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administration of 5.0μM and 10.0μM (three times daily for three days) significantly reduced keratitis. The mean clinical scores were 3.3±0.58 and 2.3±0.50 for 5.0μM and 10.0μM treatments respectively compared to 4.0±0.0 for the vehicle control ($p<0.001$). In addition, 4.3±0.6 $\log_{10}$ and 3.9±1.3 $\log_{10}$ viable *P. aeruginosa* was recovered from the 5.0μM and 10.0μM VR18 treated eyes respectively compared to 6.1±0.9 $\log_{10}$ recovered from the control eyes ($p<0.001$). Overall, our findings indicate that VR18 is a promising candidate for the development as a novel topical antibiotic against *Pseudomonas* keratitis.

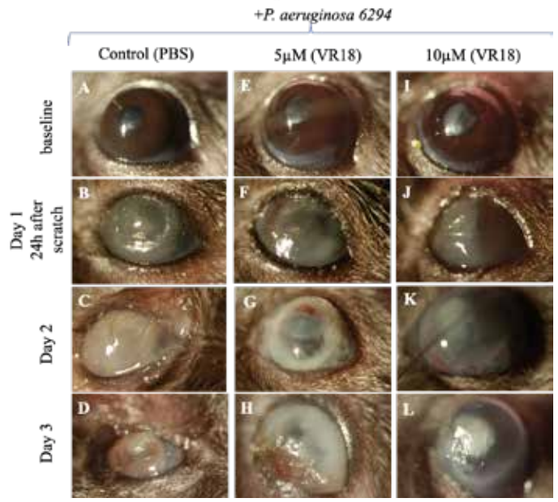

Figure 1: Representative photographs of the development of corneal infection following scratch and instillation of P. aeruginosa 6294 bacteria in the (A-D) vehicle control, (E-H) 5μM VR18 and (I-L) 10 μM VR18 treatment groups at day three.

1. Mohid, S.A.; Ghorai, A.; Ilyas, H.; Mroue, K.H.; Narayanan, G.; Sarkar, A.; Ray, S.K.; Biswas, K.; Bera, A.K.; Malmsten, M., et al. Colloids Surf B Biointerfaces 2019, 176, 360-370, doi:10.1016/j.colsurfb.2019.01.020.

R7: Antimicrobial properties of cell penetrating peptides against *Mycobacterium smegmatis*: Potential therapeutics for tuberculosis disease management

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Cell penetrating peptides (CPPs) are a class of cationic peptides that are able to translocate themselves across cellular membranes and also carry cargo molecules with them, much larger than their own size. They have been reported to possess dual characteristics of cell penetration as well as antimicrobial activity [1]. The mode of action of these peptides can be direct membrane lysis or intracellular targeting of crucial metabolic pathways. Tuberculosis (TB) is one of the most devastating diseases in the world with a global disease burden of 10 million people. It is a matter of grave concern since available antibiotics are becoming ineffective for treatment due to emergence of drug resistant strains [2]. Among developing countries, India has the highest incidence rate of TB. It also comes under 30 high burden countries listed with occurrences from all the three categories: TB, multi drug resistant-TB and TB-HIV co-infection. In the present study, antimicrobial activity of two CPPs, Tachyplesin and CyLoP-1 (derived from marine horseshoe crab and snake venom respectively) was evaluated against *Mycobacterium smegmatis*. The antimicrobial potency of peptides against *M. smegmatis* was determined by Minimum inhibitory concentration (MIC) evaluation. The peptides significantly reduced the bacterial count at their specific concentrations and time points. The peptides were also assessed for their qualitative and quantitative uptake in macrophage cells and their biocompatibility towards them. Furthermore, mechanisms of antimicrobial action of CPPs were determined using various microscopic and fluorescence techniques as well as membrane depolarization, ROS production assays. By analyzing all these aspects of CPPs antimicrobial action against *M. smegmatis*, a potential alternative therapeutics can be developed for effective tuberculosis disease management.

[1] Budagavi, D. P. & Chugh, A. European Journal of Pharmaceutical.115, 43–49 (2018); [2] Floyd, K., Glaziou, P., Zumla, A. & Raviglione, M. The Lancet Respiratory Medicine 6, 299–314 (2018).

L32: Discovery and development of SPR206: a next generation polymyxin analogue

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Polymyxins are an important class of antibiotics for therapy of Gram-negative infections, but their efficacy is limited by toxicities, especially renal toxicity. The main physicochemical drivers for toxicity are the same as the drivers for efficacy i.e. charge and lipophilicity. Nevertheless, the molecular mechanisms for activity and toxicity are undoubtedly different and careful empirical dissection of the structure-activity relationships for each has allowed the development of next generation analogues with retained, or improved, antimicrobial activity and reduced toxicity. One promising analogue, SPR206, exhibits reduced renal toxicity compared to polymyxin B in *Cynomolgus* monkey and is currently undergoing a phase I trial in human volunteers.

L33: Snake cathelicidins are highly effective against antimicrobial resistant bacteria and particular tumours

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Bacterial infections that are hard-to-treat due to antimicrobial resistance (AMR), as well as cancer, are major public health threats worldwide. Novel agents to combat these threats are urgently needed. As animal venoms have been the origin of several major drugs with anticancer and/or antimicrobial activity, we mined the genomes of 19 snake species for a class of antimicrobial peptides, i.e. cathelicidins, and synthesized 37 different snake peptides. To select the most promising peptides, we first tested all peptides for their bactericidal activity against AMR *Staphylococcus aureus* (MRSA), *Acinetobacter baumannii* and *Pseudomonas aeruginosa* and for their haemolytic activity. Further testing of the two peptides most promising in having excellent antimicrobial activity and negligible cytotoxicity revealed that they rapidly and effectively (sub-μM concentrations) kill AMR Gram-negative bacteria without affecting the viability of human erythrocytes (therapeutic index ≥500). The lead peptides were highly effective in killing AMR ESKAPE bacteria and colistin-resistant *Escherichia coli* in whole human blood. Importantly, these peptides did not induce resistance in vitro in AMR *E. coli* and *A. baumannii* using 40 serial cycles, were more effective in degrading AMR *A. baumannii* biofilms than MRSA biofilms and prevented biofilm formation by both bacteria in a dose-dependent fashion. Furthermore, lead peptides dose-dependently eliminated MRSA and AMR *A. baumannii* from wounded ex vivo human skin models. Finally, lead snake peptides were selectively cytotoxic to melanoma, renal cell carcinoma, and cervical carcinoma cell lines compared to normal melanocytes and fibroblasts, but not against ovarian epithelial cell carcinomas, colorectal carcinoma and breast ductal carcinoma cell lines compared to normal fibroblasts. Additional experiments indicated that snake peptides induce apoptosis, as measured by caspase 3/7 activation, in tumour cell lines. Together, these in vitro data indicate that lead snake peptides have potential as novel agents for systemic and topical treatment of patients with AMR infections and some tumours.

Poster Abstracts

P1: A placenta derived fragment of β-hemoglobin with antibacterial activity

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Human hemoglobin is a rich source of bioactive cryptides with antimicrobial activity [1]. To identify and characterize bioactive human peptides, peptide libraries of placenta tissue were generated by chromatography and screened for antimicrobial activity against various bacterial pathogens. Fractions with the highest antimicrobial activity harboured large amounts of a fragment of the human β-hemoglobin [HBB(111-146)], a bioactive peptide that has previously been characterized [2]. The purified HBB(111-146) showed a concentration dependent activity against different *Pseudomonas aeruginosa* stains of clinical origin and it was able to inhibit the growth of a Carbapenem resistant *P. aeruginosa* isolate that harbors a KPC-2 carrying plasmid [3]. Growth inhibition could already be observed at a peptide concentration of 15.63 µg ml-1, a concentration markedly lower than the described concentration of HBB(111-146) in the placenta (280-740 µg ml-1) [4]. Moreover we could demonstrate that HBB(111-146) permeabilizes the cell membrane of *P. aeruginosa* using Sytox green staining and we could observe membrane disintegration in electron microscopic investigations. In the presence of low pH the aspartyl protease Cathepsin D generates HBB(111-146) from the precursor hemoglobin. Under similar acidic conditions the peptide exhibits its highest antimicrobial activity against *P. aeruginosa* in liquid medium. Thus, we propose that under acidic conditions, a hallmark of inflammation and infection, HBB(111-146) can be generated from the precursor hemoglobin and exhibits broad antimicrobial activity potentially restricting diaplacental transmission of bacterial pathogens.

1 Mak, P. Front Biosci 13, 6859-6871 (2008); 2 Liepke, C. et al. J Chromatogr B Analyt Technol Biomed Life Sci 791, 345-356 (2003); 3 Hagemann, J. B., Pfennigwerth, N., Gatermann, S. G., von Baum, H. & Essig, A. J Antimicrob Chemother 73, 1812-1814, doi:10.1093/jac/dky105 (2018); 4 Standker, L. et al. Anal Biochem 401, 53-60, doi:10.1016/j.ab.2010.02.019 (2010).

P2: A novel antimicrobial peptide produced by the environmental bacterial isolate Paenibacillus polymyxa displays broad-spectrum activity in the Galleria mellonella larvae model

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Introduction: The *Galleria mellonella* (*G.mellonella*) larvae infection model is a reliable tool for early in vivo screening of antimicrobial compounds without the ethical constraints of mammalian models [1]. This model was therefore used to test an as yet unidentified antimicrobial peptide (36A90) isolated from the environmental bacterial isolate *Paenibacillus polymyxa*, which had previously shown broad-spectrum activity *in vitro*.

Methods: To assess toxicity, *G.mellonella* larvae were injected with purified 36A90 at a dose of 24mg/kg. To assess antimicrobial efficacy, larvae were first infected with the LD50 of either *Escherichia coli* (*E.coli*) or methicillin-resistant *Staphylococcus aureus* (MRSA), followed by treatment with 36A90 at a dose of 24mg/kg at intervals of 15 minutes-2 hours post infection. For both the toxicity and efficacy experiments, all larvae were incubated at 37ºC, with survival monitored for 120 hours. The *in vivo* stability of the compound was measured by assessing the antimicrobial activity of the extracted haemolymph of treated larvae over incubation periods of 0-24 hours.

Results: Preliminary compound characterisation, and examination of the draft genome sequence of the producing strain indicates that 36A90 is a novel peptidic compound. The 24mg/kg dose significantly improved the survival rate of *E.coli* infected larvae at all post-infection treatment intervals, and the MRSA infected larvae at a treatment interval of up to 30 minutes. 36A90 showed in vivo stability in the haemolymph assay for up to 4 hours.

Conclusion: This model has demonstrated the early potential of compound 36A90. Cell toxicity studies are ongoing, as is identification and structural characterisation.

[1] Andrea, A., Krogfelt, K., and Jenssen , H. (2019) Microorganisms, 7(85)

P3: Study on the influence of bacterial lipopolysaccharides on the secondary structure of antimicrobial peptides, in free-form and conjugated with polymeric linkers, using circular dichroism spectroscopy.

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Antimicrobial peptides (AMPs) are a part of the innate immune system's response to foreign organisms in the body [1]. Being a part of the innate immune system, these peptides are non-specific and hence a solution to antimicrobial resistance. Therefore, they can be used to target many different pathogenic organisms such as bacteria, fungi and viruses. After an initial lack of interest by the pharmaceutical industry, several AMPs are now being investigated in clinical trials [2-3]. In free-form, AMPs can recognise and disrupt microbial membranes through numerous mechanisms [4]. However, AMPs in free-form can be readily metabolized within the body and lose their antimicrobial activity [5]. Conjugation of AMPs with polymeric linkers can improve the AMPs' life-time and stability in the physiological environment but, may affect the secondary structure and thus activity of the conjugated AMP [6]. Previous studies highlight the significance of the secondary structure of AMPs, relating to their antimicrobial activity and cytotoxicity [7]. Therefore, the effect of polymer conjugation and interfacial interactions between AMPs and microbial membranes needs to be understood. In order to study these interactions, bacterial lipopolysaccharides are added to AMP solutions and analysed with circular dichroism spectroscopy – using AMPs in free-form and conjugated with polymeric linkers. This study is fundamental to design AMPs with desirable secondary structures, improving their performance in future developments.

[1] Diamond, G., Beckloff, N., Weinberg, A., & Kisich, K. O. (2009). The roles of antimicrobial peptides in innate host defense. Current pharmaceutical design, 15(21), 2377-2392; [2] E Greber, K., & Dawgul, M. (2017). Antimicrobial peptides under clinical trials. Current topics in medicinal chemistry, 17(5), 620-628; [3] Mahlapuu, M., Håkansson, J., Ringstad, L., & Björn, C. (2016). Antimicrobial peptides: an emerging category of therapeutic agents. Frontiers in cellular and infection microbiology, 6, 194; [4] Guilhelmelli, F., Vilela, N., Albuquerque, P., Derengowski, L., Silva-Pereira, I., & Kyaw, C. (2013). Antibiotic development challenges: the various mechanisms of action of antimicrobial peptides and of bacterial resistance. Frontiers in microbiology, 4, 353; [5] Marr, A. K., Gooderham, W. J., & Hancock, R. E. (2006). Antibacterial peptides for therapeutic use: obstacles and realistic outlook. Current opinion in pharmacology, 6(5), 468-472; [6] Bagheri, M., Beyermann, M., & Dathe, M. (2009). Immobilization reduces the activity of surface-bound cationic antimicrobial peptides with no influence upon the activity spectrum. Antimicrobial agents and chemotherapy, 53(3), 1132-1141; [7] Mai, X. T., Huang, J., Tan, J., Huang, Y., & Chen, Y. (2015). Effects and mechanisms of the secondary structure on the antimicrobial activity and specificity of antimicrobial peptides. Journal of Peptide Science, 21(7), 561-568.

P4: Identification of a new antifungal peptide against grapevine downy mildew

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The biggest challenge global agricultural production is currently fac-

ing is the secure and sustainable supply of food for a constantly growing human population. Increasing agricultural productivity through the prevention of crop yield losses due to biotic stresses has therefore assumed considerable urgency. Modern agriculture strongly relies on pesticides to avoid losses and produce high-quality foods. Fungicides in particular represent the majority of plant protection products, with viticulture representing one of the first sector in terms of fungicide usage which has a serious negative impact on the environment and on human/animal health. As a consequence, crop protection is now oriented towards a rational use of pesticides and the development of eco-friendly pesticide alternatives. Among the available biotechnological solutions, peptide aptamers, i.e. artificial, high-affinity short peptides that specifically inhibit a target molecule through protein interference, are emerging as novel molecular tools.

Grapevine downy mildew, caused by the oomycete *Plasmopara viticola* (Berk. & Curt.) Berl. & de Toni, is a worldwide destructive disease of primary importance for viticulture. Within the frame of GraptaResistance research project (https://sites.unimi.it/graptaresistance/), we identified a novel peptide aptamer, named No*Pv*1 (No *P. viticola* 1), able to counteract *P. viticola* infection ex vivo (leaf disks) and in vivo (potted plants). No*Pv*1 has been isolated from a peptide library, through the yeast two-hybrid strategy, using the PvCesA2 protein (*P. viticola* cellulose synthase 2) as a bait. In addition, we demonstrated that No*Pv*1 targets specifically *P. viticola* as well as *Phytophtora infestans*, without affecting off-target organisms and being toxic for human cells.

P5: Design and development of short and stable pepR-derived antibacterial peptides

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The increasing number of bacterial infections and resistance to existing antibiotics has become a global health problem. Although antimicrobial peptides (AMPs) have been considered promising alternatives to conventional antibiotics, one of the main limitations hindering their use as therapeutic drugs is the high susceptibility to proteolytic degradation. Here, we used pepR, a peptide with known antibacterial activity, as a starting sequence to develop short and stable antibacterial peptides. Twelve pepR-derived peptides were obtained through sequential truncations performed on each terminus of pepR sequence. The deletion of amino acid residues at the C-terminus significantly impaired the antibacterial activity, which is correlated with the lack of ability of the peptides to adopt an alpha-helical conformation. The shortest peptide with highest antibacterial activity – N6-pepR, caused a fast killing of *Staphylococcus aureus* through bacterial membrane permeabilization. However, antibacterial activity was lost after only 1 h incubation in presence of human serum. Through the use of rational design two new peptides able to retain antibacterial activity even after 24 h incubation with serum were obtained. Overall, the work developed not only resulted on the discovery of two peptides with high antibacterial activity and proteolytic resistance, but also gives insights on the physicochemical properties that can be engineered to improve the application of AMPs as therapeutic drugs.

P6: Integrated evolutionary analysis reveals antimicrobial peptides with limited resistance

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Antimicrobial peptides (AMPs) are promising novel antimicrobials, however, the potential of bacterial resistance is a major concern. Here we systematically study the evolution of resistance to a chemically diverse set of AMPs and small-molecule antibiotics in *Escherichia coli*. Four lines of evidence indicate that, compared to antibiotics, the evolution of resistance against some AMPs is frequently limited. First, no clinically significant resistant mutants have emerged in laboratory evolution tests against certain AMPs. Second, drug-resistant bacteria have displayed no cross-resistance to these AMPs. Third, gene amplification, an important genetic source of antibiotic resistance, does not influence resistance to these AMPs. Finally, genomic fragments derived from a wide range of soil bacteria confer no detectable resistance against these AMPs when introduced into native host bacteria on plasmids. We have found that simple physicochemical features dictate bacterial propensity to evolve resistance against AMPs. Our work could serve as a promising source for the development of AMP-based new therapeutics less prone to resistance, a feature necessary to avoid any possible interference with our innate immune system.

P7: The investigation of bacteria isolated from deep-sea sponges for the development of novel antibiotics

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Bacteria isolated from deep-sea creatures, like sponges, have a high potential to produce novel antibiotics as the deep-sea environment is relatively unexplored with very different conditions to those on the earth's surface. After the bacterial isolation from a deep-sea sponge of the genus *Pheronema*, one of the isolates showed antimicrobial activity against gram-positive and gram-negative bacteria and was therefore selected for further experiments. The gram-positive isolate was characterised by 16S rDNA sequencing and a possible relation to the genus *Domibacillus* was shown. The antimicrobial, purified by chromatography, was identified as a protein and its thermostability between -20°C and 56°C was shown. The antimicrobial compound was successfully tested against multiple clinically relevant indicator strains, including meticillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant enterococci. Additionally, the minimal inhibitory concentration was determined for *Klebsiella pneumonia*, *Salmonella typhimurium* and *Escherichia coli* as 29 µg/ml and for *S. aureus* and MRSA as 7.25 µg/ml. Toxicity testing in *Galleria mellonella* showed that the compound is non-toxic in a dose of at least 2.45 mg/kg. Therefore, the antimicrobial compound has shown potential to be a promising candidate for a novel antibiotic, which could be used to tackle the antimicrobial resistance crisis.

P8: First Confirmation of Nisin P Structure

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Nisin is a small peptide with antimicrobial activity against a wide range of pathogenic bacteria. Nisin is classified as a bacteriocin class I, because it is ribosomally synthesised and post-translationally modified. More specifically, nisin is considered a lantibiotic because it contains lanthionine (Lan), an unusual amino acid formed by two alanine residues linked by a sulphur atom through their β-carbon. Other unusual amino acids present in nisin are dehydroalanine (Dha), dehydrobutyrine (Dhb) and β-methyl-lanthionine [1]. Nisin has nine natural variants that have been reported at the moment, including nisin P. The Nisin P operon was previously identified in the genomes of two *Streptococcus* species [2, 3]. However, its production has not been reported before. We found that nisin P is produced by another *Streptococcus sp* and here, and we analysed its structure by nanoLC-MS/MS. The trypsinization of nisin P gave 13 exclusive unique spectra of 78 total spectra, with 19 out of 31 amino acids being represented with 99% probability. Using this technique, we confirm the presence of rings A, C and D, while rings B and E were found in approximately 50% of the chemical species, based on the presence of carbamido-methylations that were observed in modified cysteine residues. Thus, we confirmed the previously predicted structure of nisin P.

1. Gharsallaoui, A., N. Oulahal, and C. Joly. Crit Rev Food Sci Nutr, 2016. 56; 2. Zhang, Q., et al. Proceedings of the National Academy of Sciences of the United States of America, 2012. 109 (45): p. 18361-18366; 3. Wu, Z., et al. Gene, 2014. 535(2): p. 156-164.

P9: Isolation of antimicrobial compounds from three bacteria recovered from deep-sea glass sponges

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The holobionts of deep-sea sponges are attracting attention as a source of novel antimicrobial compounds, with cultivation-dependent approaches yielding promising results [1,2]. In screening biological samples from hard-to-reach habitats, such as the deep-sea, it may possible to improve the discovery of unique compounds. In this project, two species of deep-sea sponge were sampled from the North Atlantic at 1.5 – 2.8 km deep.

Three bacterial isolates (PB091, RC230 and RE697) were recovered on R2a medium and were selected for detailed analysis. Compounds were purified using liquid chromatography (hydrophobic interaction chromatography and size exclusion) and investigated to identify the minimum inhibitory concentration against Gram-positive and -negative bacteria and in and Greater Wax moth larvae assays. Finally, a draft genome obtained for PB091 was assembled and annotated using Geneious Prime and analysed using the AntiSMASH-5.0 mining tools.

Compounds produced by strain PB091 demonstrated activity towards various methicillin resistant *Staphylococcus aureus*, while RC230 and RE697 recovered compounds displayed broad spectrum activity against MRSA and *Escherichia coli*. Analysis of the draft genome of PB091 (6.7 Mbp) indicates that it is a member of the Delftia genus. AntiSMASH hits suggest the presence of antimicrobial peptide encoding gene clusters.

Bacteria isolated from deep-sea sponges are a possible source of novel antimicrobial agents. Characterisation of the three isolates recovered here and the antimicrobial compounds thus far purified from them may reveal hits that warrant further development. Draft genomes will be obtained for all three isolates and metagenomic analysis of the sponge holobiome will facilitate identification of the uncultivable bacterial taxa and may reveal relevant antimicrobial production gene clusters.

[1] Xin YJ, Kanagasabhapathy M, Janussen D, Xue S & Zhang W (2011) Phylogenetic diversity of Gram-positive bacteria cultured from Antarctic deep-sea sponges. Polar Biol 34: 1501-1512.; [2] Xu D, Han L, Li C, et al. (2018) Bioprospecting deep-sea Actinobacteria for novel anti-infective natural products. Frontiers in microbiology 9: 787.

P10: Plant foods as a source of antimicrobial peptides for food preservation

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According to the World Health Organization (WHO), 350,000 deaths per year worldwide are related to foodborne diseases caused by bacteria, viruses, parasites, and/or toxins due to contaminated food [1]. Therefore, the identification of alternative preservatives affecting a wide range of microorganisms is of high importance.

The aim of the project was to obtain antimicrobial peptides from plant foods, which can be used as preservatives in food production. Since the antimicrobial peptides are derived from a food source, they are considered as non-toxic and compatible with the clean label strategy.

Thus, storage proteins were extracted from chickpeas and peptides were then generated by enzymatic hydrolysis with chymotrypsin. The resulting peptide profiles were comprehensively analyzed by ultra-performance micro-LC-ESI-TripleTOF-MS/MS. Antimicrobial candidates were identified by virtual screening using the Antimicrobial Peptide Database and structure prediction software like I-Tasser and Helical Wheel Projection. Main selection criteria were a positive net charge, the hydrophobic content, the ability to form an amphipathic helical structure and the water solubility. 21 antimicrobial peptide candidates were identified in the peptide profile of the chickpea storage protein legumin. Afterwards, the antimicrobial activity of the synthesized peptides was experimentally tested against 14 different pathogens. The peptides leg1 (RIKTVTSFDLPALRFLKL) and leg2 (RIKTVTSFDLPALRWLKL) showed the lowest minimal inhibitory concentration (MIC) of 62.5 µM against *Escherichia coli*, 15.6 µM against *Bacillus subtilis* and 125 µM against *Clostridium perfringens*, Extended-Spectrum-Betalactamase building *E. coli* and *Pseudomonas putida*.

Consequently, leg1 and leg2 are antimicrobial peptides generated from chickpea legumin, which may be suitable to protect against food spoilage and foodborne infections.

"This PhD project is promoted by the Adalbert-Raps-Stiftung, Kulmbach"

[1] Infectious Disease Epidemiology Annual Report 2016; Robert Koch-Institut Berlin

P11: Synthesising and Screening Antimicrobial Peptides against Multidrug Resistant *Escherichia coli*

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In 2016, the O'Neil report on antimicrobial resistance (AMR) reported 50,000 deaths in Europe and the United States due to AMR bacterial infections [1]. There is an ever-growing gap between the available therapeutics and the increase in microbial resistance, particularly in gram negative bacteria. It is predicted that by 2050, there will be around 10 million deaths a year due to AMR infections [2]. *E. coli* is the most common gram negative bacterial infection in humans and in 2017, *Enterobacteriaceae*, mainly *E. coli*, comprised 84.4% of antibiotic resistant bloodstream infections [3]. Experimentally validated antibacterial peptides, up to 24mer in length, were selected from CAMP [4] and DRAMP [5] databases. Peptides were synthesised by SPOT-synthesis and screened against a multidrug resistant strain of *Escherichia coli*, with and without 10% human serum. High throughput screening was done with resazurin and fluorescence was measured. Results were analysed by Gait-cad [6] and peptides were put into 4 different activity levels depending on their IC75 when compared to a control peptide. 12 peptide sequences were selected for solid-phase resin synthesis, fully active in 10% serum, fully active without serum and peptides with weak activity to validate screening. Here we report results from minimum inhibitory concentrations assays, with 0%, 10% and 25% human serum, against a clinical isolate of multidrug resistant *E. coli* and other strains of multidrug resistant bacteria and time kill