), on a Luna C18(2), 250 $\times$ 4.6 mm, 100 Å, 5 μm column (Phenomenex) at 1 mL/min, using a gradient from 32% to 42% ACN in 22 min, and to 100% ACN in 1 min.

Peptide purification was monitored upon testing the antibacterial activity of the collected fractions against two reference indicator strains (Table S4). Purity of the collected microcins was checked by analytical HPLC and LC-MS (Fig. S1) and determined as $\geq$ 95%. Quantification of the microcins was obtained using BCA and Lowry assays.

Liquid chromatography–mass spectrometry. The purified peptides were analyzed by liquid chromatography–mass spectrometry (LC-MS) on a high-resolution electrospray—quadrupole—time of flight (ESI-Q-TOF) instrument, using either a 1290 Infinity II UPLC (chromatography system connected to a hybrid ion mobility Q-TOF instrument (6560, Agilent), for MccJ25, McC and MccB17, or an Ultimate 3000-RSLC system (Thermo Fisher Scientific) connected to a Maxis II ETD ESI-Q-TOF instrument (Bruker Daltonics), for MccE492. For the former LC-MS system, the separation was achieved on a Poroshell 120 EC-C18 column (2.1 $\times$ 100 mm, 2.7 μm, Agilent) at a flow rate of 400 μL/min, using an A2/ACN gradient from 10% to 100% ACN over 15 min. For the latter system, the separation was achieved on a Polar Advantage II Acclaim column (2.2 μm, 120 Å, 2.1 $\times$ 100 mm, Thermo Fisher Scientific) at a flow rate of 300 μL/min, using an A2/ACN gradient from 10% to 100% ACN over 15 min. The MS detection was performed in positive mode.

Antibacterial assays. (i) Agar diffusion assays. The tested strains were cultured overnight in LB medium at 37°C and 200 rpm before being inoculated at 1% into soft agar LB medium (0.75%). Wells were dug out and 80 μL of microcin were added in each well and the plates were incubated at 37°C overnight.

(ii) Measurement of minimal inhibitory and bactericidal concentrations (MIC and MBC). MIC determination was carried out using the broth microdilution assay in 96-well plates and following the Clinical and Laboratory Standards Institute (CLSI) guidelines. Two-fold serial dilutions of antibiotic were obtained starting from stock solutions at 100 μg/mL. Plates were incubated at 37°C, and growth was measured as absorbance at 600 nm over a period of 18 h. The MIC was determined as the lowest concentration that completely inhibited the bacterial growth. The MBC was determined by inoculating a MH agar surface with 10 μL from wells showing complete inhibition and incubating for 24 h at 37°C.

Antibiotic stock solutions were prepared following the CLSI guidelines and aliquoted in MilliQ water for –20°C storage. Microcins were aliquoted in MilliQ water at 200 μg/mL and stored at –20°C.

(iii) Measurement of FIC indexes. Interdependent effects analysis for all the tested combinations was performed in triplicates against the two indicator strains *E. coli* ATCC 25922 and *S.* Newport ATCC 6962 (Tables S4 to S6), using the microdilution checkerboard method following the CLSI guidelines. The FIC index was interpreted as follows (47): synergetic effect FIC $\leq$ 0.5, partial synergy 0.5 $<$ FIC $\leq$ 0.75, additivity 0.75 $<$ FIC $<$ 1, neutral 1 $\leq$ FIC $\leq$ 4, and antagonism FIC $>$ 4. For the MccJ25, MccB17, and MccE492 combinations with antibiotics, *E. coli* ATCC 25922 was used as indicator strain. For McC and antibiotics combinations, *S.* Newport ATCC 6962 was used. Concerning the pairwise microcin-microcin combinations, *S.* Newport ATCC 6962 strain was used for McC/MccJ25 and McC/MccE492 while *E. coli* ATCC 25922 was used for the other combinations.

Statistical analysis. All statistical analyses were performed in R software version 4.1.1. Chi-square test for independence was used to test for independence between antibiotic and microcin susceptibility. Multivariate factorial analysis (MFA) was performed using FactoMineR (69) and graphical representations were constructed using factoextra (70) and plotly (71) packages. Two groups of variables were considered: (i) microcin susceptibility, categorized into high (MIC $\leq$ 10 μg/mL), low (10 $<$ MIC $\leq$ 50 μg/mL) and no (MIC $>$ 50 μg/mL) activity and (ii) antibiotic susceptibility, categorized into sensible (S) and resistant (R). The bacterial species was considered as supplementary variable.

SUPPLEMENTAL MATERIAL

Supplemental material is available online only.

SUPPLEMENTAL FILE 1, PDF file, 1.2 MB.

ACNOWLEDGMENTS

We acknowledge financial support received for this work from the National Science and Engineering Research Council of Canada (NSERC) and the International Development research Center (IDRC). The mass spectra were acquired at the « Plateau technique de spectrométrie de masse bio-organique », UMR 7245 Molécules de Communication et d'Adaptation des Microorganismes, Muséum National d'Histoire Naturelle (MNHN), Paris, France and at the « Laboratoire de Transformation Alimentaire et Procédés Electromembranaires » (LTA) from Laval University, Québec, Canada. We thank Arul Marie and Rémy Puppo at the MNHN and Laurent Bazinet and Jacinthe Thibodeau at LTA for access to the instruments.

REFERENCES

1. Bengtsson-Palme J, Kristiansson E, Larsson DGJ. 2018. Environmental factors influencing the development and spread of antibiotic resistance. FEMS Microbiol Rev 42:fux053.
2. Ventola CL. 2015. The antibiotic resistance crisis: part 1: causes and threats. P T 40:277–283.
3. Hu Y, Yang X, Qin J, Lu N, Cheng G, Wu N, Pan Y, Li J, Zhu L, Wang X, Meng Z, Zhao F, Liu D, Ma J, Qin N, Xiang C, Xiao Y, Li L, Yang H, Wang J, Yang R, Gao GF, Wang J, Zhu B. 2013. Metagenome-wide analysis of antibiotic resistance genes in a large cohort of human gut microbiota. Nat Commun 4:2151. https://doi.org/10.1038/ncomms3151.
4. Brown EEF, Cooper A, Carrillo C, Blais B. 2019. Selection of multidrug-resistant bacteria in medicated animal feeds. Front Microbiol 10:456. https://doi.org/10.3389/fmicb.2019.00456.
5. Sun J, Liao XP, D'Souza AW, Boolchandani M, Li SH, Cheng K, Luis Martinez J, Li L, Feng YJ, Fang LX, Huang T, Xia J, Yu Y, Zhou YF, Sun YX, Deng XB, Zeng ZL, Jiang HX, Fang BH, Tang YZ, Lian XL, Zhang RM, Fang ZW, Yan QL, Dantas G, Liu YH. 2020. Environmental remodeling of human gut microbiota and antibiotic resistome in livestock farms. Nat Commun 11:1427. https://doi.org/10.1038/s41467-020-15222-y.
6. Hassoun-Kheir N, Stabholz Y, Kreft JU, de la Cruz R, Romalde JL, Nesme J, Sorensen SJ, Smets BF, Graham D, Paul M. 2020. Comparison of antibiotic-resistant bacteria and antibiotic resistance genes abundance in hospital and community wastewater: A systematic review. Sci Total Environ 743: 140804. https://doi.org/10.1016/j.scitotenv.2020.140804.
7. O'Brien S. 2015. Meeting the societal need for new antibiotics: the challenges for the pharmaceutical industry. Br J Clin Pharmacol 79:168–172. https://doi.org/10.1111/bcp.12401.
8. Bax R, Green S. 2015. Antibiotics: the changing regulatory and pharmaceutical industry paradigm. J Antimicrob Chemother 70:1281–1284. https://doi.org/10.1093/jac/dku572.
9. Checcucci A, Trevisi P, Luise D, Modesto M, Blasioli S, Braschi I, Mattarelli P. 2020. Exploring the animal waste resistome: The spread of antimicrobial resistance genes through the use of livestock manure. Front Microbiol 11. https://doi.org/10.3389/fmicb.2020.01416.
10. WHO. 2017. The world is running out of antibiotics, WHO report confirms. https://www.who.int/news/item/20-09-2017-the-world-is-running-out-of-antibiotics-who-report-confirms.
11. Okeke IN, Aboderin OA, Byarugaba DK, Ojo KK, Opintan JA. 2007. Growing problem of multidrug-resistant enteric pathogens in Africa. Emerg Infect Dis 13:1640–1646. https://doi.org/10.3201/eid1311.070674.
12. Calbo E, Freixas N, Xercavins M, Riera M, Nicolás C, Monistrol O, Solé MDM, Sala MR, Vila J, Garau J. 2011. Foodborne nosocomial outbreak of SHV1 and CTX-M-15-producing *Klebsiella pneumoniae*: epidemiology and control. Clin Infect Dis 52:743–749. https://doi.org/10.1093/cid/ciq238.
13. Gieraltowski L, Higa J, Peralta V, Green A, Schwensohn C, Rosen H, Libby T, Kissler B, Marsden-Haug N, Booth H, Kimura A, Grass J, Bicknese A, Tolar B, Defibaugh-Chavez S, Williams I, Wise M, *Salmonella* Heidelberg Investigation T. 2016. National outbreak of multidrug resistant Salmonella Heidelberg infections linked to a single poultry company. PLoS One 11: e0162369. https://doi.org/10.1371/journal.pone.0162369.
14. Yamaji R, Rubin J, Thys E, Friedman CR, Riley LW, Diekema DJ. 2018. Persistent pandemic lineages of uropathogenic *Escherichia coli* in a college community from 1999 to 2017. J Clin Microbiol 56:e01834-17. https://doi.org/10.1128/JCM.01834-17.
15. Zgurskaya HI, Lopez CA, Gnanakaran S. 2015. Permeability barrier of Gram-negative cell envelopes and approaches to bypass It. ACS Infect Dis 1:512–522. https://doi.org/10.1021/acsinfecdis.5b00097.
16. Masi M, Refregiers M, Pos KM, Pages JM. 2017. Mechanisms of envelope permeability and antibiotic influx and efflux in Gram-negative bacteria. Nat Microbiol 2:17001. https://doi.org/10.1038/nmicrobiol.2017.1.
17. Donia MS, Fischbach MA. 2015. Small molecules from the human microbiota. Science 349:1254766. https://doi.org/10.1126/science.1254766.
18. Garcia-Gutierrez E, Mayer MJ, Cotter PD, Narbad A. 2019. Gut microbiota as a source of novel antimicrobials. Gut Microbes 10:1–21. https://doi.org/10.1080/19490976.2018.1455790.
19. Rebuffat S. 2012. Microcins in action: amazing defence strategies of Enterobacteria. Biochem Soc Trans 40:1456–1462. https://doi.org/10.1042/BST20120183.
20. Baquero F, Lanza VF, Baquero MR, Del Campo R, Bravo-Vazquez DA. 2019. Microcins in *Enterobacteriaceae*: peptide antimicrobials in the eco-active intestinal chemosphere. Front Microbiol 10:2261. https://doi.org/10.3389/fmicb.2019.02261.
21. Telhig S, Ben Said L, Zirah S, Fliss I, Rebuffat S. 2020. Bacteriocins to thwart bacterial resistance in Gram negative bacteria. Front Microbiol 11:586433. https://doi.org/10.3389/fmicb.2020.586433.
22. Arnison PG, Bibb MJ, Bierbaum G, Bowers AA, Bugni TS, Bulaj G, Camarero JA, Campopiano DJ, Challis GL, Clardy J, Cotter PD, Craik DJ, Dawson M, Dittmann E, Donadio S, Dorrestein PC, Entian K-D, Fischbach MA, Garavelli JS, Göransson U, Gruber CW, Haft DH, Hemscheidt TK, Hertweck C, Hill C, Horswill AR, Jaspars M, Kelly WL, Klinman JP, Kuipers OP, Link AJ, Liu W, Marahiel MA, Mitchell DA, Moll GN, Moore BS, Müller R, Nair SK, Nes IF, Norris GE, Olivera BM, Onaka H, Patchett ML, Piel J, Reaney MJT, Rebuffat S, Ross RP, Sahl H-G, Schmidt EW, Selsted ME, 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. https://doi.org/10.1039/c2np20085f.
23. Montalbán-López M, Scott TA, Ramesh S, Rahman IR, van Heel AJ, Viel JH, Bandarian V, Dittmann E, Genilloud O, Goto Y, Grande Burgos MJ, Hill C, Kim S, Koehnke J, Latham JA, Link AJ, Martínez B, Nair SK, Nicolet Y, Rebuffat S, Sahl H-G, Sareen D, Schmidt EW, Schmitt L, Severinov K, Süssmuth RD, Truman AW, Wang H, Weng J-K, van Wezel GP, Zhang Q, Zhong J, Piel J, Mitchell DA, Kuipers OP, van der Donk WA. 2021. New developments in RiPP discovery, enzymology and engineering. Nat Prod Rep 38:130–239. https://doi.org/10.1039/d0np00027b.
24. Gordon DM, O'Brien CL. 2006. Bacteriocin diversity and the frequency of multiple bacteriocin production in *Escherichia coli*. Microbiology (Reading) 152:3239–3244. https://doi.org/10.1099/mic.0.28690-0.
25. Gordon DM, Oliver E, Littlefield-Wyer J. 2007. The diversity of bacteriocins in Gram-negative bacteria, p 5–18. In Riley MA, Chavan MA (ed), Bacteriocins. Springer Berlin Heidelberg, Berlin, Heidelberg.
26. Sassone-Corsi M, Nuccio S-P, Liu H, Hernandez D, Vu CT, Takahashi AA, Edwards RA, Raffatellu M. 2016. Microcins mediate competition among *Enterobacteriaceae* in the inflamed gut. Nature 540:280–283. https://doi.org/10.1038/nature20557.
27. Zschuttig A, Zimmermann K, Blom J, Goesmann A, Pohlmann C, Gunzer F. 2012. Identification and characterization of microcin S, a new antibacterial peptide produced by probiotic Escherichia coli G3/10. PLoS One 7: e33351. https://doi.org/10.1371/journal.pone.0033351.
28. Card T, Logan RF, Rodrigues LC, Wheeler JG. 2004. Antibiotic use and the development of Crohn's disease. Gut 53:246–250. https://doi.org/10.1136/gut.2003.025239.
29. Frohlich EE, Farzi A, Mayerhofer R, Reichmann F, Jacan A, Wagner B, Zinser E, Bordag N, Magnes C, Frohlich E, Kashofer K, Gorkiewicz G, Holzer P. 2016. Cognitive impairment by antibiotic-induced gut dysbiosis: Analysis of gut microbiota-brain communication. Brain Behav Immun 56: 140–155. https://doi.org/10.1016/j.bbi.2016.02.020.
30. Wipperman MF, Fitzgerald DW, Juste MAJ, Taur Y, Namasivayam S, Sher A, Bean JM, Bucci V, Glickman MS. 2017. Antibiotic treatment for tuberculosis induces a profound dysbiosis of the microbiome that persists long after therapy is completed. Sci Rep 7:10767. https://doi.org/10.1038/s41598-017-10346-6.
31. Bistoletti M, Caputi V, Baranzini N, Marchesi N, Filpa V, Marsilio I, Cerantola S, Terova G, Baj A, Grimaldi A, Pascale A, Frigo G, Crema F, Giron MC, Giaroni C. 2019. Antibiotic treatment-induced dysbiosis differently affects BDNF and TrkB expression in the brain and in the gut of juvenile mice. PLoS One 14: e0212856. https://doi.org/10.1371/journal.pone.0212856.
32. Kang K, Imamovic L, Misiakou M-A, Bornakke Sørensen M, Heshiki Y, Ni Y, Zheng T, Li J, Ellabaan MMH, Colomer-Lluch M, Rode AA, Bytzer P, Panagiotou G, Sommer MOA. 2021. Expansion and persistence of antibiotic-specific resistance genes following antibiotic treatment. Gut Microbes 13: 1–19. https://doi.org/10.1080/19490976.2021.1900995.
33. Riboulet-Bisson E, Sturme MHJ, Jeffery IB, O'Donnell MM, Neville BA, Forde BM, Claesson MJ, Harris H, Gardiner GE, Casey PG, Lawlor PG, O'Toole PW, Ross RP. 2012. Effect of Lactobacillus salivarius Bacteriocin Abp118 on the mouse and pig intestinal microbiota. PLoS One 7:e31113. https://doi.org/10.1371/journal.pone.0031113.
34. Le Lay C, Fernandez B, Hammami R, Ouellette M, Fliss I. 2015. On Lactococcus lactis UL719 competitivity and nisin (Nisaplin((R))) capacity to inhibit *Clostridium difficile* in a model of human colon. Front Microbiol 6: 1020.
35. Fernandez B, Savard P, Fliss I. 2016. Survival and metabolic activity of pediocin producer *Pediococcus acidilactici* UL5: Its impact on intestinal microbiota and *Listeria monocytogenes* in a model of the Human terminal ileum. Microb Ecol 72:931–942. https://doi.org/10.1007/s00248-015-0645-0.

36. Umu OC, Bauerl C, Oostindjer M, Pope PB, Hernandez PE, Perez-Martinez G, Diep DB. 2016. The potential of class ii bacteriocins to modify gut microbiota to improve host health. PLoS One 11:e0164036. https://doi.org/10.1371/journal.pone.0164036.
37. Coates ARM, Hu Y, Holt J, Yeh P. 2020. Antibiotic combination therapy against resistant bacterial infections: synergy, rejuvenation and resistance reduction. Expert Rev Anti Infect Ther 18:5–15. https://doi.org/10.1080/14787210.2020.1705155.
38. Metlitskaya A, Kazakov T, Kommer A, Pavlova O, Praetorius-Ibba M, Ibba M, Krasheninnikov I, Kolb V, Khmel I, Severinov K. 2006. Aspartyl-tRNA synthetase is the target of peptide nucleotide antibiotic Microcin C. J Biol Chem 281:18033–18042. https://doi.org/10.1074/jbc.M513174200.
39. Rosengren KJ, Clark RJ, Daly NL, Goransson U, Jones A, Craik DJ. 2003. Microcin J25 has a threaded sidechain-to-backbone ring structure and not a head-to-tail cyclized backbone. J Am Chem Soc 125:12464–12474. https://doi.org/10.1021/ja0367703.
40. Adelman K, Yuzenkova J, Porta AL, Zenkin N, Lee J, Lis JT, Borukhov S, Wang MD, Severinov K. 2004. Molecular mechanism of transcription inhibition by peptide antibiotic microcin J25. Mol Cell 14:753–762. https://doi.org/10.1016/j.molcel.2004.05.017.
41. Li Y-M, Milne JC, Madison LL, Kolter R, Walsh CT. 1996. From peptide precursors to oxazole and thiazole-containing peptide antibiotics: microcin B17 synthase. Science 274:1188–1193. https://doi.org/10.1126/science.274.5290.1188.
42. Yorgey P, Lee J, Kordel J, Vivas E, Warner P, Jebaratnam D, Kolter R. 1994. Posttranslational modifications in microcin B17 define an additional class of DNA gyrase inhibitor. Proc Natl Acad Sci U S A 91:4519–4523. https://doi.org/10.1073/pnas.91.10.4519.
43. Parks WM, Bottrill AR, Pierrat OA, Durrant MC, Maxwell A. 2007. The action of the bacterial toxin, microcin B17, on DNA gyrase. Biochimie 89:500–507. https://doi.org/10.1016/j.biochi.2006.12.005.
44. Thomas X, Destoumieux-Garzón D, Peduzzi J, Afonso C, Blond A, Birlirakis N, Goulard C, Dubost L, Thai R, Tabet JC, Rebuffat S. 2004. Siderophore peptide, a new type of post-translationally modified antibacterial peptide with potent activity. J Biol Chem 279:28233–28242. https://doi.org/10.1074/jbc.M400228200.
45. Huang K, Zeng J, Liu X, Jiang T, Wang J. 2021. Structure of the mannose phosphotransferase system (man-PTS) complexed with microcin E492, a pore-forming bacteriocin. Cell Discov 7:20. https://doi.org/10.1038/s41421-021-00253-6.
46. Pankey GA, Sabath LD. 2004. Clinical relevance of bacteriostatic versus bactericidal mechanisms of action in the treatment of Gram-positive bacterial infections. Clin Infect Dis 38:864–870. https://doi.org/10.1086/381972.
47. Eliopoulos G, Moellering R. 1996. Antimicrobial combinations, p 330–396. In Lorian V (ed), Antibiotics in laboratory medicine. The Williams & Wilkins Co, Baltimore, MD.
48. Falagas ME, Lourida P, Poulikakos P, Rafailidis PI, Tansarli GS. 2014. Antibiotic treatment of infections due to carbapenem-resistant *Enterobacteriaceae*: systematic evaluation of the available evidence. Antimicrob Agents Chemother 58:654–663. https://doi.org/10.1128/AAC.01222-13.
49. Goyal D, Dean N, Neill S, Jones P, Dascomb K. 2019. Risk factors for community-acquired extended-spectrum beta-lactamase–producing *Enterobacteriaceae* infections—a retrospective study of symptomatic urinary tract infections. Open Forum Infect Dis 6. https://doi.org/10.1093/ofid/ofy357.
50. Heilbronner S, Krismer B, Brötz-Oesterhelt H, Peschel A. 2021. The microbiome-shaping roles of bacteriocins. Nat Rev Microbiol 19:726–739. https://doi.org/10.1038/s41579-021-00569-w.
51. D'Onofrio A, Crawford JM, Stewart EJ, Witt K, Gavrish E, Epstein S, Clardy J, Lewis K. 2010. Siderophores from neighboring organisms promote the growth of uncultured bacteria. Chem Biol 17:254–264. https://doi.org/10.1016/j.chembiol.2010.02.010.
52. Ran R, Zeng H, Zhao D, Liu R, Xu X. 2017. The novel property of heptapeptide of microcin C7 in affecting the cell growth of *Escherichia coli*. Molecules 22:432. https://doi.org/10.3390/molecules22030432.
53. Ben Said L, Emond-Rheault JG, Soltani S, Telhig S, Zirah S, Rebuffat S, Diarra MS, Goodridge L, Levesque RC, Fliss I. 2020. Phenomic and genomic approaches to studying the inhibition of multiresistant *Salmonella enterica* by microcin J25. Environ Microbiol 22:2907–2920. https://doi.org/10.1111/1462-2920.15045.
54. Delgado MA, Rintoul MR, Farias RN, Salomon RA. 2001. *Escherichia coli* RNA polymerase is the target of the cyclopeptide antibiotic microcin J25. J Bacteriol 183:4543–4550. https://doi.org/10.1128/JB.183.15.4543-4550.2001.
55. Bellomio A, Vincent PA, de Arcuri BF, Farías RN, Morero RD. 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. https://doi.org/10.1128/JB.00206-07.
56. Baquero F, Levin BR. 2021. Proximate and ultimate causes of the bactericidal action of antibiotics. Nat Rev Microbiol 19:123–132. https://doi.org/10.1038/s41579-020-00443-1.
57. Falagas ME, Kasiakou SK. 2005. Colistin: the revival of polymyxins for the management of multidrug-resistant gram-negative bacterial infections. Clin Infect Dis 40:1333–1341. https://doi.org/10.1086/429323.
58. Nierhaus D, Nierhaus KH. 1973. Identification of the chloramphenicol-binding protein in *Escherichia coli* ribosomes by partial reconstitution. Proc Natl Acad Sci U S A 70:2224–2228. https://doi.org/10.1073/pnas.70.8.2224.
59. Schroeder R, Waldsich C, Wank H. 2000. Modulation of RNA function by aminoglycoside antibiotics. EMBO J 19:1–9. https://doi.org/10.1093/emboj/19.1.1.
60. Ma B, Bs C. 1990. Single protein omission reconstitution studies of tetracycline binding to the 30S subunit of *Escherichia coli* ribosomes. Biochemistry 29:5374–5379.
61. Mathavan I, Zirah S, Mehmood S, Choudhury HG, Goulard C, Li Y, Robinson CV, Rebuffat S, Beis K. 2014. Structural basis for hijacking siderophore receptors by antimicrobial lasso peptides. Nat Chem Biol 10:340–342. https://doi.org/10.1038/nchembio.1499.
62. Ferguson AD, Kodding J, Walker G, Bos C, Coulton JW, Diederichs K, Braun V, Welte W. 2001. Active transport of an antibiotic rifamycin derivative by the outer-membrane protein FhuA. Structure 9:707–716. https://doi.org/10.1016/s0969-2126(01)00631-1.
63. Yuzenkova J, Delgado M, Nechaev S, Savalia D, Epshtein V, Artsimovitch I, Mooney RA, Landick R, Farias RN, Salomon R, Severinov K. 2002. Mutations of bacterial RNA polymerase leading to resistance to microcin j25. J Biol Chem 277:50867–50875. https://doi.org/10.1074/jbc.M209425200.
64. Campbell EA, Korzheva N, Mustaev A, Murakami K, Nair S, Goldfarb A, Darst SA. 2001. Structural mechanism for rifampicin inhibition of bacterial RNA polymerase. Cell 104:901–912. https://doi.org/10.1016/s0092-8674(01)00286-0.
65. Lavina M, Pugsley AP, Moreno F. 1986. Identification, mapping, cloning and characterization of a gene (sbmA) required for microcin B17 action on *Escherichia coli* K12. J Gen Microbiol 132:1685–1693. https://doi.org/10.1099/00221287-132-6-1685.
66. Paulsen VS, Mardirossian M, Blencke HM, Benincasa M, Runti G, Nepa M, Haug T, Stensvag K, Scocchi M. 2016. Inner membrane proteins YgdD and SbmA are required for the complete susceptibility of *Escherichia coli* to the proline-rich antimicrobial peptide arasin 1(1–25). Microbiology (Reading) 162:601–609. https://doi.org/10.1099/mic.0.000249.
67. Kaatz GW, Seo SM, Barriere SL, Albrecht LM, Rybak MJ. 1989. Ciprofloxacin and rifampin, alone and in combination, for therapy of experimental *Staphylococcus aureus* endocarditis. Antimicrob Agents Chemother 33:1184–1187. https://doi.org/10.1128/AAC.33.8.1184.
68. Wells CM, Beenken KE, Smeltzer MS, Courtney HS, Jennings JA, Haggard WO. 2018. Ciprofloxacin and rifampin dual antibiotic-loaded biopolymer chitosan sponge for bacterial inhibition. Mil Med 183:433–444. https://doi.org/10.1093/milmed/usx150.
69. Lê S, Josse J, Husson F. 2008. FactoMineR: An R package for multivariate analysis. J Stat Softw 25:18.
70. Kassambara A MF. 2017. Practical guide to principal component methods in R: PCA, M (CA), FAMD, MFA, HCPC, factoextra. https://CRAN.R-project.org/package=factoextra.
71. Ploty Inc. 2015. Collaborative data science publisher: Plotly Technologies Inc. https://plotly.com.
72. Guijarro JI, González-Pastor JE, Baleux F, Millán JLS, Castilla MA, Rico M, Moreno F, Delepierre M. 1995. Chemical structure and translation inhibition studies of the antibiotic microcin C7. J Biol Chem 270:23520–23532. https://doi.org/10.1074/jbc.270.40.23520.