Management of *Staphylococcus* Mediated Systemic Infection by Enhancing the Resurging Activity of Co-trimoxazole in Presence of Cryptdin-2

Amrita Kaur¹ · Shiv Kumar Chabba² · Ujjwal Jit Kaur¹ · Arashdeep Kaur¹ · Simran Preet³ · Praveen Rishi¹

Received: 19 July 2017 / Accepted: 6 September 2017 / Published online: 9 September 2017
© Association of Microbiologists of India 2017

Abstract Resurgence of sensitivity of the antibiotics, to which the pathogen had developed resistance in the past, requires special attention for strengthening the reservoir of antimicrobial compounds. Reports in the recent past have suggested that co-trimoxazole (COT) has regained its activity against methicillin resistant *Staphylococcus aureus* (MRSA). The present study exploited the use of COT in the presence of an antimicrobial peptide (AMP), cryptdin-2 (a murine Paneth cell alpha defensin), in order to reduce the selective pressure of the antibiotic on the pathogen. *In vitro* antibacterial activity and in vivo efficacy of the combination was ascertained against MRSA induced systemic infection using a murine model. Observations of the present study might help in restoring the regained activity of conventional antibiotics, such as COT, when used in combination with novel antimicrobial molecules like AMPs. This might prove as a viable strategy to eliminate the chances of re-occurrence of resistance due to their multi-prong targeting and synergistically combating infections caused by these resistant pathogens.

Keywords Alpha-defensins · Antibiotic resistance · Co-trimoxazole (COT) · Cryptdin-2 · Methicillin resistant *Staphylococcus aureus* (MRSA)

Introduction

Staphylococcus aureus, having developed resistance to the first generation antibiotics initially, is gradually emerging not only as methicillin resistant but is also showing decreased sensitivity towards vancomycin [1, 2]. Thus, methicillin resistant *S. aureus* (MRSA) and vancomycin intermediate *S. aureus* (VISA)/vancomycin resistant *S. aureus* (VRSA) mediated hospital or community acquired infections are posing a serious problem for their management. Therefore, there is a need for establishing effective treatment options to combat such infections [3]. This can be made possible, either by revisiting the therapeutic armamentarium or by exploring other agents that can be used alone or in combination with conventional antibiotics. In this context, studies have been carried out using previously efficacious antibiotics, including co-trimoxazole (COT) and clindamycin [4]. COT, a combination of trimethoprim/sulphamethoxazole, an antibiotic in use earlier for several decades, gradually becoming inactive in the recent past, has again demonstrated in vitro effectiveness against *S. aureus*, as well as resistant MRSA isolates [5, 6]. Studies conducted in Israel indicated a heightened sensitivity of methicillin resistant isolates to COT over the years from 73% in 1994–1998 to 95% in 2001–2004, with a similar trend observed in the USA as well [7–9]. The efficacy of COT as a substitute to vancomycin has also been reported against MRSA mediated bacteremia [10, 11]. In view of these reports, it is the right time to make efforts to retain the re-gained efficacy of COT against MRSA. In

✉ Praveen Rishi
rishipraveen@yahoo.com

¹ Department of Microbiology, Basic Medical Sciences Block-1, Panjab University, Sector-25, South Campus, Chandigarh, India

² Department of Pathology, MVJ Medical College and Research Hospital, NH4, Dandupalya, Kolathur Post, Hoskote, Bangalore, India

³ Department of Biophysics, Basic Medical Sciences Block-2, Panjab University, Sector-25, South Campus, Chandigarh, India

this context, world-wide, antimicrobial peptides (owing to their direct antibacterial and immunomodulatory properties) are being exploited alone [12–21] as well as in conjunction with antibiotics against various pathogens including *S. aureus* [22–26] in order to reduce the selective pressure of the antibiotic on the pathogen, thereby eliminating the risk of reversal of the sensitive phenotype to the resistant one yet again. As per our previous experience, while exploring the activity of cryptdin-2 (a murine Paneth cell α -defensin) against various pathogens, the peptide was found to exhibit a pronounced activity against *S. aureus* [22]. All these observations prompted us to validate the potential of COT-cryptdin-2 combination against MRSA mediated systemic infection in mice.

Materials and Methods

Bacterial Strains

Two staphylococcal strains, i.e. *S. aureus* ATCC 9144 (procured from the Institute of Microbial Technology, Chandigarh, India) and a clinical methicillin resistant strain of *S. aureus*, MRSA P.E. 9050 (procured from Government Medical College and Hospital, Sector 32, Chandigarh) were employed in the present study. The strains were maintained on nutrient agar slants and plates.

Animals

BALB/c mice of either sex, 4–6 weeks old (18–22 g), procured from Central Research Institute, Kasauli (India) were used in the present study. Before the start of all experiments, animals were adapted to the conditions of animal room for a week. All the animals received standard pellet diet and water ad libitum. The experimental protocols were approved by the Institutional Animal Ethics Committee, Panjab University, Chandigarh, India (Approval Number-IAEC/411 dated 11/09/2013) and guidelines issued by Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India, on animal experimentation, were strictly followed.

Synthetic Cryptdin-2 and COT

Chemically synthesized cryptdin-2 with amino acid sequence LRLVLCYCRRTRGCKRRERMNGTCKRGHLMYTLCCR, identical to the sequence of mouse Paneth cell cryptdin-2 with disulphide linkages between Cys^I–Cys^{VI}, Cys^{II}–Cys^{IV}, Cys^{III}–Cys^V was procured from Genpro Biotech, New Delhi, India. It was suspended in 0.01% acetic acid, stored as a stock solution of 1 g/L at –20 °C

and was used within 3 weeks. The commercially available COT consisting of trimethoprim and sulfamethoxazole in the ratio of 1:5 was used in the current study.

In-Vitro Activity of COT and Cryptdin-2

Minimum Inhibitory Concentration (MIC)

The MICs of both, cryptdin-2 and COT, against both the staphylococcal strains were determined by broth dilution technique as described by us previously [16].

Fractional Inhibitory Concentration (FIC)

To evaluate synergy between the two antibacterial agents, FIC index was determined by checkerboard microtitre test, using an 8-by-8 well configuration at 64 different combinations as described by us previously [25].

Scanning Electron Microscopic Analysis (SEM)

The ultrastructural changes induced by cryptdin-2 and COT, alone as well as in combination, on staphylococcal cells incubated with these agents were studied by electron microscopy. Samples were processed and examined for any change in morphology by a scanning electron microscope (JEOL JEM 1600 model) as described by us earlier [25].

Evaluation of Efficacy of the Combination using *Staphylococcus* Mediated Systemic Infection Model

Encouraging results obtained with the cryptdin-2 and COT combination prompted us to study the in vivo efficacy of the combination using a murine model. For standardization of infection model, 0.2 mL of bacterial suspension containing 10⁷CFU/mL of MRSA P.E. 9050 was administered intravenously [27] and systemic infection was confirmed on the basis of isolation and enumeration of bacterial load from livers, spleens and kidneys of infected animals post 6 h of infection. Furthermore, to investigate the efficacy of the agents used, mice were randomly divided into six groups containing 6–8 mice per group. Group 1: Mice in this group served as control and were administered 0.2 mL of PBS. Group 2: Mice were infected with MRSA P.E. 9050 via intravenous route (0.2 ml PBS containing 10⁷CFU/mL). Group 3: Mice were treated with a single dose of cryptdin-2 (5 µg/mouse). Group 4: Mice were treated with COT (9.6 mg/kg body weight). Group 5: Mice were treated with cryptdin-2 (5 µg/mouse) + COT (9.6 mg/kg body weight). Group 6: Mice were treated with half the respective doses of cryptdin-2 (2.5 µg/mouse) + COT (4.8 mg/kg body weight). COT and cryptdin-2 were administered intravenously. All mice were sacrificed

12–14 h post therapy and their livers, spleens and kidneys were removed aseptically for assessment of bacterial load in terms of CFU per organ.

Histological Studies

Liver, spleen and kidney tissue samples were fixed in 10% buffered formalin and processed for histological examination by staining with hematoxylin–eosin and thereafter viewing under the light microscope.

Estimation of Biochemical Parameters

Extent of Lipid Peroxidation (LPO)

Lipid peroxidation was estimated as described by Wills [28]. The amount of malondialdehyde-thiobarbituric acid (MDA-TBA) chromophore, an indicator of extent of lipid peroxidation, was estimated using an extinction coefficient of $1.56 \times 10^5 \text{ M}^{-1} \text{ cm}^{-1}$. The results were expressed as nanomoles of MDA-TBA chromophore formed per mg of protein.

Estimation of Nitric Oxide Levels

Nitrite levels were measured by a slight modification of the method of Green et al. [29] and served as an indicator of nitric oxide production. The optical density was measured at 546 nm using a standard graph of sodium nitrite.

Estimation of Catalase Activity

The catalase activity was assayed by the method of Luck [30]. Change in absorbance was recorded spectrophotometrically at 240 nm.

Determination of Superoxide Dismutase (SOD) Activity

SOD activity was measured by the method of Kono [31]. Change in absorbance was read at 560 nm for 3 min at 30 s interval. SOD activity was expressed as units of SOD/mg of protein where one unit of activity is defined as the amount of SOD required to inhibit the rate of reduction of nitro blue tetrazolium (NBT) by 50%.

Statistical Analysis

Data were expressed as mean $\pm$ standard deviation of three independent experiments. Statistical analysis was done by Student's two sample *t* test and one way analysis of variance (ANOVA) followed by pair wise comparison procedures (Tukey test) using Jandel Sigma Stat Statistical

Software, version 2.0. In all cases, statistical significance was defined as *p* value of at least <0.05 .

Results

In-Vitro Activity of COT and Cryptdin-2

Minimum Inhibitory Concentration (MIC)

The MIC of cryptdin-2, both against *S. aureus* ATCC 9144 as well as MRSA P.E. 9050, was found to be 15 mg/L. The MIC of COT was found to be 0.5 mg/L for *S. aureus* ATCC 9144 and 16 mg/L for MRSA P.E. 9050.

Fractional Inhibitory Concentration (FIC)

Sharp reduction in optical density was observed in the combination wherein the concentration of cryptdin-2 was 3.75 mg/L and COT was 1 mg/L. The FIC index was found to be 0.3125, which indicated in vitro synergy between the two agents.

Scanning Electron Microscopy

To investigate the possible mechanism by which cryptdin-2 exerts its anti-staphylococcal activity, morphology of peptide-treated bacteria was examined. After 60 min of incubation with sub-MIC values of cryptdin-2, COT and a combination of cryptdin-2 + COT, all the staphylococcal cells revealed membrane wrinkling and probable leakage of cytoplasmic contents through the damaged cytoplasmic membrane (Fig. 1). Maximum damage was observed in bacterial cells treated with the combination.

Therapeutic Potential of the Combination of Cryptdin-2 and COT Against Systemic MRSA Infection

Enumeration of Bacterial Load

Therapeutic efficacy of cryptdin-2 and COT when administered alone, as well as in combination, was investigated after 12–14 h of chemotherapy in terms of reduction in the bacterial load in various target organs of mice infected with MRSA P.E. 9050. In the livers of mice in the treatment group, log unit decrease in MRSA count was found to be 0.94 in group 4 but was found to decrease by 1.43 log units in case of Group 3 in comparison to the infected mice in Group 2. The most significant decrease was found in Group 5 with a value decreasing up to 3.01 log units in comparison to the infected group. Similarly, in spleens of treated mice belonging to Group 3, Group 4 and Group 5, 0.95,

Fig. 1 Scanning Electron Micrographs: **a** Untreated (control) cells of *S. aureus* revealing characteristic grape like appearance, **b** *S. aureus* treated with sub-MIC concentration of cryptdin-2 for 60 min with visibly distorted cellular surface, **c** *S. aureus* treated with sub-MIC

concentration showing damaged cellular structure, **d** *S. aureus* cells showing maximally distorted cellular architecture when treated with a combination of cryptdin-2 and COT

1.03 and 2.71 fold log unit reduction was observed respectively. A similar decrease in log units was also observed in case of kidneys with a decrease of 0.5, 0.67 and 2.17 in case of Group 3, Group 4 and Group 5, respectively. Bacterial load reduction in Group 6 (wherein the mice were administered both the agents at half the concentration) was found to be of the same order as in Group 5 (Fig. 2).

Histo-Architectural Studies

The photomicrographs of livers, spleens and kidneys of mice in all the groups have been shown in the Fig. 3, 4 and 5 respectively. Mice liver cells in Group 1 depicted mild cellular vacuolation and the spleen samples of the same group showed mild white pulp expansion, both of which were not considered significant. The kidneys also depicted normal morphology. On the contrary, liver tissue samples of the infected group showed multiple foci of inflammation along with signs of fatty change which lead us to conclude that the organ was heavily infected. The spleen of infected

Fig. 2 Log₁₀ CFU of MRSA in livers, spleens and kidneys in cryptdin-2 treated, cryptdin-2 + COT treated mice versus control animals. Values are expressed as mean $\pm$ SD of six different observations. * p < 0.05 versus infected, # p < 0.05 versus infected + cryptdin-2, \$ p < 0.05 versus infected + cotrimoxazole alone

Fig. 3 Photomicrographs of mouse liver samples (×400) showing, **a** control (normal morphology), **b** infected (multiple inflammatory foci), **c** cryptdin-2 treated (mild vacuolar degeneration), **d** COT treated (lymphocytic infiltration), **e** cryptdin-2 + COT (mild inflammation only)

Fig. 4 Photomicrographs of mouse spleen samples (×400) showing **a** control (normal morphology), **b** infected (enlarged lymphoid tissue), **c** cryptdin-2 treated (mild reactive follicular spleen), **d** COT

treated (non-reactive follicular spleen), **e** cryptdin-2 + COT (very mild inflammation only)

animals showed enlarged lymphoid tissue encroaching upon the red pulp area and the kidney showed a small focus containing lymphocytes, thereby confirming the systemic spread of infection. When cryptdin-2 was administered to

mice post infection, liver, spleen and kidney tissue samples of the treated group indicated recovery. The liver had minor vacuolar degeneration and the spleen had minor follicular reactivity. The kidneys were found to be normal.

Fig. 5 Photomicrographs of mouse kidney samples ($\times 400$) showing **a** control (normal morphology), **b** infected (various foci of inflammation), **c** cryptdin-2 treated (normal morphology), **d** COT treated (small focus of inflammation), **e** cryptdin-2 + COT (normal kidney)

On being treated with COT, the liver, spleen and kidney sections showed lymphocytic infiltration along with multiple foci of inflammation but the reaction observed was less severe than that of the infected group. The most significant results were obtained in Group 5 and 6 as the liver and spleen showed minor inflammation and the kidney depicted normal histological architecture.

Estimation of MDA as an Indicator of ROS Generation

A significant reduction in the amount of MDA, in comparison to the infected group, was observed in the livers, spleens and kidneys of mice treated with cryptdin-2 and cotrimoxazole alone ($p < 0.05$). However, further blunting in the levels of MDA were observed ($p < 0.05$) when mice were administered cryptdin-2 and COT, simultaneously. No significant difference was observed between Groups 5 and 6 when compared with the infected group (Fig. 6a).

Estimation of Nitrite Levels

Infected mice that were administered cryptdin-2 and COT, demonstrated a significant decrease in nitrite levels, as observed in the liver tissue samples ($p < 0.05$). Additionally, a significant reduction in the levels of nitrite were observed in liver tissues treated with cryptdin-2 and COT in combination ($p < 0.05$). No significant difference was

observed between Groups 5 and 6 when compared with the infected group (Fig. 6b).

Estimation of Catalase Activity

It was observed that COT and cryptdin-2, when given alone, resulted in significant rise in the levels of catalase ($p < 0.05$). However, no significant rise was obtained in Group 5 and 6 wherein the combination of these two antimicrobial agents was administered (Fig. 6d).

Measurement of SOD Activity

Change in the functional status of SOD during *Staphylococcus* infection in mouse model was evaluated and it was found that a significant increase in the SOD levels was observed in the livers, spleens and kidney samples of cryptdin-2 and COT treated groups ($p < 0.05$). The most significant results ($p < 0.05$) were obtained in Group 5 and 6 (Fig. 6c).

Discussion

Indiscriminate use of antibiotics along with non-compliance of patients in following the strict antibiotic regime prescribed by the physician is considered as one of the

Fig. 6 Effect of cryptdin-2 alone, COT alone and both in combination on **a** MDA levels (nanomoles of MDA/mg protein), **b** nitrite levels (mmoles of nitrite/mg protein), **c** superoxide dismutase activity (units/mg protein), **d** catalase levels (units/mg protein) in mice

infected with *S. aureus*. All values are expressed as mean $\pm$ SD of six different observations. * $p < 0.05$ versus control, # $p < 0.05$ versus infected, $^{\$}p < 0.05$ versus infected + cryptdin-2 alone, % $p < 0.05$ versus infected + COT alone

factors resulting in emergence of resistance in pathogens. This has out-paced the development of strategies for combating infections caused by these deadly pathogens [32]. In the face of this problem, scientists all over the world are being forced to look for other viable treatment options against these shrewd organisms [33]. A recent trend depicting the resurgence of efficacy of conventional antibiotics has intrigued the scientific community and in the current scenario, it becomes imperative to come up with strategies to at least, maintain the resurging activities of conventional antibiotics [34]. This being the hypothesis of the present study, COT was chosen on the basis of preliminary in vitro studies wherein the MRSA strain was observed to be intermediately sensitive to it. To further mitigate the chances of occurrence of resistance, previous studies have indicated the synergistic action of various peptides (e.g., alpha-helical peptide p18, HE2, colistin, cathelicidins, etc.) with commonly used antibiotics against many pathogenic microorganisms [22, 35, 36]. Therefore, the present study was designed to explore the efficacy of

the combination of COT and cryptdin-2 against systemic MRSA infection in a mouse model.

Interestingly, cryptdin-2 displayed the same MIC value against both, the standard *S. aureus* strain as well as the MRSA isolate indicating its equal effectiveness against both the strains at the same concentration, which can be attributed to the multi-prong approach exhibited by the peptide in its antibacterial mechanism. FIC index obtained using microtitre checkerboard assay substantiated the synergism between the two agents, wherein the FIC value of COT was observed to be sixteen times lesser than the value recorded when COT was used alone. Likewise, a fivefold decrease in the FIC value of cryptdin-2 was also observed. This clearly indicated that the combination was more effective at lower concentrations of the respective agents against the MRSA strain as compared to higher dose of the agents when used individually. This could be attributed to the fact that cryptdin-2 might have resulted in pore formation in the cell membrane of the target organism thereby allowing more penetration of COT which is known

to interfere with the bacterial folate synthesis pathway resulting in cell death [37]. Additionally, it can be hypothesised that the presence of cryptdin-2 might lead to suppression of chromosomally transmitted *mecA* gene responsible for producing a protein PBP-2a which is not affected by the β -lactamase resistant penicillins [38]. All these mechanisms might have acted in a co-operative manner with each other, thereby reducing the dose of the respective agents. Further, reduction in the effective concentration of the antibiotic might also result in reduction of the associated side effects. In comparison to our earlier studies, cryptdin-2 appears to be more potent against *S. aureus* (Gram-positive organisms) as compared to *Salmonella* (Gram-negative organisms) which may be attributed to the difference in the membrane structures of the Gram-positive and Gram-negative organisms [39]. The efficacy of the combination was also reflected in the morphological alterations evaluated by SEM.

Further, in vivo studies were carried out to validate the above mentioned observation using *Staphylococcus* mediated systemic infection model. The study indicated a higher clearance of staphylococci in all the target organs when both the agents were used in combination. Furthermore, histological analysis of the target organs reflected restoration of normal histo-architecture in the adjunct group. Previously, combination studies employing RP-I along with vancomycin have demonstrated heightened efficacy against *S. aureus* infection model [40]. Similarly, maganin-II, cecropin A and vancomycin have also been reported to be effective against *Staphylococcus* infection in vivo [41]. It was also observed in our study that concentration of cryptdin-2 required for in vivo efficacy was much lower than the MIC recorded in vitro. This could be due to the inherent property of the peptide in causing immunomodulation in addition to possessing direct antibacterial activity [42–44]. Therefore, it is possible that exogenously administered cryptdin-2 might have been recognised as an effector molecule of innate immunity.

During the infection process, *S. aureus* encounters oxidative stress within the host tissues. This is exhibited in the form of oxidative burst resulting in production of a large number of toxic by-products and oxidative intermediates. It was interesting to note that treatment with the combination decreased the levels of oxidants, MDA (in all the tissue homogenates) and NO (particularly in liver homogenate). These findings have been further augmented by a previous study suggesting the role of antibacterial agents such as defensins in quenching the free radicals produced due to the interaction between the phagocyte and the bacterium [45]. It is also possible that direct killing of the peptide might have reduced the number of surviving bacteria resulting in quorum quenching and simultaneously proving insufficient to activate histiocytes. In case of

antioxidants, maximum increase in SOD levels was found in the combination group, which points towards the immunomodulatory activity of cryptdin-2. However, no significant increase in the level of catalase was observed in the combination group which is in concordance with our earlier studies against *Salmonella* [22]. By this observation, it can be hypothesised that cryptdin-2 might operate by increasing the SOD levels rather than the catalase levels, for restoring the antioxidant activity of the host.

It can be concluded from the study that cryptdin-2 and COT combination might help in rejuvenating the use of conventional anti-staphylococcal antibiotics in the treatment of multidrug resistant strains of *S. aureus*. Therefore, the present study is indicative of rational use of previously efficacious antibiotics in combination with other effective molecules in order to maintain the sensitivity and to combat the emerging resistance in pathogens like *S. aureus*.

Acknowledgements Financial support from the Department of Microbiology, Panjab University, Chandigarh and Department of Science and Technology (DST-PURSE) is gratefully acknowledged.

References

- Appelbaum PC (2006) The emergence of vancomycin-intermediate and vancomycin-resistant *Staphylococcus aureus*. Clin Microbiol Infect 12:16–23. doi:[10.1111/j.1469-0691.2006.01344.x](https://doi.org/10.1111/j.1469-0691.2006.01344.x)
- Zadrazilova I, Pospisilova S, Masarikova M, Imramovsky A, Ferriz JM, Vinsova J, Cizek A, Jampilek J (2015) Salicylanilide carbamates: promising antibacterial agents with high in vitro activity against methicillin-resistant *Staphylococcus aureus* (MRSA). Eur J Pharm Sci 77:197–207. doi:[10.1016/j.ejps.2015.06.009](https://doi.org/10.1016/j.ejps.2015.06.009)
- Holmes AH, Moore LSP, Sundsfjord A, Steinbakk M, Regmi S, Karkey A, Guerin PJ, Piddock LJV (2016) Understanding the mechanisms and drivers of antimicrobial resistance. Lancet 387:176–187. doi:[10.1016/S0140-6736\(15\)00473-0](https://doi.org/10.1016/S0140-6736(15)00473-0)
- Frei CR, Miller ML, Lewis JS, Lawson KA, Hunter JM, Ormasionwu CU, Talbert RL (2010) Trimethoprim-sulphamethoxazole or clindamycin for community-associated MRSA (CA-MRSA) skin infections. J Am Board Fam Med 23:714–719. doi:[10.3122/jabfm.2010.06.090270](https://doi.org/10.3122/jabfm.2010.06.090270)
- Kaka AS, Rueda AM, Shelburne SA, Hulten K, Hamill RJ, Musher DM (2006) Bactericidal activity of orally available agents against methicillin-resistant *Staphylococcus aureus*. J Antimicrob Chemother 58:680–683. doi:[10.1093/jac/dkl283](https://doi.org/10.1093/jac/dkl283)
- Elwell LP, Wilson HR, Knick VB, Keith BR (1986) In vitro and in vivo efficacy of the combination trimethoprim-sulphamethoxazole against clinical isolates of methicillin resistant *Staphylococcus aureus*. Antimicrob Agent Chemother 29:1092–1094. doi:[10.1128/AAC.29.6.1092](https://doi.org/10.1128/AAC.29.6.1092)
- Bishara J, Pitlik S, Samra Z, Levy I, Paul M, Leibovici L (2003) Co-trimoxazole sensitive, methicillin-resistant *Staphylococcus aureus*, Israel. Emerg Infect Dis 9:1988–1997. doi:[10.3201/eid0909.020666](https://doi.org/10.3201/eid0909.020666)
- Styers D, Sheehan DJ, Hogan P, Sahm DF (2006) Laboratory-based surveillance of current antimicrobial resistance patterns

- and trends among *Staphylococcus aureus*: 2005 status in the United States. *Ann Clin Microbiol Antimicrob* 5:2. doi:[10.1186/1476-0711-5-2](https://doi.org/10.1186/1476-0711-5-2)
9. Tsuji BT, Rybak MJ, Cheung CM, Amjad M, Kaatz GW (2007) Community and health care associated methicillin-resistant *Staphylococcus aureus*: a comparison of molecular epidemiology and antimicrobial activities of various agents. *Diagn Microbiol Infect Dis* 58:41–47. doi:[10.1016/j.diagmicrobio.2006.10.021](https://doi.org/10.1016/j.diagmicrobio.2006.10.021)
  10. Frame PT, McLaurin RL (1984) Treatment of CSF shunt infections with intrashunt plus oral antibiotic therapy. *J Neurosurg* 60:354–360. doi:[10.3171/jns.1984.60.2.0354](https://doi.org/10.3171/jns.1984.60.2.0354)
  11. Goldberg E, Paul M, Talker O, Samra Z, Raskin M, Hazzan R, Leibovici L, Bishara J (2010) Co-trimoxazole versus vancomycin for the treatment of methicillin-resistant *Staphylococcus aureus* bacteraemia: a retrospective cohort study. *J Antimicrob Chemother* 65:1779–1783. doi:[10.1093/jac/dkq179](https://doi.org/10.1093/jac/dkq179)
  12. Harder J, Glaser R, Schroder JM (2007) Human antimicrobial proteins-effector of innate immunity. *J Endotoxin Res* 13:317–338. doi:[10.1177/0968051907088275](https://doi.org/10.1177/0968051907088275)
  13. Bahrndorf S, Gill C, Lowenberger C, Skovgård H, Hald B (2014) The effects of temperature and innate immunity on transmission of *Campylobacter jejuni* (Campylobacterales: Campylobacteraceae) between life stages of *Musca domestica* (Diptera: Muscidae). *J Med Entomol* 51:670–677. doi:[10.1603/ME13220](https://doi.org/10.1603/ME13220)
  14. Midorikawa K, Ouhara K, Komatsuzawa H, Kawai T, Yamada S, Fujiwara T, Yamazaki K, Sayama K, Taubman MA, Kurihara H, Hashimoto K, Sugai M (2003) *Staphylococcus aureus* susceptibility to innate antimicrobial peptides, β -defensins and CAP18, expressed by human keratinocytes. *Infect Immun* 71:3730–3739. doi:[10.1128/IAI.71.7.3730-3739.2003](https://doi.org/10.1128/IAI.71.7.3730-3739.2003)
  15. Abraham P, George S, Kumar KS (2014) Novel antibacterial peptides from the skin secretion of the Indian bicoloured frog *Clinotarsus curtipes*. *Biochimie* 97:144–151. doi:[10.1016/j.biochi.2013.10.005](https://doi.org/10.1016/j.biochi.2013.10.005)
  16. Preet S, Verma I, Rishi P (2010) Cryptdin-2: a novel therapeutic agent for experimental *Salmonella* Typhimurium infection. *J Antimicrob Chemother* 65:991–994. doi:[10.1093/jac/dkq066](https://doi.org/10.1093/jac/dkq066)
  17. Gupta R, Srivastava S (2014) Antifungal effect of antimicrobial peptides (AMPs LR14) derived from *Lactobacillus plantarum* strain LR/14 and their applications in prevention of grain spoilage. *Food Microbiol* 42:1–7. doi:[10.1016/j.fm.2014.02.005](https://doi.org/10.1016/j.fm.2014.02.005)
  18. Sharma D, Mandal SM, Manhas RK (2014) Purification and characterization of a novel lipopeptide from *Streptomyces amritsarensis* sp. nov. active against methicillin-resistant *Staphylococcus aureus*. *AMB Express* 4:50. doi:[10.1186/s13568-014-0050-y](https://doi.org/10.1186/s13568-014-0050-y)
  19. Shireen T, Singh M, Das T, Mukhopadhyay K (2013) Differential adaptive responses of *Staphylococcus aureus* to in vitro selection with different antimicrobial peptides. *Antimicrob Agents Chemother* 57:5134–5137. doi:[10.1128/AAC.00780-13](https://doi.org/10.1128/AAC.00780-13)
  20. Gautam N, Sharma N, Ahlawat OP (2014) Purification and characterization of bacteriocin produced by *Lactobacillus brevis* UN isolated from Dhulliachar: a traditional food product of north east India. *Indian J Microbiol* 54:185–189. doi:[10.1007/s12088-013-0427-7](https://doi.org/10.1007/s12088-013-0427-7)
  21. Hernández-Saldaña OF, Valencia-Posadas M, Norma M, Bideshi DK, Barboza-Corona JE (2016) Bacteriocinogenic bacteria isolated from raw goat milk and goat cheese produced in the center of Mexico. *Indian J Microbiol* 56:301–308. doi:[10.1007/s12088-016-0587-3](https://doi.org/10.1007/s12088-016-0587-3)
  22. Rishi P, Preet S, Bharran S, Verma I (2011) *In vitro* and in vivo synergistic effects of cryptdin-2 and ampicillin against *Salmonella*. *Antimicrob Agents Chemother* 55:4176–4182. doi:[10.1128/AAC.00273-11](https://doi.org/10.1128/AAC.00273-11)
  23. Singh AP, Prabha V, Rishi P (2014) Efficacy of cryptdin-2 as an adjunct to antibiotics from various generations against *Salmonella*. *Indian J Microbiol* 54:323–328. doi:[10.1007/s12088-014-0463-y](https://doi.org/10.1007/s12088-014-0463-y)
  24. Giacometti A, Cirioni O, Kamysz W, D'Amato G, Silvestri C, Del Prete MS, Lukasiak J, Scalise G (2004) *In vitro* activity and killing effect of the synthetic hybrid cecropin A-melittin peptide CA(1-7)M(2-9)NH(2) on methicillin-resistant nosocomial isolates of *Staphylococcus aureus* and interactions with clinically used antibiotics. *Diagn Microbiol Infect Dis* 49:197–200. doi:[10.1016/j.diagmicrobio.2004.02.008](https://doi.org/10.1016/j.diagmicrobio.2004.02.008)
  25. Singh AP, Prabha V, Rishi P (2013) Value addition in the efficacy of conventional antibiotics by nisin against *Salmonella*. *PLoS ONE* 8:e76844. doi:[10.1371/journal.pone.0076844](https://doi.org/10.1371/journal.pone.0076844)
  26. Randhawa HK, Gautam A, Sharma M, Bhatia R, Varshney GC, Raghava GPS, Nandanwar H (2016) Cell-penetrating peptide and antibiotic combination therapy: a potential alternative to combat drug resistance in methicillin-resistant *Staphylococcus aureus*. *Appl Microbiol Biotechnol* 100:4073–4083. doi:[10.1007/s00253-016-7329-7](https://doi.org/10.1007/s00253-016-7329-7)
  27. Takeuchi O, Hoshino K, Akira S (2000) Cutting edge: TLR2-deficient and MyD88-deficient mice are highly susceptible to *Staphylococcus aureus* infection. *J Immunol* 165:5392–5396. doi:[10.4049/jimmunol.165.10.5392](https://doi.org/10.4049/jimmunol.165.10.5392)
  28. Wills ED (1996) Mechanisms of lipid peroxide formation in animal tissues. *Biochem J* 99:667–676
  29. Green LC, Wagner DA, Glogowski J, Skipper PL, Wishnok JS, Tannenbaum SR (1982) Analysis of nitrate, nitrite and ^{15}N nitrate in biochemical fluids. *Anal Biochem* 126:131–138. doi:[10.1016/0003-2697\(82\)90118-X](https://doi.org/10.1016/0003-2697(82)90118-X)
  30. Luck H (1971) Catalase. In: Bergmeyer H (ed) *Methods of enzymatic analysis*. Academic press, New York, pp 885–894
  31. Kono Y (1978) Generation of superoxide radical during auto-oxidation of hydroxylamine and an assay for superoxide dismutase. *Arch Biochem Biophys* 186:189–195. doi:[10.1016/0003-9861\(78\)90479-4](https://doi.org/10.1016/0003-9861(78)90479-4)
  32. Blaskovich MA, Butler MS, Cooper MA (2017) Polishing the tarnished silver bullet: the quest for new antibiotics. *Essays Biochem* 61:103–114. doi:[10.1042/EBC20160077](https://doi.org/10.1042/EBC20160077)
  33. Kalia VC (2014) Microbes, antimicrobials and resistance: the battle goes on. *Indian J Microbiol* 54:1–2. doi:[10.1007/s12088-013-0443-7](https://doi.org/10.1007/s12088-013-0443-7)
  34. Maheshwari R (2007) Combating antibiotic resistance in bacteria. *Indian J Microbiol* 47:181–183. doi:[10.1007/s12088-007-0036-4](https://doi.org/10.1007/s12088-007-0036-4)
  35. Cassone M, Otvos L Jr (2010) Synergy among antibacterial peptides and between peptides and small-molecule antibiotics. *Expert Rev Anti Infect Ther* 8:703–716. doi:[10.1586/eri.10.38](https://doi.org/10.1586/eri.10.38)
  36. Yenuga S, Narmadha G (2010) The human male reproductive tract antimicrobial peptides of the HE2 family exhibit potent synergy with standard antibiotics. *J Pept Sci* 16:337–341. doi:[10.1002/psc.1246](https://doi.org/10.1002/psc.1246)
  37. Wormser GP, Keusch GT, Heel RC (1982) Co-trimoxazole (trimethoprim-sulfamethoxazole): an updated review of its antibacterial activity and clinical efficacy. *Drugs* 24:459–518. doi:[10.2165/00003495-198224060-00002](https://doi.org/10.2165/00003495-198224060-00002)
  38. Lowy FD (2003) Antimicrobial resistance: the example of *Staphylococcus aureus*. *J Clin Invest* 111:1265–1273. doi:[10.1172/JCI200318535](https://doi.org/10.1172/JCI200318535)
  39. Rishi P, Singh AP, Arora S, Garg N, Kaur IP (2013) Revisiting eukaryotic anti-infective biotherapeutics. *Crit Rev Microbiol* 40:281–292. doi:[10.3109/1040841X.2012.749210](https://doi.org/10.3109/1040841X.2012.749210)
  40. Yeaman MR, Kupferwasser D, Yount NY, Waring AJ, Ruchala P (2009) Efficacy of intravenous platelet kinocidin congener RP-1 in a murine model of *Staphylococcus aureus* (SA) biofilm infection. In: Presented at 49th interscience conference on antimicrobial agents and chemotherapy (ICAAC), San Francisco
  41. Cirioni O, Silvestri C, Ghiselli R, Giacometti A, Orlando F, Mocchegiani F, Chiodi L, Della Vittoria A, Saba V, Scalise G

- (2006) Experimental study on the efficacy of combination of α -helical antimicrobial peptides and vancomycin against *Staphylococcus aureus* with intermediate resistance to glycopeptides. *Peptides* 27:2600–2606. doi:[10.1016/j.peptides.2006.05.004](https://doi.org/10.1016/j.peptides.2006.05.004)
42. Huang Y, Huang J, Chen Y (2010) Alpha-helical cationic antimicrobial peptides: relationships of structure and function. *Protein Cell* 1:143–152. doi:[10.1007/s13238-010-0004-3](https://doi.org/10.1007/s13238-010-0004-3)
43. Bowdish DM, Davidson DJ, Lau YE, Lee K, Scott MG, Hancock RE (2005) Impact of LL-37 on anti-infective immunity. *J Leukoc Biol* 77:451–459. doi:[10.1189/jlb.0704380](https://doi.org/10.1189/jlb.0704380)
44. Hancock REW, Sahl HG (2006) Antimicrobial and host-defense peptides as new anti-infective therapeutic strategies. *Nat Biotechnol* 24:1551–1557. doi:[10.1038/nbt1267](https://doi.org/10.1038/nbt1267)
45. Labro MT (2000) Interference of antibacterial agents with phagocyte functions: immunomodulation or immune-fairy tales. *Clin Microbiol Rev* 13:615–650. doi:[10.1128/CMR.13.4.615-650.2000](https://doi.org/10.1128/CMR.13.4.615-650.2000)