Development of a new ten amino acid antimicrobial peptide (AMP) against extensively drug-resistant bacteria, *Pseudomonas aeruginosa* and *Acinetobacter baumannii*

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ABSTRACT

Growing microbial resistance to conventional antimicrobial agents reveals the need for development of novel and alternative therapeutic strategies. In the present study, we developed a new ten amino acid antimicrobial peptide (AMP) and evaluated its antibacterial activity against extensively drug-resistant bacteria, *Pseudomonas aeruginosa* (*P. aeruginosa*) and *Acinetobacter baumannii* (*A. baumannii*). After synthesis of the AMP, minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were tested using different concentrations (2 to 128 ng/mL) of the AMP. Afterwards, the cell viability and toxicity of the AMP were investigated on primary fibroblast cells using MTT and trypan blue exclusion assays with different concentrations (2 to 128 ng/mL) of the AMP, respectively. The AMP showed MICs≥0.023 and 0.046 ng/mL for *P. aeruginosa* and MICs≥0.18 ng/mL for *A. baumannii*. In addition, the AMP exhibited MBCs≥0.05 and 0.09 ng/mL for *P. aeruginosa* and MBCs≥0.04 and 0.045 ng/mL for *A. baumannii*. Results from the disk diffusion assay showed 12 and 16 mm for *P. aeruginosa* and 9 mm for *A. baumannii*. *In vitro* cell cytotoxicity and hemolytic activity assays confirmed the safety profile of the AMP (no toxicity or hemolytic activities at concentrations ≤8 ng/mL). Our results suggested that the AMP developed in this study is a reliable and safe antimicrobial agent for the treatment of extensively drug-resistant bacteria, *P. aeruginosa* and *A. baumannii*.

Key words: antibacterial peptide (AMP); antibiotic resistance; cytotoxicity; *Pseudomonas aeruginosa* (*P. aeruginosa*); *Acinetobacter baumannii* (*A. baumannii*)

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Introduction

Antimicrobial resistance (AMR) or drug resistance, including antibiotic resistance, remains one of the most serious public health problems (BYARUGABA, 2004; AYUKEKBONG et al., 2017; MAHMOUDI et. al., 2019). Any use of antimicrobials, even for appropriate and justified reasons, contributes to the development of microbial resistance. AMR is growing considerably, particularly in developing countries, due to gross abuse of antimicrobials. In developing countries, the misuse of antimicrobials and self-medication are facilitated through their simple availability, without prescription and by unregulated supply chains. Furthermore, non-compliance in the use of antimicrobials has significant repercussions for resistance, and poverty is a main root factor of antimicrobial misuse in developing countries (BYARUGABA 2004; OKEKE et al., 2005a; 2005b; AYUKEKBONG et al., 2017). On the other hand, a great number of patients miss doses either by mistake or deliberately, particularly in cases where signs and symptoms begin to subside after an initial favorable therapeutic response (HART and KARUIKI, 1998; AYUKEKBONG et al., 2017). In other conditions including an acute side effect, patients abandon their treatment and return to the hospital only with a recurring infection by a more virulent and resistant strain of the pathogen. This leads to the exposure of surviving pathogens to subtherapeutic concentrations of antimicrobials, resulting in increased chances of them acquiring resistance (OKEKE et al., 1999; AYUKEKBONG et al., 2017). An increasing prevalence of infections caused by multidrug-resistant (MDR) isolates has been reported in many countries (ZAVASCKI et al., 2010).

Antimicrobial peptides (AMPs), a diverse class of small proteins, are host defense molecules produced by the innate immunity, that play an important role in the fight against a wide variety of microorganisms, including bacteria, fungi, parasites, and viruses. AMPs, as potential next-generation antibiotics, have been demonstrated to be a promising multifunctional therapeutic agent against a wide variety of pathogens, including the opportunistic pathogens *Pseudomonas aeruginosa* (*P. aeruginosa*; a Gram-negative, aerobic, non-spore forming rod bacillus) and *Acinetobacter baumannii* (*A. baumannii*; a Gram-negative, aerobic, pleomorphic, non-motile bacillus) (VANZOLINI et al., 2001; SAJJAN et al., 2001; MOGHADDAM et al., 2014; 2018; BEN HUR et al., 2022).

P. aeruginosa and *A. baumannii*, as major nosocomial pathogens worldwide, are non-fermentative Gram-negative bacteria which share worrisome characteristics, including intrinsic resistance to a wide range of antibiotics, as well as the ability to continuously develop extraordinary resistance mechanisms to most classes of antimicrobial agents. Thus, these pathogens can become easily resistant to almost or even all commercially available antimicrobial agents (ZAVASCKI et al., 2010).

In the light of the above, the present study aimed to develop a novel ten amino acid AMP and assess its antibacterial effect against MDR *P. aeruginosa* and *A. baumannii* strains.

Materials and methods

Peptide design and synthesis. The AMP used in this study was designed using data recorded from databases, such as the Antimicrobial Peptide Database, the Collection of Anti-microbial Peptides (CAMP), and the Peptaibol database. In brief, Database Filtering Technology was used to screen AMPs with activity against Gram-negative bacteria. The selected AMPs were then optimized using search filters based on their physical and chemical properties, such as positive net charge, flexibility, size, hydrophobicity, and amphipathicity. After this, a series of proposed peptides were chosen as templates, on the basis of which the target peptide was designed by substituting or displacing amino acids (Template-based design). Lastly, the peptide (KWKLFKKIVL) was chemically synthesized (GenScript USA, Inc.) and purified to >95%. High-performance liquid chromatography (HPLC) was used to confirm peptide identity.

AMP preparation. A stock aliquot of the AMP was prepared in sterile distilled water (pH 7.2) to obtain a concentration of 1 mg/mL by UV spectroscopy, and then stored at -20°C until use. Serial 2-fold

dilutions ranging from 2 to 128 ng/mL (2, 4, 8, 16, 32, 64, and 128 ng/mL) were made using this stock solution diluted with sterile distilled water.

Antibacterial Activity

Bacterial strains and culture conditions. *P. aeruginosa* PAO1, extensively drug-resistant (XDR) *P. aeruginosa*, *Staphylococcus aureus* (*S. aureus*) ATCC 25923, extensively drug-resistant (XDR) *A. baumannii*, and *A. baumannii* ATCC 19606 strains were obtained from the Department of Medical Microbiology, Shahid Beheshti University of Medical Sciences. Tehran, Iran. Bacterial strains were cultured in trypticase soy agar (TSA) overnight at 37°C.

Antimicrobial susceptibility testing. The minimum inhibitory concentration (MIC) was tested using the broth microdilution method (CLSI M07-A8) to determine the antimicrobial resistance profile of *P. aeruginosa* and *A. baumannii* (CLSI, 2018). Specific antibiotics were selected according to the Clinical and Laboratory Standards Institute CLSI 2010, formerly the National Committee for Clinical Laboratory Standards guidelines (NCCLS, 2023).

Determination of minimum inhibitory concentration (MIC). The MIC of the AMP was determined by the broth microdilution method (CLSI M07-A8) (CLSI, 2018), using serial two-fold dilutions of the AMP, in concentrations ranging from 2 to 128 ng/mL, with bacterial concentrations adjusted to 10^5 CFU/mL in Mueller Hinton Broth (MHB), in a shaking bath for 18 h at 37°C. MHB media with only the tested bacteria, and with no bacterial suspension were used as positive and negative controls, respectively. Finally, the MIC value was determined as the lowest concentration that completely inhibited bacterial growth after 24 h of incubation at 37°C. The experiments were repeated in triplicate for each strain.

Determination of minimum bactericidal concentration (MBC). The minimum bactericidal concentration (MBC) was used to determine the lowest concentration that resulted in killing 99.9% of the initial bacterial population. For determination of the MBC, aliquots of 10 µL were taken from each tube with no visible bacterial growth, then seeded on MHA plates, and incubated for 24 h at 37°C. The experiments were repeated in triplicate for each strain.

Disk diffusion assay. The antibacterial activity of the AMP was evaluated using the Kirby-Bauer disc diffusion method on Cation-Adjusted Mueller Hinton agar (Merck, Germany), according to the clinical and laboratory standards institute guidelines (CLSI, 2018). Briefly, bacterial suspensions adjusted to 0.5 McFarland (1.5×10^8 CFU/mL, 20 µL) were cultured on MHA plates, the prepared disks (6 mm in diameter, containing 100 µg of the studied AMP in sterile distilled water) were placed in the center of the plates, and the plates were incubated at 37°C for 24 h. The inhibition zone of bacterial growth was reported using a ruler and compared between samples. In addition, the radial diffusion assay was used to determine the potency of the AMP, as described previously (WADE et al., 2019).

In-vitro cell cytotoxicity assay. The effects of the various AMP concentrations (2 to 128 ng/mL) were evaluated on the cell viability and cytotoxicity of primary human skin fibroblasts by MTT and Trypan blue exclusion assays, respectively. For this purpose, the cells were exposed to AMP for 24, 48 and 72 h. MTT and trypan blue exclusion assays were carried out, as described earlier (ABDOLLAHPOUR-ALITAPPEH et. al., 2018; 2019).

Culture of skin fibroblasts. Primary human skin fibroblasts were derived from the foreskins of 9 to 12 year-old boys after circumcision. Written informed consent was obtained from the parents of all the specimen donors. Skin fibroblasts were isolated and cultured as previously described (MOGHADDAM et al., 2018). Briefly, the samples were collected aseptically and washed several times with 75% alcohol and phosphate-buffered saline (PBS) containing 1% antibiotic (100 U/mL penicillin/streptomycin) and antimycotic (100 U/mL amphotericin) solution. Full-thickness skin was treated with 4 mg/mL of dispase (Gibco, USA) for 1 h at 37°C. Next, the epidermis was peeled from the dermis, which was then cut into small pieces and transferred into a falcon tube containing 0.03% collagenase type I solution (Gibco, USA). Pure dermis was digested in the incubator shaker at 37°C for 6 h. The fibroblast cells were cultured in Dulbecco's modified Eagle medium (DMEM) supplemented with 10% fetal bovine se-

rum (FBS), penicillin 100 U/mL and streptomycin 100 μg/mL, in 5% CO2, with 95% humidity at 37°C. After 5-6 days, the cultured fibroblasts were harvested by trypsinization, and the cultures were passaged into T25 culture flasks at 1:4. When the subcultures reached 80-90% confluence, serial passaging was performed by trypsinization. Cells from passages 5-8 were used in subsequent experiments when the cells reached the logarithmic growth phase.

Trypan blue exclusion assay. After morphological assessment, the cell viability was simultaneously assessed by trypan blue dye exclusion assay. Briefly, cells were treated with 2 to 128 ng/mL of the AMP for 24, 48 and 72 h. The cells were trypsinized with 0.25% trypsin-EDTA solution, resuspended in PBS, and stained with the same volume of 0.4% trypan blue dye solution (v/v in PBS). Within 2 min, the cells were loaded into a Neubauer chamber, and after 2-3 min the number of viable and non-viable cells per 1x1 mm square was counted under an inverted microscope. The dead cells retained the blue dye and hence were colored, whereas live cells remained unstained. The cells/mL was calculated as the average cell count × dilution factor $\times 10^4$ cells/mL. The cell viability percentage was determined as [(number of viable cells/total number of viable + non-viable cells) ×100. The percentage of growth inhibition was represented as (cell viability (control) - cell viability (with AMP)).

MTT assay. MTT was used to evaluate the *in vitro* cytotoxicity of the AMP on fibroblast cells. In brief, the cells (1×10^4 cells/well) were seeded into 96-well tissue culture plates (Greiner, Frickenhausen, Germany) and incubated overnight at 37°C, 5% CO2. At 80% confluency, the cells were treat- ed with the AMP at increasing concentrations (2 to 128 ng/mL) for 1, 2, and 3 days at 37°C under standard conditions (95% humidity and 5% CO2) in triplicate. Cells with no treatment and those treated with DMSO were used as negative and positive controls, respectively. After 24-, 48- and 72-hour incubation, the medium was aspirated, cell monolayers were washed twice with PBS, and 100 μl/well MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide) solution (Sigma-Aldrich, 5 mg/mL in PBS) was added to each well. After 4-hour incubation at 37°C, the media were aspirated, the formazan crystals in the cells were dissolved in 100 μl of dimethyl sulfoxide (DMSO, Sigma Aldrich, USA), and the plates were incubated for 15 minutes in the dark. Subsequently, the plates were incubated on a rotary shaker at 37°C for one hour to solubilize the formations of purple crystal formazan, and the absorbance was measured at 570 nm. The OD value of samples was normalized to that of the blank wells with cell culture media, and no cells. The absorbance of the untreated control cells was considered to be 100% survival. The cytotoxicity rate was calculated using the following formula: cytotoxicity (%) = 100 - ((At-Ab)/ (Ac-Ab)) × 100, where At = Absorbance value of the test compound, Ab = Absorbance value of the blank, and Ac = Absorbance value of the negative control.

Hemolytic activity assay. The hemolytic activity of the AMP was evaluated on human erythrocytes. A 20% (v/v) solution of erythrocytes in PBS was preincubated for 15 min at 37°C, and then solution was diluted to 10% by adding 2 to 128 ng/mL of AMP. After an additional 15 min at 37°C, the cells were centrifuged and the absorption of the supernatant was measured at 415 nm. The hemolysis percentage was calculated as $A_{peptide} - A_{media} / A_{100} - A_{media} \times 100$; where A_{100} is the absorbance of erythrocyte suspension with 100% hemolysis. Complete lysis was obtained by suspending erythrocytes in PBS containing 0.2% Triton X-100.

Statistical analysis. All the experiments were conducted in triplicate or with at least three different samples, and the mean of each was recorded. The data were analyzed using GraphPad Prism v8.0.2.263 software. Where appropriate, an independent samples t test or One-way ANOVA was applied. The data are expressed as mean ± SD. P values less than 0.05 were considered to be significant.

Results

Bacterial antibiotic susceptibility. An antibiogram was used to determine the antibiotic resistance of *P. aeruginosa* and *A. baumannii*. The results of the MIC showed that *P. aeruginosa* and *A. baumannii* were resistant to Imipenem (carbapenem), Meropenem (carbapenem), Ceftazidime (cephalosporins), and Ciprofloxacin (fluoroquinolone), thereby

Table 1. Minimum inhibitory concentrations (MIC; μg/mL) of different antibiotics against extensively drug-resistant (XDR) *Pseudomonas aeruginosa* (*P. aeruginosa*) and *Acinetobacter baumannii* (*A. baumannii*)

Bacteria	Imipenem (carbapenem)	Meropenem (carbapenem)	Ceftazidime (cephalosporins)	Ciprofloxacin (fluoroquinolone)	Ciprofloxacin (fluoroquinolone)
P. aeruginosa	64	128	256	128	1
A. baumannii	32	64	256	64	1

proving to be XDR. Importantly, both *P. aeruginosa* and *A. baumannii* were highly resistant to carbapenem (imipenem and meropenem; MIC≥32 μg/mL). More importantly, the carbapenem-resistant bacteria were also resistant to ciprofloxacin and ceftazidime (MICs≥64 μg/mL) (Table 1).

AMP characterization. HPLC confirmed the purity of the AMP≥95.0%. In addition, the theoretical molecular mass of the peptide was determined to be 1302.70 Da. The chromatogram of the AMP is shown in Fig. 1.

MIC, MBC, and disk diffusion of the AMP. Table 2 shows the MIC, MBC and disk diffusion values of the AMP for *P. aeruginosa* and *A. baumannii*. Our findings show that the AMP had MICs≥0.023 and 0.046 ng/mL for *P. aeruginosa* and MICs≥0.18 ng/mL for *A. baumannii*. In addition, the AMP exhibited MBCs≥0.05 and 0.09 ng/mL for *P. aeruginosa* and

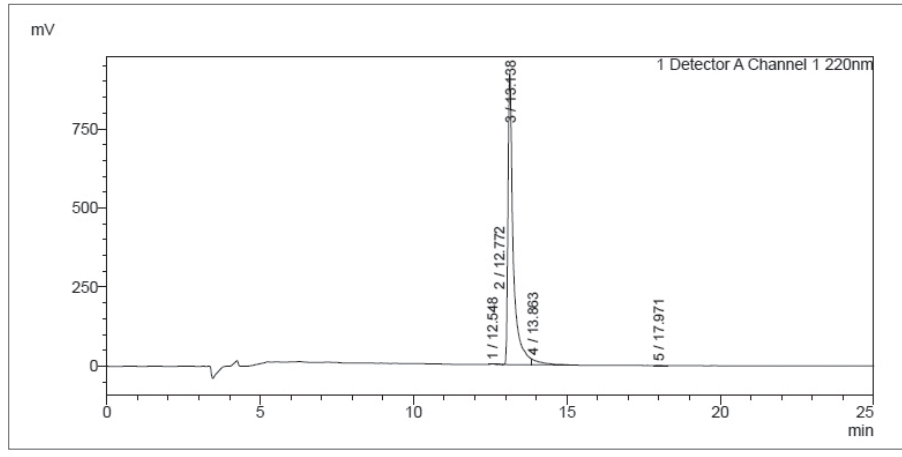

Fig. 1. Chromatogram of the peptide (KWKLFKKIVL) used in this study

Table 2. Minimum inhibitory concentrations (MIC), minimum bactericidal concentration (MBC) and disk diffusion methods of the AMP against *Pseudomonas aeruginosa* (*P. aeruginosa*), *Acinetobacter baumannii* (*A. baumannii*), and *Staphylococcus aureus* (*S. aureus*)

Bacteria	MIC (ng/mL)	MBC (ng/mL)	Disk Diffusion Method (mm)
P. aeruginosa PAO1	0.023	0.05	16
P. aeruginosa XDR (imp. positive)	0.046	0.09	12
S. aureus 15923	0.092	0.19	9
A. baumannii (MDR)	0.18	0.4	9
A. baumannii atcc 19606	0.18	0.45	9

Fig. 2. The antibacterial effect of the AMP against *Pseudomonas aeruginosa* (*P. aeruginosa*) and *Acinetobacter baumannii* (*A. baumannii*)

MBCs≥0.04 and 0.045 ng/mL for *A. baumannii*. The results from the disk diffusion assay showed 12 and 16 mm for *P. aeruginosa* and 9 mm for *A. baumannii* (Fig. 2). Taken together, our interesting findings revealed that the AMP has efficient antibacterial activity against *P. aeruginosa* and *A. baumannii* in its safe concentration.

Cell viability and cytotoxicity. At 24- and 48-h post-incubation, no difference in cell viability and cytotoxicity levels was observed at AMP concentrations ≤16 ng/mL, while cytotoxicity effects were found at AMP concentrations ≥32 ng/mL (P≤0.05). After 72-h post-incubation, no significant cytotoxicity was detected at AMP concentrations ≤8 ng/mL against the tested cells (P≤0.05). At concentrations ≥ 8 ng/mL (P≤0.05) the AMP did not affect the viability of fibroblasts at three incubation times (24, 48, and 72 h), showing a high biocompatibility profile (Fig. 3).

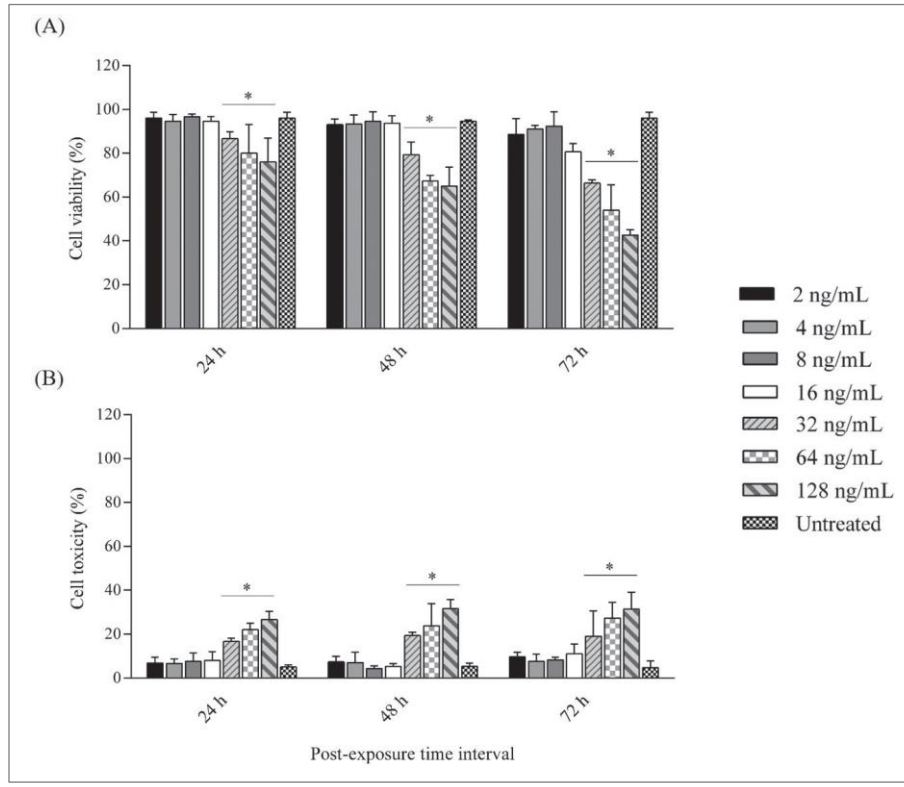

Fig. 3. Trypan blue exclusion and MTT assay. The effects of various concentrations of the AMP (2 to 128 ng/mL) on the cell viability (A) and cytotoxicity (B) of fibroblasts (P≤0.05)

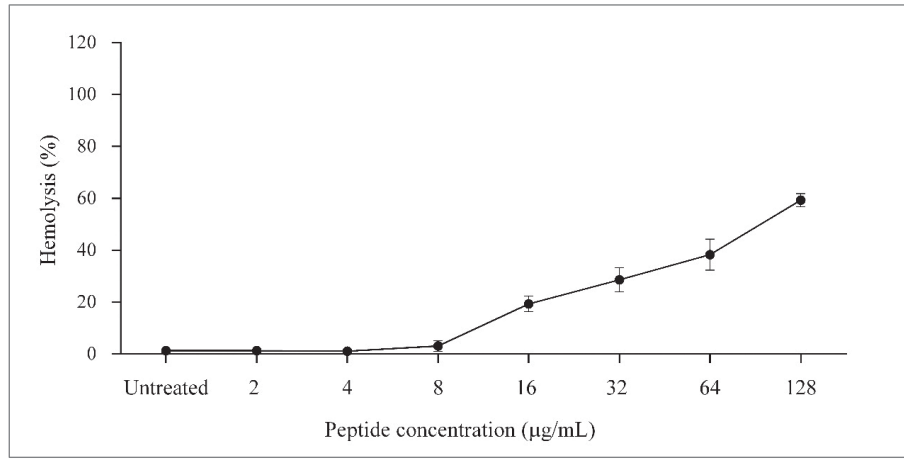

Fig. 4. The cytotoxicity effect of the AMP on erythrocyte cells using hemolysis assay Dose-dependent hemolysis was assessed using various concentrations of AMP (2 to 128 ng/mL) ($P \leq 0.05$)

Hemolysis effect of AMP on erythrocytes. We investigated the hemolytic activity of the AMP on human erythrocytes. Fig. 4 indicates dose-dependent hemolysis results. The HD50 value (the 50% hemolytic dose) of the AMP was 16 ng/mL.

Discussion

The overuse of antibiotics has led to the emergence of antimicrobial resistance and, as a result, AMPs are receiving significant attention as an alternative. Identification of effective AMPs from natural sources in a laboratory is a cost-intensive and time-consuming process (SHARMA et al., 2021).

P. aeruginosa and *A. baumannii* are non-fermentative Gram-negative bacteria intrinsically resistant to a wide range of antibiotics (ZAVASCKI et al., 2010), as documented in the present study; Our results clearly demonstrated that XDR *P. aeruginosa* and *A. baumannii* are resistant to carbapenem, cephalosporins, and fluoroquinolone. The resistance mechanisms of *P. aeruginosa* and *A. baumannii* include the production of β-lactamases, efflux pumps, and target-site or outer membrane modifications. Resistance to multiple antibiotics usually originates from the combination of different mechanisms in a single isolate, or the action of a single potent resistance mechanism (ZAVASCKI et al., 2010). There are important challenges in the treatment of MDR *P. aeruginosa* and *A. baumannii*, highlighting the dire need for the development of new antimicrobial agents. AMPs are potential multifunctional therapeutic agents, which are effective against a broad spectrum of microorganisms.

The majority of natural AMPs contain 10-50 amino acids, with net charges 0 to +7 and hydrophobic contents of 31-70% (MISHRA et al., 2017). AMPs exert their mechanism of action through disrupting bacterial cell membranes, modulating the immune response, and regulating inflammation (HANEY et al., 2019; MAGNA et al., 2020), leading to rapid eradication of invading pathogens, with little or no ability of the bacteria to develop resistance. There is a wide variety of unconventional sources for AMPs, including unculturable soil and marine bacteria, as well as approaches available for the production of vast libraries of derivatives (MAGNA et al., 2020).

In the present study, we developed a new AMP using a template-based design, and assessed its antibacterial effects against *P. aeruginosa* and *A. baumannii*. In general, the damaging properties of AMPs depend on the charge, hydrophobicity, amphipathicity, stereochemistry, and propensity of peptides to form barrels (MOGHADDAM et al., 2018). Furthermore, the sensitivity of eukaryotic cells to AMPs is dependent on variations in the

membrane lipid compositions, hydrophobicity, and metabolic activity of the cells (MOGHADDAM et al., 2014; 2018).

Our results clearly indicated that the newly-synthesized AMP has an efficient antibacterial activity against XDR *P. aeruginosa* and *A. baumannii* in its safe concentration. Our AMP showed MICs≥0.023 and 0.046 ng/mL for *P. aeruginosa*, and MICs≥0.18 ng/mL for *A. baumannii*, showing a suitable potency for bacterial killing, which is consistent with previously-published studies. GIACOMETT et al. (2004) showed that the CM15 peptide has antibacterial activity (MICs between 1 and 16 mg/L) on clinical isolates of *S. aureus*. MOOSAZADEH MOGHADDAM et al. (2014; 2018) demonstrated the antibacterial activity of the CM11 peptide (WKLFKKILKVLNH2), a short cecropin–melittin hybrid peptide, with MICs of 8-16 and 4-16 mg/L for antibiotic-resistant strains of *Klebsiella pneumonia* and *Salmonella typhimurium*, respectively, and MICs ≥ 16 μg/mL for clinical isolates, including *P. aeruginosa* and *A. baumannii* strains. In another study, RODRÍGUEZ-HERNÁNDEZ et al. (2006) showed the MIC ranges of 2-8, 2-4, 2-8 and 4-4 mg/L for CA(1-8)M(1-18), and three of its shortened analogues, namely CA(1-7)M(2-9), its N^{α}-terminal octanoylated analogue and CA(1-7)M(5-9) against A. baumannii strains, respectively. In addition, the AMP developed in this study exhibited MBCs ≥ 0.05 and 0.09 ng/ mL for *P. aeruginosa* and MBCs≥0.04 and 0.045 ng/mL for *A. baumannii*, which is consistent with other studies (MOGHADDAM et al., 2014; 2018; RODRIGUEZ-MELCON et al., 2021).

The results from the cytotoxicity studies showed the promising safety profile of our AMP, as concentrations ≤ 8 ng/mL had no toxicity and produced no change in cell viability in fibroblasts. In addition, the sensitivity of erythrocyte cells to the AMP was low in comparison with the cell line. Our AMP demonstrated little hemolytic activity in 8 ng/mL, and concentrations ≤ 8 ng/mL had no hemolytic activity. The high biocompatibility of antibacterial agents is very critical and guarantees their safety.

However, our study had limitations that warrant further studies to evaluate the effect of the developed AMP on a larger number of bacterial strains, using clinically-isolated bacteria as well as mouse models of infections. In addition, syner- gic studies to assess the effects of the developed AMP in combination with other antibiotics may represent a promising approach for the use of the AMP.

Conclusions

In this study, we developed a novel ten amino acid AMP using a template-based design, followed by antibacterial assessment. The newly-developed AMP exhibited significant activity and a favorable safety profile *in vitro* against XDR *P. aeruginosa* and *A. baumannii* strains. Nevertheless, further studies are required to find its full potential and limitations in the treatment of the infections caused by such bacteria.

Ethics approval

This study was approved by Gerash University of Medical Sciences (IR.GERUMS.REC.1398.012). All procedures performed in this study involving human participants were in accordance with the ethical standards of the Gerash Research Committee and were performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments, or comparable ethical standards.

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Declaration of competing interest

The authors declare that there are no potential conflicts of interest.

Authorship contribution statement

YM and AP conceived and designed the study. AHM, YM and ZA designed and optimized the AMP. MJ, MM, HH, ER, and SAK performed the experiments. AHM and BFM analyzed the data and

interpreted the results. AHM, BFM, MJ, MM, HH, SAK, and ZA prepared the manuscript. YM and AP reviewed and edited the manuscript, and provided detailed feedback. All the authors have read and agreed to the published version of the manuscript.

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SAŽETAK

Rastuća antimikrobna rezistencija na konvencionalna antimikrobna sredstva upućuje na potrebu za razvojem novih i alternativnih terapijskih strategija. U istraživanju je razvijen novi antimikrobni peptid (AMP) s 10 aminokiselina, te je procijenjen njihov antibakterijski učinak protiv široko rezistentnih bakterija *Pseudomonas aeruginosa* (*P. aeruginosa*) i *Acinetobacter baumannii* (*A. baumannii*). Nakon sinteze AMP-a minimalna inhibicijska koncentracija (MIK) i minimalna baktericidna koncentracija (MBK) analizirane su primjenom različitih koncentracija (2 - 128 ng/mL) AMP-a. Nakon toga vijabilnost i toksičnost AMP-a analizirane su na primarnim fibroblastnim stanicama, upotrebom MTT-a i testa *trypan blue exclusion* s različitim koncentracijama AMP-a (2 - 128 ng/mL). AMP je pokazao vrijednosti MIK-a ≥0,023 i 0,046 ng/mL za *P. aeruginosa* te ≥0,18 ng/mL za *A. baumannii*. Osim toga, AMP je pokazao vrijednosti MBK-a ≥0,05 i 0,09 ng/mL za *P. aeruginosa* te vrijednosti MBK-a ≥0,04 i 0,045 ng/mL za *A. baumannii*. Rezultati disk-difuzijskog testa pokazali su 12 i 16 mm za *P. aeruginosa* i 9 mm za *A. baumannii*. *In vitro* testovi stanične toksičnosti i hemolitičke aktivnosti potvrdili su sigurnosni profil AMP-a (nije bilo toksičnosti ni hemolitičke aktivnosti pri koncentracijama ≤8 ng/mL). Rezultati pokazuju da je AMP razvijen u ovom istraživanju pouzdano i sigurno antimikrobno sredstvo za liječenje bakterija širokog opsega rezistencije *P. aeruginosa* i *A. baumannii*.

Ključne riječi: antibakterijski peptid (AMP); antibiotska rezistencija; citotoksičnost; *Pseudomonas aeruginosa*; *Acinetobacter baumannii*