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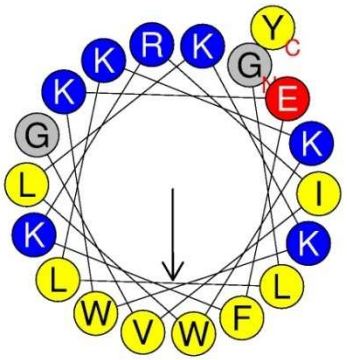


Helical wheel projection of peptide 2

**Hemolitic activity**

**Antibacterial activity**

**Structural characterization by circular dichroism**

*De novo* designed alpha helical cationic peptides with antimicrobial actvity against multi-resistant clinical isolates

# Antimicrobial activity of *de novo* designed cationic peptides against multi-resistant clinical isolates.

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**Abstract.**

Antibiotic resistance is one of the main problems concerning public health or clinical practice. Antimicrobial peptides appear as good candidates for the development of new therapeutic drugs. In this study we *de novo* designed a group of cationic antimicrobial peptides, analyzed its physicochemical properties, including its structure by circular dichroism and studied its antimicrobial properties against a panel of clinical isolates expressing different mechanisms of resistance. Three cationic alpha helical peptides exhibited antimicrobial activity comparable to, or even better than the comparator omiganan (MBI‐226).

**Keywords.**

Peptides; cationic; antimicrobial; resistance

## Introduction

Antimicrobial peptides (AMPs) are naturally occurring molecules of the innate immune system that play an important role in the host defense of animals and plants [1]. In recent years, natural or designed AMPs have attracted considerable interest as potential candidates for the development of novel antibiotics [2-3]. The main reason for this interest is that its particular mechanism of action is unlikely to induce drug resistance, in part because resistance against AMPs cannot be selected without bacterial cell wall undergoing profound structural changes [4]. However, pathogens can eventually respond to AMPs reducing the negative charge of their cell envelope with specific surface modifications and subvert mechanisms of AMPs [5]. Bacteria are capable of adapting and resisting AMPs, through the production of peptidases and proteases that degrade antimicrobial peptides, and the production of compounds that inhibit the action of AMPs [6]

The broad activity spectrum and the relative selectivity towards microbial membranes are also two important features that drive the interest of researchers on AMPs as new antibiotic molecules.

The cationic AMP omiganan (MBI‑226), an analogue of indolicidin, is one of the most studied AMPs and it has recently finished Phase II trials (ClinicalTrials.gov identifier: NCT00608959). Omiganan showed activity against gram-positive and gram-negative bacteria but also *Candida* spp. isolates [7-8]. Therefore, the objectives of this work were to design a group of new peptide sequences, and analyze their physicochemical properties and antimicrobial activities against 82 bacterial strains, including wild type and drug resistant clinical isolates. Omiganan was used in this study as comparator for these peptides.

## Materials and methods

*Peptides design and synthesis*

The sequences were designed using a combined rational and computer assisted approach. Cationic alpha helical peptides were designed identifying short putative active regions from AMP databases. Then, these regions were combined or modified in order to have cationic sequences with different physicochemical parameters, like alpha helix content and hydrophobicity. For this purpose we used multiple alignment tools and simulators of physicochemical properties like ClustalX, HeliQuest [9] and HydroMCalc [10]. We established specific amino acid positions and identified functionally relevant motifs in natural or designed

peptides. Considering all these diverse parameters, a group of peptides was synthesized with or without C terminus amidation. The purity grade of all peptides was >95% by HPLC (GenScript Co., Piscataway, NJ 08854, USA). The peptide sequences: Peptide 1: WPKWWKWKRRWGRKKAKKRRG; peptide2: GLLKKWLKKWKEFKRIVGY; peptide3: FGKEKKAWWRRRKWLK ; peptide5: RIVQRIKKWLLKWKKLGY.

*Bacterial Strains*

The panel analyzed included 82 previously well characterized isolates collected at the National Reference Laboratory (INEI) with different mechanisms of resistance: 39 Gram-positive (*van*A, *van*B, *van*C, *mec*A, *erm*A, *erm*C, *msr*A, *lnu*A genes) and 43 Gram-negative bacteria ($bla_{VIM}$, $bla_{IMP}$, $bla_{SPM}$, $bla_{KPC-2}$, $bla_{OXA-23}$, $bla_{OXA-58}$, $bla_{CTX-M-2}$, $bla_{PER-2}$, $bla_{GES}$, $bla_{VEB-1}$, $bla_{TEM-1}$, $bla_{CMY}$, $bla_{CIT}$, $bla_{SHV-1}$, $bla_{OXA-9}$). The panel includes *P. aeruginosa* ATCC27853, *E. coli* ATCC25922, *S. aureus* ATCC29213, *E. faecalis* ATCC51299 and *E. faecalis* ATCC29212 reference strains.

*Antimicrobial activity*

Minimal inhibitory concentration was determined by standard microdilution assay according to CLSI recommendations [11], using Mueller Hinton Broth (DIFCO) supplemented with $Ca^{2+}$ (20-25mg/L) and $Mg^{2+}$ (10-12.5mg/L). Omiganan® was used as comparator.

*Hemolytic assay*

The citotoxic activity of the peptides was evaluated according to the method described previously [12]. Briefly, a volume of heparinized human whole blood was diluted 3x in phosphate-buffered saline and then centrifuged 10 min. at 1500 rpm. This procedure was repeated three times. The cellular pellet was resuspended in phosphate-buffered saline to a final dilution of 10% (v/v). The stock cell suspension was further diluted to about 0.5% (v/v). Peptides were then added at different concentrations and incubated at 37 ºC for 30 min. Afterwards, tubes were centrifuged and the absorbance of the supernatant was measured at 550 nm. The percentage of lysis was then calculated relative to 0% lysis with buffer and 100% lysis with water. The absorbance measurement was repeated three times, and the averaged values were used.

*Circular dichroism in the far UV*

We studied the secondary structure content by circular dichroism spectroscopy in the far UV, using a JASCO J computer 810 (Jasco Corp., Tokyo, Japan) acid calibrated with (+) 10

camphorsulfonic acid. The measurements were performed under nitrogen gas flow of 8 l/h at a temperature of 20 °C, controlled by a Peltier system (JASCO).

Spectra were recorded between 185 and 320 nm, using a 0.1 cm cell path length. The peptide concentrations were 40 uM, dissolved in sodium phosphate buffer pH 7.0 or 10 mM in the same buffer with sodium dodecyl sulphate (SDS) 10 mM. The sensitivity was 100 millidegrees. We used a scan speed of 50 nm/min, a response time of 1 s and a bandwidth of 1 nm. We performed an average of five assays for each sample spectra. The average absorption was corrected by buffer and then baseline to zero using the average of readings between 290 and 320 nm. Finally, the data were smoothed using a Golay polynomial Savizky fourth grade, with a window of ten points. The spectra were converted to molar ellipticity residue half by using the relationship: $[\theta] = \theta / (10 \times c \times n \times d)$, where [$\theta$] is the molar ellipticity (in degrees × cm2 × dmol-1), $\theta$ the ellipticity in millidegrees, $n$ is the number of residues of the peptide and c its molar concentration, d the length of the cell in centimeters.

The mean hydrophobicity ($H$) and the mean hydrophobic moment ($\mu H$) were calculated from the amino acid sequences, using the Eisenberg scale for hydrophobicity by the HydroMCalc applet [10]

**Results.**

*Structural analysis of the peptides*. The circular dichroism spectra of peptides in aqueous solution shows that they are all unstructured in aqueous buffer, with a characteristic minimum at approximately 200 nm (Figure 1A). With the addition of SDS micelles (Figure 1B), conformational changes occured in peptides 2 and 5 that are consistent with the formation of alpha-helix structure with two characteristic minima near 208 and 222 nm. Peptide 1 also underwent such a transition, although the acquired structure level was lower than the one seen for peptides 2 and 5. The circular dichroism spectrum of peptide 3 is almost invariable with the addition of SDS micelles, indicative of the persistence of a disordered conformation. For omiganan, the spectrum was significantly modified in the presence of SDS, the 200 nm band was attenuated and a new band near 230 nm appeared; which could be the result of the interaction between the side chains of tryptophan. Figure 1C shows the helical wheel projection of the peptides, depicting the amphipatic residues and their relative position in the alpha helix.

Peptides were designed in order to have different alpha helical content and different amphipathicity, the latter calculated as the hydrophobicity and mean hydrophobic moment with specific software (HydroMCalc and Heliquest). Helical conformation was monitored in SDS micelles, which are generally employed as a simple membrane-mimetic environment. Table 1 summarizes the structural analyses and hemolytic activity of the peptides.

*Hemolytic activity.* The peptides (C-terminus amidated) were incubated with human red blood cells in order to evaluate their hemolytic activity. Table 1 shows the results as a relative value to 100% hemolysis of human red blood cells treated with distilled water. Peptides 1, 3, 4 and 5 showed little or negligible hemolytic activity, similarly with omiganan. Peptide 2 displayed an hemolysis of red blood cells almost 4-times higher than omiganan and peptide 5.

*Antimicrobial activity of the peptides*. Antimicrobial activity of C-terminus amidated and non amidated peptides was evaluated by microdilution test against a first panel with 8 isolates. The panel included 5 clinical (*S. warnerii* M6823, *S. cohnii* M6767, *S. aureus* M6794, *P. aeruginosa* M13513 and *K. pneumoniae* M13540) and 3 ATCC isolates (*S. aureus* ATCC29213, *P. aeruginosa* ATCC27853 and *E. coli* ATCC25922). MICs values of those peptides with amidated C-terminus were equal or lower (up to 3 dilutions) than those peptides with non amidated C-terminus, for the 8 isolates tested (data not shown). Peptide 3, with the lowest hydrophobic moment and helicity, did not show significant antimicrobial activity, except for coagulase negative staphylococci (MIC of 8 and 4 mg/L, respectively). Peptide 4 showed no antimicrobial activity for all the eight isolates tested. On the other hand peptides 1, 2 and 5 showed antimicrobial activity comparable to, or in some cases better than, omiganan.

Considering these results, together with the lower hemolytic activity of C-terminus amidated peptides, the antimicrobial activity of the C-terminus amidated peptides 1, 2 and 5 was evaluated against a large panel of 82 well-characterized bacterial isolates, including the 8 isolates used in the first panel. Table 2 displays MIC values of peptides 1, 2 and 5 and omiganan against a panel containing 43 gram-negative and 39 gram-positive isolates. This panel included isolates expressing clinically relevant resistance mechanisms to antibiotics, like carbapenemase-producing enterobacteria and *P. aeruginosa*, methicillin-resistant *S. aureus* or vancomycin-resistant enterococci (Table 2). Peptide 1 showed $MIC_{90}$ values of 128 mg/L for all gram-negative isolates except for *K. pneumoniae* strains (MIC $\geq$1024 mg/L). Peptides 2 and 5 showed similar performance against gram-negative bacteria with $MIC_{90}$ values between 32 and

128 mg/L, and slightly lower than peptide 1. Peptides 1, 2 and 5 showed a similar activity for each gram-positive species (Table 2). *E. faecalis* isolates displayed higher MIC values than other enterococci species for the three analyzed peptides and omiganan (Table 2). No association between mechanism of resistance and MIC values was observed, similar results were reported for omiganan by Sader *et al.* [7]

The omiganan MIC ranges obtained herein were slightly higher (up to three dilutions) than previous reports [7-8]. Omiganan MIC values for ATCC control strains were into the range described by Anderegg *et al.* [13], but on the upper border (Table 2). We suspect that the difference of our results of MIC range for omiganan could be associated to: i) a smaller number of isolates included in our panel, ii) our isolates collection could be strongly biased with antimicrobial resistant strains, and/or iii) intrinsic differences of each population of isolates.

*Concluding remarks*. We designed a group of peptides with different physicochemical characteristics, and tested their antimicrobial activity against a panel of clinical bacterial isolates. At least seven structural or physical parameters could be considered critical for biological activity: size, sequence, charge, degree of structuring (helicity), hydrophobicity, amphipathicity and angles subtended by hydrophobic and hydrophilic faces of the formed helix [14].

Some authors [15] argue that the secondary structure and biological activity are not coupled, and AMPs do not form pores in membranes but rather destabilize them disturbing the organization of the lipids, consistent with the idea that physical chemical and interfacial properties are the critical factors for determining the biological activity; this theory would suit omiganan that is not structured as alpha helix. In any case, helicity seems to be an important parameter for antimicrobial activity in our peptides, since the three peptides that displayed alpha helical content in SDS micelles also showed antibacterial activity. However, other parameters may be involved, for example peptide 1 and 5, although having different helicity, they showed similar antimicrobial activity against Gram-positive strains. But, on the other hand, these two peptides showed different activity when tested on Gram-negative bacteria, especially in *K. pneumoniae* specie (Table 2).

Peptide 2 and 5 had similar physicochemical properties, like alpha helix content, amphipathicity and net charge, but also antimicrobial activity, however peptide 2 was highly hemolytic to human red blood cells. Furthermore peptide 1 showed antimicrobial activity

against gram-positive and -negative strains, although it did not show high alpha helix content in contact with SDS micelles.

Also interesting was the relative low activity of all these peptides against *E. faecalis* isolates (64-256 mg/L), compared to another *Enterococci* species, like *E. faecium* and *E. gallinarum.* This low activity was also observed for omiganan [7], indicating a possible different cell wall composition in *E. faecalis* specie. It is evident that certain differences within the bacterial cell wall are probably associated with these different sensitivities to AMPs.

Peptides 1, 2 and 5 showed good antibacterial activity against a broad spectrum of clinical isolates, although peptide 2 displayed high cytotoxicity. These three peptides could become good templates for topical use.

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**Figure 1.** Graphical analysis of the peptides structure.

**Footnote:** Circular dichroism of peptides in aqueous solution (panel A) and in SDS micelles (panel B). Helical wheel projection diagrams of the peptides, considering the first 18 amino acids (panel C). omi, omiganan; p1, peptide 1; p2, peptide 2; p3, peptide 3; p5, peptide 5.

**Table 1**. Physicochemical properties, structural analyses and hemolytic activity of the peptides

**Footnote:** The values for hydrophobicity (H) and mean hydrophobic moment (μH) were obtained from HydroMCalc software. The percent helix values were determined based on circular dichroism spectra calculated as the mean residue molar ellipticity at 222 nm, in SDS micelles. * Isolelectric point and net charge were calculated for the acidic C terminus version of the peptides. Hemolytic activity is shown as a percentage (%) of hemolysis compared to distilled water (100% hemolysis). One representative experiment. Ne: not evaluated

**Table 2.** Antimicrobial activity of three designed peptides and omiganan against gram-negative and gram-positive bacteria.

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**Table 1.**

| | Peptide 1 | Peptide 2 | Peptide 3 | Peptide 4 | Peptide 5 |
|---|---|---|---|---|---|
| **Mean Hydrophobicity (*H* )** | -0.67 | -0.23 | -0.54 | -0.12 | -0.28 |
| **Mean hydrophobic moment (μ*H* )** | 0.24 | 0.35 | 0.14 | 0.17 | 0.41 |
| **Helicity (%Helix)** | 27.86 | 64.2 | 0.54 | 8.81 | 88.43 |
| ***Isoelectric Point** | 13.10 | 10.89 | 12.25 | 9.85 | 11.75 |
| ***Net Charge** | +12 | +6 | +7 | +3 | +7 |
| **Hemolytic activity** | 3.4 | 39.8 | 1.5 | 1.72 | 9.5 |

| Omiganan |
| --- |
| -0.31 |
| 0.28 |
| 10.15 |
| 12.79 |
| +4 |
| 10 |

**Table 2 continued,**

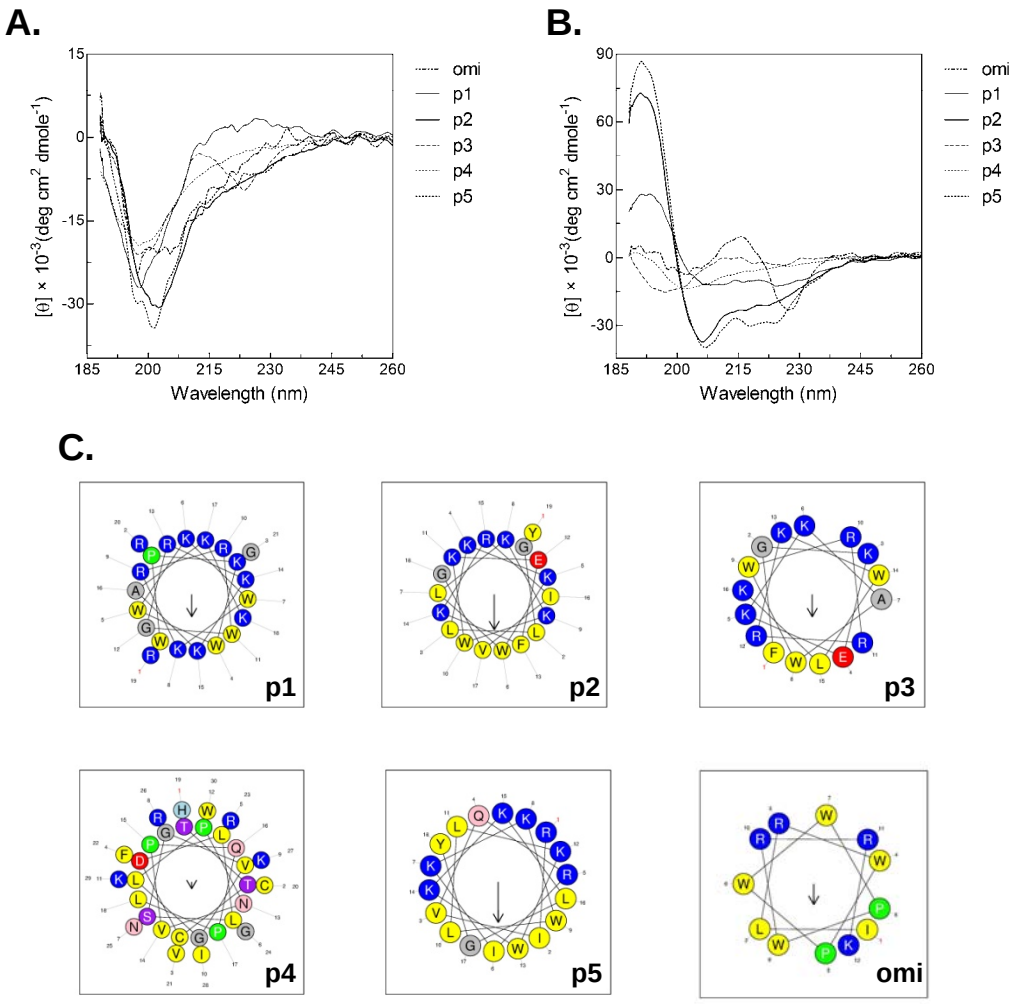
| Specie | Strain | Genes | MIC (mg/L) | | | |
|---|---|---|---|---|---|---|
| | | | Peptide 1 | Peptide 2 | Peptide 5 | Omiganan |
| | M5306 | *bla*$_{PER-2}$ + *bla*$_{CTX-M-2}$ + bla$_{TEM-1}$ | 256 | 4 | 64 | 64 |
| | M9209 | *bla*$_{KPC-2}$ | 128 | 64 | 128 | 64 |
| *S. aureus* (11) | | | | | | |
| | ATCC29213 | none | 32 | 64 | 32 | 32 |
| | P33 | *msr*A | 32 | 128 | 64 | 32 |
| | P28 | *erm*A | 32 | 64 | 64 | 32 |
| | P204 | *erm*A | 64 | 64 | 32 | 64 |
| | M6276 | *erm*A + *lnu*A | 16 | 128 | 64 | 32 |
| | P239 | *erm*C | 32 | 64 | 64 | 32 |
| | M6794 | *mec*A | 32 | 64 | 64 | 64 |
| | M2832 | *mec*A | 32 | 128 | 64 | 64 |
| | M4046 | *mec*A | 32 | 128 | 32 | 32 |
| | M6820 | *mec*A | 64 | 128 | 64 | 128 |
| | M6784 | *mec*A | 32 | 32 | 32 | 32 |
| *S. epidermidis* (4) | | | | | | |
| | M2923 | none | 16 | 16 | 16 | 8 |
| | M2931 | none | 16 | 16 | 8 | 16 |
| | M2919 | *mec*A | 16 | 16 | 8 | 8 |
| | M2921 | *mec*A | 8 | 8 | 8 | 8 |
| *S. saprophyticus* (2) | | | | | | |
| | M4070 | *mec*A | 16 | 32 | 8 | 8 |
| | M2981 | *mec*A | 16 | 8 | 8 | 8 |
| *S. haemolyticus* (2) | | | | | | |
| | M2976 | *mec*A | 16 | 8 | 8 | 4 |
| | M3014 | none | 16 | 8 | 8 | 4 |
| *S. hominis* (2) | | | | | | |
| | M2973 | *mec*A | 4 | 4 | 4 | 4 |
| | M2967 | *mec*A | 8 | 8 | 8 | 4 |
| *S. warnerii* (1) | | | | | | |
| | M6823 | *mec*A | 8 | 8 | 8 | 8 |
| *S. cohnii* (1) | | | | | | |
| | M6767 | *mec*A | 16 | 16 | 8 | 4 |
| *E. faecalis* (8) | | | | | | |
| | ATCC 29212 | none | 64 | 128 | 128 | 128 |
| | ATCC 51299 | *van*B | 256 | 256 | 256 | 256 |
| | M4899 | *van*B | 256 | 128 | 256 | 256 |
| | M6534 | *van*B | 128 | 256 | 256 | 256 |
| | M4992 | *van*A | 128 | 128 | 128 | 128 |
| | M6383 | *van*A | 128 | 128 | 128 | 128 |
| | M4449 | *van*A | 128 | 128 | 128 | 128 |
| | M6983 | *van*A | 64 | 128 | 128 | 128 |
| *E. faecium* (6) | | | | | | |
| | PZAP95 | none | 32 | 16 | 16 | 16 |
| | M6261 | none | 32 | 16 | 16 | 16 |
| | M2619 | *van*B | 32 | 16 | 16 | 16 |
| | M2481 | *van*B | 32 | 16 | 16 | 16 |
| | M2304 | *van*A | 16 | 16 | 8 | 4 |
| | M2664 | *van*A | 16 | 8 | 8 | 8 |
| *E. gallinarum* (2) | | | | | | |
| | M2723 | *van*C1 + *van*A | 32 | 32 | 16 | 16 |
| | M2685 | *van*C1 + *van*A | 16 | 16 | 16 | 16 |

**Table 2.**

| Specie | Strain | Genes | MIC (mg/L) Peptide 1 | Peptide 2 | Peptide 5 | Omiganan |
|---|---|---|---|---|---|---|
| *P. aeruginosa* (12) | | | | | | |
| | ATCC 27853 | none | 64 | 64 | 64 | 256 |
| | PCOS12 | none | 64 | 64 | 64 | 512 |
| | M5470 | none | 128 | 64 | 64 | 512 |
| | M7907 | $bla_{PER}$ | 128 | 64 | 64 | 128 |
| | M13513 | $bla_{KPC-2}$ | 32 | 64 | 64 | 256 |
| | M11005 | $bla_{KPC-2}$ | 64 | 64 | 128 | 512 |
| | M7723 | $bla_{KPC-2}$ | 64 | 64 | 32 | 256 |
| | M7728 | $bla_{IMP}$ | 128 | 64 | 64 | 512 |
| | M5109 | $bla_{VIM}$ + $bla_{GES-1}$ | 128 | 64 | 64 | 256 |
| | M5200 | $bla_{VIM}$ + $bla_{GES-1}$ | 64 | 64 | 64 | 512 |
| | M7525 | $bla_{SPM}$ | 64 | 64 | 64 | 512 |
| | M7712 | $bla_{SPM}$ | 64 | 64 | 64 | 512 |
| *Acinetobacter sp.* (10) | | | | | | |
| | M13523 | $bla_{OXA-51}$ | 64 | 4 | 64 | 32 |
| | M9665 | $bla_{OXA-51}$ | 128 | 32 | 128 | 4 |
| | M5282 | $bla_{OXA-51}$ | 64 | 8 | 64 | 8 |
| | M5179 | $bla_{OXA-51}$ | 64 | 32 | 64 | 32 |
| | M7489 | $bla_{OXA-51}$ + $bla_{TEM}$ | 64 | 16 | 64 | 8 |
| | PFAV1 | $bla_{OXA-51}$ + $bla_{OXA-58}$ + $bla_{PER}$ | 64 | 16 | 64 | 16 |
| | M5277 | $bla_{PER}$ | 64 | 8 | 64 | 32 |
| | M5949 | $bla_{OXA-23}$ + $bla_{OXA-GVI}$ | 256 | 16 | 256 | 32 |
| | M7978 | $bla_{IMP-1}$ | 64 | 8 | 64 | 16 |
| | M9013 | $bla_{OXA-51}$ + $bla_{IMP}$ | 32 | 8 | 32 | 32 |
| *K. pneumoniae* (12) | | | | | | |
| | PFAV3 | none | 1024 | 128 | 128 | 128 |
| | M9140 | $bla_{CIT}$ | 1024 | 32 | 32 | 64 |
| | M9491 | $bla_{MOX}$ | 1024 | 64 | 64 | 128 |
| | M9170 | $bla_{OXA-GIII}$ | >1024 | 32 | 64 | 128 |
| | M5825 | $bla_{GES-3}$ + $bla_{CTX-M-2}$ | >1024 | 8 | 32 | 64 |
| | M9310 | $bla_{CTX-M-2}$ + $bla_{TEM-1}$ + $bla_{SHV-1}$ | >1024 | 16 | 32 | 64 |
| | M9375 | $bla_{CTX-M-2}$ + $bla_{TEM-1}$ + $bla_{SHV-1}$ | 1024 | 64 | 16 | 32 |
| | M1803 | $bla_{PER-2}$ + $bla_{CTX-M-2}$ + $bla_{TEM-1}$ + $bla_{SHV}$ + $bla_{OXA-9}$ | >1024 | 32 | 64 | 1024 |
| | M7647 | $bla_{VIM}$ + $bla_{CTX-M-2}$ + $bla_{TEM-1}$ + $bla_{SHV-1}$ | >1024 | 32 | 32 | 1024 |
| | M13540 | $bla_{KPC-2}$ | >1024 | 16 | 64 | 256 |
| | M9885 | $bla_{KPC-2}$ | >1024 | 64 | 32 | 256 |
| | M11245 | $bla_{KPC-2}$ + $bla_{PER-2}$ | 1024 | 16 | 8 | 32 |
| *E. coli* (9) | | | | | | |
| | ATCC 25922 | none | 128 | 32 | 32 | 64 |
| | M9884 | none | 128 | 32 | 32 | 64 |
| | M7859 | $bla_{CIT}$ | 128 | 32 | 16 | 64 |
| | PNEU23 | $bla_{OXA-GIII}$ + $bla_{TEM-1}$ | 128 | 32 | 32 | 32 |
| | PCOS15 | $bla_{PER-2}$ + $bla_{TEM-1}$ | 128 | 64 | 64 | 64 |
| | PABC11 | $bla_{CTX-M-2}$ | 128 | 32 | 32 | 64 |
| | PLCA1 | $bla_{CTX-M-2}$ + $bla_{TEM-1}$ | 128 | 64 | 64 | 64 |

| | | | | | | |
|---|---|---|---|---|---|---|
| | M5306 | *bla*$_{PER-2}$ + *bla*$_{CTX-M-2}$ + bla$_{TEM-1}$ | 256 | 4 | 64 | 64 |
| | M9209 | *bla*$_{KPC-2}$ | 128 | 64 | 128 | 64 |
| *S. aureus* (11) | | | | | | |
| | ATCC29213 | none | 32 | 64 | 32 | 32 |
| | P33 | *msr*A | 32 | 128 | 64 | 32 |
| | P28 | *erm*A | 32 | 64 | 64 | 32 |
| | P204 | *erm*A | 64 | 64 | 32 | 64 |
| | M6276 | *erm*A + *lnu*A | 16 | 128 | 64 | 32 |
| | P239 | *erm*C | 32 | 64 | 64 | 32 |
| | M6794 | *mec*A | 32 | 64 | 64 | 64 |
| | M2832 | *mec*A | 32 | 128 | 64 | 64 |
| | M4046 | *mec*A | 32 | 128 | 32 | 32 |
| | M6820 | *mec*A | 64 | 128 | 64 | 128 |
| | M6784 | *mec*A | 32 | 32 | 32 | 32 |
| *S. epidermidis* (4) | | | | | | |
| | M2923 | none | 16 | 16 | 16 | 8 |
| | M2931 | none | 16 | 16 | 8 | 16 |
| | M2919 | *mec*A | 16 | 16 | 8 | 8 |
| | M2921 | *mec*A | 8 | 8 | 8 | 8 |
| *S. saprophyticus* (2) | | | | | | |
| | M4070 | *mec*A | 16 | 32 | 8 | 8 |
| | M2981 | *mec*A | 16 | 8 | 8 | 8 |
| *S. haemolyticus* (2) | | | | | | |
| | M2976 | *mec*A | 16 | 8 | 8 | 4 |
| | M3014 | none | 16 | 8 | 8 | 4 |
| *S. hominis* (2) | | | | | | |
| | M2973 | *mec*A | 4 | 4 | 4 | 4 |
| | M2967 | *mec*A | 8 | 8 | 8 | 4 |
| *S. warnerii* (1) | | | | | | |
| | M6823 | *mec*A | 8 | 8 | 8 | 8 |
| *S. cohnii* (1) | | | | | | |
| | M6767 | *mec*A | 16 | 16 | 8 | 4 |
| *E. faecalis* (8) | | | | | | |
| | ATCC 29212 | none | 64 | 128 | 128 | 128 |
| | ATCC 51299 | *van*B | 256 | 256 | 256 | 256 |
| | M4899 | *van*B | 256 | 128 | 256 | 256 |
| | M6534 | *van*B | 128 | 256 | 256 | 256 |
| | M4992 | *van*A | 128 | 128 | 128 | 128 |
| | M6383 | *van*A | 128 | 128 | 128 | 128 |
| | M4449 | *van*A | 128 | 128 | 128 | 128 |
| | M6983 | *van*A | 64 | 128 | 128 | 128 |
| *E. faecium* (6) | | | | | | |
| | PZAP95 | none | 32 | 16 | 16 | 16 |
| | M6261 | none | 32 | 16 | 16 | 16 |
| | M2619 | *van*B | 32 | 16 | 16 | 16 |
| | M2481 | *van*B | 32 | 16 | 16 | 16 |
| | M2304 | *van*A | 16 | 16 | 8 | 4 |
| | M2664 | *van*A | 16 | 8 | 8 | 8 |
| *E. gallinarum* (2) | | | | | | |
| | M2723 | *van*C1 + *van*A | 32 | 32 | 16 | 16 |
| | M2685 | *van*C1 + *van*A | 16 | 16 | 16 | 16 |

# Figure 1.




Circular dichroism of peptides in aqueous solution (panel A) and in SDS micelles (panel B). Projection of helical wheel of the peptides (panel C). omi, omiganan; p1, peptide 1; p2, peptide 2; p3, peptide 3; p4, peptide 4; p5, peptide 5.

# Antimicrobial activity of *de novo* designed cationic peptides against multi-resistant clinical isolates.

**Highlights.**

- *de novo* designed cationic peptides

- physicochemical properties of peptides

- antimicrobial resistant bacteria