The characteristics and roles of antimicrobial peptides as potential treatment for antibiotic-resistant pathogens: A review

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Abstract

The emergence of antibiotic resistant bacteria has become a significant and ever-increasing threat to global public health, increasing both morbidity and mortality rates, and the financial burden on health services. Infection by drug-resistant bacteria is anticipated to contribute to the demise of millions of people in the future unless a competent and effective response is devised to engage with this issue. The emergence and spread of resistance is commonly caused by the excessive or inappropriate use of antibiotics and substandard pharmaceuticals. It arises when pathogens adapt to different conditions and develop self-defence mechanisms. Currently, novel antimicrobial peptides (AMPs) have been reported to be the sole cure for some clinical cases of infectious diseases such as sepsis and skin infections, although these agents may, on occasion, require administration together with an adjunctive low-dose antibiotic. Although AMPs are a promising alternative form of anti-microbial therapy and easily applied in the medical sector, they still have limitations that should not be taken lightly. Hence, this review explores the characteristics, advantages and disadvantages of AMPs for their potential in treating antibiotic-resistant pathogens.

Introduction

39 Antibiotics are anti-bacterial medications that inhibit or kill the growth of bacteria. It is usually

40 competent in the treatment of pathogenic bacteria. However, antibiotics constantly lose their anti-

41 bacterial strength as drug-resistant bacteria emerge with the misuse of the antibiotics (Gould &

42 Bal, 2013; Sengupta, Chattopadhyay & Grossart, 2013; Wright, 2014). The antibiotic resistant

43 bacteria are evolving worldwide, threatening the effectiveness of antibiotics, which have

44 previously recovered millions of lives from infectious diseases (Golkar, Bagasra & Pace, 2014;

45 Gould & Bal, 2013; Sengupta, Chattopadhyay & Grossart, 2013; Wright, 2014). This scenario is

46 becoming an important public health problem as it will lead to a prolonged hospital stay, increasing

47 the cost of health care and risk of deaths (Golkar, Bagasra & Pace, 2014; Ventola, 2015).

48 The finding for alternative antimicrobial agents with new mechanisms of action is of urgent need.

49 Although some antibiotics are still effective in killing bacteria, long-term concerns about the good

50 and bad effects of their usage remain to be taken seriously. Worryingly, the number of deaths

51 caused by bacterial infections has risen dramatically, making it one of the leading factors of life-

52 threatening diseases (Morehead & Scarbrough, 2018; World Health Organization, 2020).This

53 problem arises due to the emergence of resistant infectious agents which is a major problem in the

54 treatment of microbial infections (Ventola 2015). The attempts to discover other substances to

55 replace the function of available antibiotics are still on going and being explored up to this day.

56 Antimicrobial peptides (AMPs) are one of the alternative components that may inhibit bacterial

57 growth, possibly replacing the function of antibiotics in the future (Pfalzgraff, Brandenburg &

58 Weindl, 2018). These peptides can bind and interact with the negatively charged bacterial cell

59   membranes, resulting in the disruption of the bacterial cell membrane. They cause damage to the

60   cellular membrane, affecting the transportation of the large molecules such as proteins and ruining

61   the morphology of the cells leading to cell death (Lei et al., 2019). Besides, some AMPs such as

62   non-lytic AMPs including buforin II, indolicin and drosocin can also translocate across bacterial

63   membrane to act on intracellular targets, including ribosomes (Cardoso et al., 2019; Le, Fang &

64   Sekaran 2017). These provide a good rationale for AMPs as a potential alternative for treatment

65   of antibiotic-resistant pathogens. This paper provides an overview on the dilemma and impact of

66   antibiotic resistance, followed by naturally occurring AMPs and synthetic AMPs analogues as a

67   potent antimicrobial agent, in view of finding solutions and improving the quality of health

68   worldwide. The advantages and disadvantages of AMPs are also discussed in relation to those of

69   antibiotics. This paper is intended for all scientists and academicians in related fields to recognize

70   the potential use of AMPs as a therapeutic agent and to serve as a reference for future related

71   studies.

Survey methodology

73   We conducted a literature search covering publications in 2011 till 2021 in relevant topics. The

74   keywords used in the search included "antimicrobial peptides", "antibiotic-resistance",

75   "mechanisms of action", "AMPs", "bacteriocins", "bacterial resistance", "mode of action", "multi-

76   drug resistance", "naturally occurring AMPs" and "synthetic AMPs" through Google Scholar,

77   Web of Science and PubMed Central platform. To ensure a comprehensive and unbiased coverage

78   of the literature, all papers were assessed for information related to the crisis of antibiotic

79   resistance; the history, sources and structure of AMPs; the bacterial resistance mechanisms and

80   mechanisms of action of AMPs; and the benefits or limitations from the use of antibiotics and

81   AMPs. The assessment of the data was performed by multiple individuals for articles in the

82   different sections, followed by compilation and revision by first and corresponding authors.

83   **The Dilemma and Impact of Antibiotic Resistance**

84   Although the world is rapidly moving towards an era of globalization, especially in the field of

85   medical technology, the problem of antibiotic resistance is something that cannot be denied when

86   the number of deaths caused by bacterial infections has risen dramatically, making it as one of the

87   leading factors of life-threatening diseases (Morehead & Scarbrough, 2018). In early 1945, during

88   the era of the discovery of penicillin, Sir Alexander Fleming had proclaimed and warned that

89   antibiotics would one day be a highly demanded drugs and an era of abuse would emerge in the

90   future (Spellberg & Gilbert, 2014). Now, many cases of the microbial resistance against antibiotics

91   are being reported year by year. For example, Gentamicin-Resistance *Enterecoccus* (GRE) was

92   first reported to be resistant to vancomycin, the drug that is in use to treat Methicillin-Resistant

93   *Staphylococcus Aureus* (MRSA) and Methicillin resistant coagulase-negative Staphylococci (MR-

94   CoNS) (Sengupta, Chattopadhyay & Grossart, 2013).

95   The pharmaceutical industries have introduced many new antibiotics in the late 1960s to 2000s

96   which include imipenem, ceftazidime, levofloxacin, linezolid, daptomycin and ceftaroline.

97   However, over time, more and more bacterial resistance appears, and the number of new drug

98   discovery steeply decreases. As a result, bacterial infection becomes a great threat to human health

99   (Ventola, 2015). Worryingly, the genetic traits for antibiotic resistance can be transferred to other

100   bacteria through horizontal gene transfer (HGT). Some resistance can also be caused by mutations

101   at the genetic level of a bacterial cell leading to expression of altered target sites which is no longer

102   recognized by the antibiotics. This has led to the difficulties in controlling bacterial infections, as

103   many antibiotics will not be able to exhibit similar effect over time and in different individuals

104   (Read & Woods, 2014).

105   To avoid the adverse effects, the use of antibiotics should follow the guidelines in managing

106   infections. Excessive use of antibiotics and misuse can lead to many complications such as

107   diarrhea, indigestion, nausea, yeast infections or digestive problems. Incomplete use of antibiotics

108   will not help killing the germs effectively but will increase the selection of resistance to strive and

109   occupy the niche left by the susceptible strains. Antibiotics can also be toxic if not taken correctly

110   and could turn out to be hazardous if taken more than the recommended doses (Ventola, 2015).

111   Previously, a high percentage of antibiotic resistance was found in farm animals and reached

112   consumers through meat products (Bartlett, Gilbert & Spellberg, 2013), resulting in the spreading

113   of bacterial resistance to human and adversely affecting human health (Centers for Disease Control

114   and Prevention, 2013). The use of antibiotics in agriculture also affects the microbial balance of

115   the environment (Bartlett, Gilbert & Spellberg, 2013). Certain amount of antibiotics ingested by

116   livestock are excreted in their stools or urine. This causes widespread dissemination to the

117   environment when their stools are used as fertilizers, which can be absorbed into groundwater and

118   even soil (Bartlett, Gilbert & Spellberg, 2013). Moreover, antibiotics namely tetracycline is used

119   as pesticides on plants. This practice causes long term adverse ecological consequences due to the

120   increase of resistant bacteria contaminating the environment (Golkar, Bagasra & Pace, 2014).

121   The crisis of antibiotic resistance which is becoming increasingly pervasive today is having a

122   deleterious effect on the disease management. In general, infections caused by resistant bacterial

123   strains are more severe than those by susceptible strains (Cosgrove & Carmeli, 2003). For instance,

124   a significantly higher fatality rate has been reported for MRSA in comparison with that for

125   methicillin-susceptible *S. aureus* infection (Cosgrove & Carmeli, 2003; Engemann et al., 2003).

126   The adverse effects of antibiotic resistance can be evaluated in accordance with several factors,

127   including an increase in patient mortality, greater resource utilisation, an escalation in the cost of

128   care and reduction in hospital activity (Friedman, Temkin & Carmeli, 2016).

129   Mortality is the most severe consequence of antibiotic resistance. A report by Centers for Disease

130   Control and Prevention (CDC) in 2019, suggest that more than 2.8 million antibiotic resistance

131   associated infections occur in the United States every year, with over 35 000 fatalities. The annual

132   increase in the number of cases has rendered the use of additional isolation rooms and consumable

133   items necessary, with comparable increases being required in nursing care, support services and

134   associated medical tests (diagnostic test and imaging), and these have placed a greater resource

135   utilisation and the overall cost of care (Friedman, Temkin & Carmeli, 2016). In the wake of this,

136   a relatively substantial amount of hospital spending, in excess of $4.6 billion annual basis, was

137   reported for the treatment of patients infected by antibiotic resistant bacteria (Centers for Disease

138   Control and Prevention, 2019). In addition, this crisis has been observed to rein in everyday

139   hospital activities, with elective operations being cancelled against a background of outbreaks of

140   antibacterial-resistant illnesses (Macraea et al., 2001).

141   ## The Archival and the Diversity of AMPs

142   AMPs have been in the focus as the alternative in addressing the problem of antibiotic resistance.

143   AMPs have been available naturally and synthetically; the history of naturally occurring AMPs

144 and the evolution of synthetics AMPs are discussed in this topic and the analogies between these

145 two forms of AMPs are compiled in Table 1.

146 1.The History of Naturally Occurring AMPs

147 Naturally occurring AMPs that act as host defences are found in nearly all forms of life, and most

148 of them have been reported to be isolated from eukaryotes, such as animals, plants and fungi

149 (Kumar, Kizhakkedathu & Straus, 2018). AMPs were also found in prokaryotic cells when

150 antimicrobial substances known as gramicidins were isolated from *Bacillus brevis* (Nakatsuji &

151 Gallo, 2012). Historically, bacteria have been among the earliest sources of AMPs, and the

152 percentage of AMPs isolated from bacteria have the potential to increase in the future (Figure 1).

153 In 1939, Dubos extracted AMPs from *Bacillus* strain in soil to protect mice from pneumococcal

154 infections (Dubos, 1939). In a previous study, gramicidin showed antibacterial activity against

155 various Gram-positive bacteria (Dubos & Hotchkiss, 1941). Gramicidin is effective in the

156 treatment of infected wounds on guinea pig skin and in the treatment of topical wounds and ulcers

157 (Van Epps, 2006; Gause. & Brazhnikova, 1944), thus demonstrating their potential as the first

158 commercially used AMPs in the health industry. After that, in 1941, other AMPs isolated from

159 bacteria, called tyrocidines, were found to be effective against Gram-negative and Gram-positive

160 bacteria (Dubos & Hotchkiss, 1941).

161 In bacteria, AMPs help particular organisms by killing other bacterial species that compete for the

162 same nutrients and ecological niche. Known as bacteriocins, bacterial AMPs can be classified into

163 two classes: lantibiotics and non-lantibiotics. Lantibiotics are AMPs comprising the non-natural

164 amino acid lanthionine. In 1947, a type of lantibiotic AMPs isolated from *Lactococcus lactis*,

165   known as nisin, was found to be active against a number of Gram-positive bacteria and historically

166   used as a preservative for many years without any noticeable growth of resistance (Mattick &

167   Hirsch, 1947). Meanwhile, non-lantibiotics are AMPs composed of thermostable peptides which

168   do not contain lanthionine and do not undergo post-translational modifications (Heng & Tagg,

169   2006). Garvicin Q (GarQ) is a type of non-lantibiotics AMPs with a relatively broad antimicrobial

170   spectrum towards *Listeria* and *Lactococcus* spp (Tymoszewska et al., 2017).

171   In plants, AMPs play an important role in their protection against the infection of bacteria or fungi.

172   In 1942, another AMPs, called purothionin, isolated from *Triticum aestivum* plants (Balls, 1942),

173   was detected to be effective against other bacteria (Ohtani et al., 1977). The thionin family is

174   among the best-studied groups of AMPs isolated from plants, apart from plant defensins and

175   cyclotides.

176   The highest diversity of AMPs was found in animals. For example, in 1956, AMPs named

177   defensins were discovered from rabbit leukocyte isolation (Hirsch, 1956). Later, in 1960, an AMP

178   called lactoferrin was successfully isolated from cow's milk (Groves, 1960), followed by the

179   synthesis of the AMPs known as bominins from epithelial cells in 1962 (Kiss & Michl, 1962).

180   Several discoveries of AMPs from leukocytes have also been documented around 1970s and

181   1980s. Among these are rabbit-human α-defensins and purothionin (Selsted, Szklarek & Lehrer

182   1984; Selsted et al., 1993). In 1980, Hultmark et al. (1980) used silk butterflies as a model system

183   to successfully demonstrate that P9A and P9B could be induced in the hemolymph by co-

184   vaccination with *Enterobacter cloacae*. Shortly thereafter, these peptides were renamed as

185   cecropin until they became known as the major α-helical AMPs (Hultmark et al., 1980). In 1987,

186   Zasloff et al. (1987) isolated and characterized cationic AMPs from the African toad frog, *Xenopus*

187   *laevis*, and named them magainin peptides (Zasloff, 1987). A few years later, β-defensin and θ-

188   defensin were characterized after isolation from bovine granulocytes and from leukocytes of the

189   rhesus monkey, respectively (Diamond et al., 1991; Tran et al., 2002).

190   In the early 1990s, there were several views that lysozyme was one of the first AMPs to exhibit

191   antimicrobial activity involving non-enzymatic mechanisms. Based on these views, AMPs are seen

192   to have a role in the immunity of human that lacks an adaptive immune system (Diamond et al.,

193   2009). In the mid-1990s, several other peptides were also discovered, such as the first anionic

194   AMPs found in *X. laevis*, while other peptides in the rumen of sheep and cattle were characterized

195   (Brogden, Ackermann & Huttner, 1997). In addition, AMPs have also been found in fruit flies,

196   called *Drosophila melanogaster*; by losing the genes encoding for AMPs in fruit flies will make

197   them susceptible to fungal infections. This shows the importance of AMPs in protecting flies from

198   microbial invasion (Lemaitre et al., 1996).

199   There are a lot of studies on AMPs that had been conducted to determine their ability to kill bacteria

200   and fight infections. AMPs exist in almost all multicellular organisms and play roles in the

201   mammalian immune system (Lemaitre et al., 1996).They have been widely identified in many

202   areas of the human body that are usually exposed to germ-like infections. AMPs are important in

203   innate modulation, as they can be produced naturally by various types of blood cells, including

204   neutrophils, eosinophils and platelets, in the event of inflammation or injury, supporting that AMPs

205   are among the agents responsible for fighting infections caused by germs (Diamond et al., 2009).

206   2. The Evolution of Synthetic AMPs

207   In general, living organisms produce gene-encoded AMPs that provide an immediate defence

208   mechanism upon invasion by pathogens (Giannamaria & Gabriele, 2020). However, the

209   application of AMPs in the clinical setting has been retsricted due to pharmaceutical limitations

210   such as poor bioavailability, susceptibility to enzymatic degradation and toxicity (Deslouches et

211   al., 2020; Costa et al., 2019). For this reason, synthetic AMPs that can maintain therapeutic

212   effectiveness with higher biological stability and a greater safety profile continue to be developed.

213   Most synthetic AMPs are designed to recapture the amphiphilic properties of natural AMPs which

214   are believed to be the primary determinants of their antibacterial activity. In other words, the

215   natural peptides will be modified to produce *de novo* scaffolds that resemble the parent peptides

216   (Azmi, Skwarczynski & Toth, 2016).

217   Historically, various methods were used to optimize AMPs from natural occurring sources,

218   thus generating synthetic variants. In 1881, the azide-coupling method was used by treating the

219   silver salt of glycine with benzoylchloride to create the first N-protected dipeptide,

220   benzoylglycylglycine. Nevertheless, in 1901, Emil Fischer reported the first synthetic dipeptides

221   by hydrolysis of the glycine diketopiperazine, known as glycylglycine (Lichtenthaler, 2002). The

222   development of temporary amino-protecting groups was required to overcome synthetic

223   challenges. In 1931, the carbobenzoxy (Cbz) group was introduced, followed by the the *ert-*

224   butyloxycarbonyl (Boc) group in 1957 (Anderson & McGregor, 1957; Grapsas, Cho &

225   Mobashery, 1994). The discovery of solid phase peptide synthesis (SPSS) was achieved in 1963,

226   when peptide sequences were synthesized on solid support (Merrifield, 1963). However, the major

227   limitations of SPSS included insufficient coupling and degradation reactions, as well as a build-up

228   of by-products (Pedersen et al., 2012; Schnölzer et al., 2009; King et al., 2009). For that reason,

229   several new techniques of protein synthesis have been developed to overcome the limitations of

230   SPPS. For example, chemical ligation and coupling two peptide fragments together were

231   introduced (Kemp et al, 1981). Other ligation methods such as native chemical ligation (NCL),

232   expressed protein ligation (EPL), and Staudinger ligation were also introduced to overcome the

233   constraint (Dawson et al., 1994).

234   To date, methods for producing synthetic AMPs are constantly being improved. For

235   example, using cationic peptides based on natural templates is becoming one of the most exciting

236   new strategies for synthesizing AMPs today. These AMPs have complex mechanisms of action

237   and do not readily lead to resistance. With their anti-inflammatory properties, as well as

238   antimicrobial synergy, they hold promises as adjunctive strategies to supplement and enhance

239   current therapies (Fjell et al., 2012). Several computational tools have also evolved in the

240   development of more economical and powerful synthetics AMPs (Cardoso et al., 2019). For

241   instances, the empirical methods, machine learning and *de novo* computational methods are being

242   used in the optimization of peptides through random processes (Porto et al., 2012). Genetic

243   algorithms also offer an alternative in the development of synthetic AMPs by identifying

244   antibacterial activity-conferring determinants through successive generations of mutations and

245   deletions in the target sequence (Kliger, 2010; Fjell et al., 2011). These candidates are refined over

246   time as lower fitness values are removed from the candidate sequences (Fjell et al., 2011).

247   Hundreds of synthetic AMPs have been produced with the help of computer aided design

248   (Wimley, 2019). Previoulsy, naturally occuring AMPs are used as templates to optimize their

249   activity and stability by mutating one or more amino acid residues; this was followed by the *de*

250   *novo* design of a variety of synthetic peptides, peptoids, peptidomimetics, oligomers and polymers

251   (Jiang et al., 2021). An example is iseganan, where protegerin is used as the template and one or

252   more amino acid residues has been mutated to other proteinogenic L-amino acids to achieve

253 antimicrobial activity against gram-negative and gram-positive bacteria (Trotti et al., 2004). There

254 are several other examples of synthetic AMPs that are produced using this approach, such as

255 omiganan, which is developed using indolicin; and pexiganan,which in turn, is developed by

256 magainin 2 (Ge et al., 1999; Gottler & Ramamoorthy, 2009; Sader et al., 2004).

257 Another way of producing synthetic AMPs is utilization of β-amino acids as the building blocks

258 or using non-natural N-substituted amino acids (Jiang et al., 2021). For example, synthesized

259 helical β-peptide that was developed from β-amino acid; and synthesized oligo-N-substituted-

260 glycine-based helical peptoid that was developed by magainin 2 amide. Both of these synthetic

261 AMPs show greater and more stable antibacterial activity compared to naturally occuring AMPs

262 (Chongsiriwatana et al., 2008; James & Annelise, 2003; Richard & Samuel 2001). Generally,

263 synthetic AMPs are more stable and possess better activity and selectivity compared to naturally

264 occuring AMPs. However, the limitations of producing synthetic AMPs include the extended time

265 required to do so and the high cost (Jiang et al. 2021).

The Structures of AMPs

267 To clearly identify the potential of AMPs, the structure of AMPs needs to be well elucidated.

268 AMPs are relatively short molecules, containing 12–100 amino acids with an amphipathic

269 structure (Hodges et al., 2011). Several databases exist that manage information and conduct

270 peptide analysis, due to the high numbers of natural, semi-synthetic and synthetic AMPs

271 (Mahlapuu et al., 2016). AMPs can be classified based on their structure, amino acid composition

272 and size. The structural features of AMPs can be divided into four main groups, (1) peptides with

273   amphipathic α-helices (2) β sheets, (3) combined α-helices and β sheet structures (αβ) known as a

274   mixed structure and (4) non–αβ structure known as extended structure (Figure 2).

275   α-helical peptides are the most widely studied types of AMPs to date. The α-helical peptide has

276   two amino acids adjacent to each other with a distance of 0.15 nm between them; the centre is

277   about 100 degrees from the top view. Among the well-known peptides studied in this group are

278   LL-37 and human lactoferricin (Epand and Vogel 1999; Hunter et al. 2005; Legrand et al. 2005;

279   Pasupuleti 2011). In addition, among the other widely studied AMPs are colistin, melittin, nisin,

280   and Cecropin A-Magainin 2 (CAMA) (Bechinger & Lohner, 2006; Kumar, Kizhakkedathu &

281   Straus, 2018).

282   β-sheet peptides are composed of at least two β strands with disulfide bonds between these sheets.

283   Interestingly, almost all β-sheet AMPs contain preserved cysteine residues and form disulphide

284   bonds such as gomesin, polyphemusin, protegerin and tachyplesin (Kumar, Kizhakkedathu &

285   Straus, 2018). However, some studies have reported short β-sheet forming AMPs that do not have

286   disulfide bonds (Cândido et al., 2019; Ong et al., 2014; Ong, Gao & Yang, 2013). For example,

287   the synthetic β-sheet AMP known as IK8-all D (irikirik-NH$_2$) which is derived from β-sheet

288   forming peptides (IRIK)$_2$-NH$_2$ (IK8-all L) has shown no formation of disulfide bonds in its design

289   (Ong et al., 2014).

290   Besides α-helical peptides and β-sheet peptides, there is a kind of AMP structure that had been

291   found with the formation of α-helices and β-sheets (αβ) (mixed structure). In this class of AMPs,

292   the two monomers are packed against each other with the β-sheet of one monomer facing the α-

293   helix of another monomer (Kovaleva et al., 2020). Human β-defensin-2 and pine defensin 1

294   (PsDef1) are among the peptides studied in this group (Jenssen, Hamill & Hancock, 2006;

295   Kovaleva et al., 2020).

296   Extended/random coil AMPs display another unique structure that has been frequently discussed.

297   This structure consist of two or more proline residues, tryptophan, arginine and histidine which

298   have the capabilities to break the secondary structure elements (Bahar & Ren, 2013). In addition,

299   many peptides such as indolicin and moricin, adopt their active structure only after they interact

300   with the target cell membrane. Indolicin is a hemolytic AMP isolated from bovine neutrophils. It

301   is effective as an antimicrobial agent because it has 13 tridecapeptide amides and an extremely

302   high tryptophan content (Cardoso et al., 2019). Indolicin changes its structural profile to a "boat-

303   like"and transmembrane orientation to translocate the bacterial membrane and act on DNA

304   (Cardoso et al., 2019). Moricin is a random coil AMP that was isolated from *Manduca sexta*. It

305   consists of one aspartic acid, two arginine and nine lysine residues and features α-helical structures

306   to perform their membrane-associated or intracellular mechanisms of action (Dai et al., 2008)

307   Apart from those, there have been progressively increasing reports in newly discovered AMPs

308   with cyclic and disulfide-rich AMPs as well as AMPs with more complex topologies in the past

309   two decades. Some studies reported these as a fifth class of AMPs (Koehbach & Craik, 2019).

310   These peptides have been identified based on the nature of the peptide's cyclic topology such as

311   "head to tail" or "head to side chain" as well as the nature of the crosslinks such as the presence

312   of disulfide or thioether bridges (Koehbach & Craik, 2019). For example, microcin J25 (lasso

313   peptides) have been found with a head-to-side chain cycle (magenta) threaded by the C-terminal

314   tail and sterically locked in place by bulky residues (cyan) (Rosengren et al., 2003).

The Bacterial Resistance Mechanisms towards Antibiotics and the Way AMPs can help

Understanding on the mechanisms of antibiotic resistance will allow the relevance of AMPs to be seen as a potential alternative for antibiotics. Antibiotic resistance mechanisms in bacteria and how AMP mechanisms aid in killing bacteria are depicted in Figure 3. This figure displays four major molecular mechanisms by which bacteria can withstand antibiotic effects. Among these are drug-target modifications, antibiotic-degrading enzymes, antibiotic-altering enzymes and antibiotic efflux pumps (Laws, Shaaban, & Rahman, 2019). These resistance mechanisms can occur in one bacterial cell simultaneously, resulting in high levels of resistance to various antibiotic compounds (Peterson & Kaur, 2018). In addition to these four major mechanisms, bacterial biofilm has also attracted a great attention in resistance mechanisms towards antibiotics. Bacteria that attach to the surface and grow as biofilm are protected from killing by antibiotics, thus makes the treatment difficult (Dincer, 2020).

Meanwhile, most of the AMPs were found to kill bacterial cells by disrupting the bilayer membrane without the interference of all the available antibiotic resistance characters that might be present in a bacterial cell. However, there are studies indicating that bacteria can resist AMPs treatment at sub-lethal doses and expel them by efficient efflux pumps (Cardoso et al., 2017). Membrane interactions are important in the direct antimicrobial activity of AMPs (Hollmann et al., 2018; Lei, J. et al., 2019). Several models have been introduced to explain the mechanism of disrupting bilayer membranes by AMPs. These models are the barrel-stave model, the toroidal-pore model, and the carpet model. In the barrel-stave model, recruitment of additional peptides placed perpendicularly into the bilayer will lead to the formation of a peptide-lined transmembrane pore. In this pore, the peptides align with the hydrophobic side facing the lipid core of the

338   membrane, while the hydrophilic regions face the interior region of the pore. In the toroidal-pore

339   model, phospholipids bend continuously from one leaflet to another due to the interaction of

340   AMPs. This then results in a pore lined by both peptides and the head groups of phospholipids.

341   For the carpet model, the mechanism is explained by the formation of micelles due to membrane

342   disruptions by the tension in the bilayer as a result of peptide accumulation (Mahlapuu et al., 2016).

343   Intracellular targeting and inhibition of protein synthesis also act as targets by which some of the

344   AMPs may interfere and express their function and ability to disrupt the cell growth. To reach the

345   cytoplasmic membrane of Gram-negative bacteria, AMPs will translocate through the outer

346   membrane via a self-promoted uptake (Le, Fang & Sekaran, 2017; Mahlapuu et al., 2016).

347   Moreover, activated AMPs cause damages to bacterial cells by attacking an internal target or

348   translocating across the membrane receptors, entering the bacterial cytoplasm and disrupting

349   intracellular targets (Jindal, Le & Yusof, 2015; Malanovic & Lohner, 2016). Bacterial destruction

350   also occurs by the interaction between the electrostatic forces of the positively charged amino

351   acids of the AMPs and the negatively charged cell surface. These create an ion-permeable channel

352   and increase membrane permeability to develop cleavages (Lin & Weibel, 2016).

353   The translocation of AMPs will not only disrupt the cellular membrane but also target some

354   important processes, such as DNA transcription and replication, RNA synthesis and protein

355   synthesis, enzymatic activity, protein folding or cell wall synthesis (Le, Fang & Sekaran, 2017).

356   For example, indolicin acts by targeting DNA and inhibiting the replication process, indirectly

357   killing the bacteria. Bacterial death caused by AMPs could be the result of multiple and

358   complementary actions. The mode of action of AMPs depends on several factors, including peptide

359   concentrations, the targeted bacterial species, tissue localization and the bacterial growth phase

360   (Kumar, Kizhakkedathu & Straus, 2018; Mahlapuu et al., 2016).

361   Bacterial cytoplasmic membranes are rich with negatively charged phospholipids, including

362   phosphatidylglycerol, cardiolipin and phosphatidylserine, all of which are highly attracted to the

363   positive charges of AMPs (Ebenhan et al., 2014). Gram-negative bacteria consist of an additional

364   lipopolysaccharide-rich outer membrane that acts as a barrier to the cytoplasmic membranes. The

365   presence of teichoic acids in the cell wall of Gram-positive bacteria also provide an additional

366   electronegative charge to the bacterial surface (Ebenhan et al., 2014). As opposed to bacteria,

367   human cells seem to be rich in neutrally charged phospholipids, such as phosphatidylethanolamine,

368   phosphatidylcholine and sphingomyelin. This fundamental difference between microbial and

369   mammalian membranes has made AMPs a highly selective agent against bacteria (Ebenhan et al.,

370   2014). The presence of cholesterol in humans affects the fluidity of the phospholipid in the

371   membranes via an increased stability of the bilayer, then, reduce the activity of AMPs via

372   stabilization of phospholipids bilayer (Subczynski et al., 2018).

The Advantages and Disadvantages of AMPs

374   In developing AMPs as the potential treatment for antibiotic-resistant pathogens, the advantages

375   as well as the limitations of AMPs should be considered and are discussed in this topic.

376   1. Advantages of AMPs

377   The long-term and overly frequent use of conventional antibiotics as antibacterial agents has the

378   potential to cause mutations in the bacterium, thereby increasing resistance to the antibiotics

379   themselves (Bahar & Ren, 2013). This issue has prompted researchers and the pharmaceutical

380   industry to focus on identifying drugs capable of replacing antibiotics. AMPs are a type of cationic

381   peptide, an agent thought to be able to fulfil the role of antibiotics. Unlike antibiotics, AMPs

382   interact with the cell membrane of bacteria by neutralizing the charge and, subsequently, causing

383   bacterial death by penetrating the membrane, thereby reducing the risk of bacterial resistance

384   (Mahlapuu et al., 2016). This ability on the part of AMPs indicates them to be more effective than

385   conventional antibiotics.

386   AMPs have demonstrated a wide range of capabilities in killing bacteria as well as fungi and

387   viruses (Amso & Hayouka, 2019; Mahlapuu et al., 2016). Interestingly, AMPs have less side

388   effects on the hosts, as their uses cause a very minimal toxicity to the body based on previous

389   studies (Zharkova et al., 2019; Lei, J. et al., 2019; Mahlapuu et al., 2016). For example, a peptide

390   known as citrus-amp1, which is isolated from citrus, exhibited low toxicity effects when tested on

391   *Galleria mellonella*, a cell line derived from the larval-fat body tissues of the wax moth, and on

392   U87 MG, a human glioblastoma cell line commonly used as a model for cytotoxicity (Kishi et al.,

393   2018). A peptide known as Nisin A also presented low toxicity effects when tested on HT29 and

394   Caco-2 cells by using MTT assay (Maher & McClean, 2006).

395   AMPs with a simple structure-activity relationship are widely used in the development of

396   medicines. They are particularly useful in this regard because they are associated with excellent

397   water stability and solubility (Dehsorkhi, Castelletto & Hamley, 2014). For example, daptomycin,

398   another type of AMPs, has been used as an anionic antibacterial peptide to treat skin infections

399   stemming from Gram-positive bacteria, thereby showing inhibitory effects on *S. aureus* and

400   typhoid bacillus *Salmonella typhi* (Lei, J. et al., 2019).

401   Additionally, AMPs have also demonstrated a good inhibition of cancer cells (Mahlapuu et al.,

402   2016). In fact, cancer cells are more sensitive to AMPs than normal cells. This is because the

403   cytoskeletons of cancer cells do not grow well when compared with those of normal cells, which

404   allows AMPs to easily enter the lipid membrane and form ion channels or pores. This process

405   eventually destroys the cancer cells by causing the leakage of the cell content (Jäkel et al., 2012;

406   Mahlapuu et al., 2016). More specifically, the content of those cationic AMPs associated with the

407   high acid phospholipids that occupy the outer surface of cancerous cell causes changes in the

408   membrane, extracellular matrix and cytoskeleton (Mahlapuu et al., 2016). The loss of

409   phospholipids asymmetry in cancer cells provides them with more negatively charged residues in

410   their upper leaflet, thus favouring electrostatic attraction of AMPs (Martín & Amelio, 2021). In

411   terms of acting as antimicrobial agents, AMPs have the potential to fight antibiotic-resistant

412   bacteria. The bactericidal effect of AMPs is generally due to the creation of pores in the bacterial

413   cytoplasmic membrane, which results in a loss of control over the flow of ions through the

414   membrane and, consequently, cell deaths. This renders the use of AMPs a promising strategy for

415   addressing the problem of antibiotic resistance through fulfilling the role of conventional

416   antibiotics (Lei, J. et al., 2019).

417   2. Disadvantages of AMPs

418   Despite the uniqueness and recognised advantages of AMPs, concerns have been raised about

419   certain disadvantages of their excessive use that may eventually lead to emergence of resistance

420   against AMPs as bacteria will always mutate for survival. Among other disadvantages include

421   several aspects such as toxicity, immunogenicity, haemolytic activity in certain type of human

422   cells, reduced activity based on salt sensitivity, and the high cost of production (Aoki & Ueda,

423   2013; Moravej et al., 2018). These characteristics render the use of AMPs in the field of medicine

424   more difficult.

425   There have been challenges in classifying the good AMPs and AMPs that can cause side effects.

426   In some cases, the use of AMPs is associated with a high risk of toxic effects in human cells. For

427   example, certain peptides such as arenicin, LTX-109 and LL-37 have been found to cause side

428   effects (itching, burning and pain) to mammalian cells in vitro and, further, to be toxic with the

429   formation of pore at the membrane, disruption of the membrane and cell lysis, when injected into

430   the bloodstream (Patrulea, Borchard & Jordan, 2020). This problem urges research to look for

431   more new AMPs compounds with less toxicity effects. In addition, although AMPs have been

432   reported not to elicit an immunogenic response (no interference from the action of the host cell),

433   immunogenicity continues to be a concern and even a serious problem in the development of the

434   peptide drugs (Mahlapuu et al., 2016). Based on previous findings, structural properties such as

435   the changes in peptide sequences (modified amino acids), glycosylation changes, the presence of

436   aggregates and other possible factors have been identified as the factors that can lead to

437   immunogenicity of AMPs (da Cunha et al., 2017; Natalia, Brendan & Sam., 2017). These factors

438   may cause the function of AMPs to be disrupted.

439   A certain number of AMPs have been reported to influence haemolytic activity. Indolicidin, for

440   example, a 13-residue cationic peptide that is rich in tryptophan, has been found to exhibit a broad

441   spectrum of anti-bacterial activity, however it exhibits haemolytic activity that limits their clinical

442   applications (Mirski et al., 2018). Some types of AMPs can interact directly with the host cell and

443   dissolve it, although most AMPs bind to the bacterial opening through electrostatic interactions.

444   The amide peptides exhibit higher antimicrobial activity than natural AMPs, although they are

445   more haemolytic. In addition, the functional analysis of AMPs has demonstrated how their high

446   amphiphilicity and high hydrophobicity contribute to their increased haemolytic capability (Aoki

447   & Ueda, 2013; Bahar & Ren, 2013). However, the haemolytic activity of several AMPs was

448   observed to be different in certain types of different species. For example, based on a previous

449   study, 24 AMPs were evaluated for their haemolytic activity in cells of four different species such

450   as human, dog, rat and bovine. Based on this study, some of the AMPs showed no or less

451   haemolytic activity towards each species, and vice versa (Greco et al., 2020). More thorough

452   studies need to be conducted to identify the most appropriate AMPs that do not cause harm to

453   human.

454   The fact that some AMPs require electrostatic interactions with microbial membranes to form a

455   skeletal structure has caused them to be more sensitive to salt, which often leads to problems with

456   clinical applications (Andersson, Hughes & Kubicek, 2016; Hollman et al., 2018). Human body

457   fluids that have a high salt concentration disrupt the function of these AMPs and, therefore,

458   deactivate those (Bastos, Ferreira & Vitorino, 2017). Thus, the identification of salt-resistant

459   AMPs is essential to improve the effectiveness of AMPs within the human body. AMPs are also

460   rapidly degraded in human body by proteases (Aoki & Ueda, 2013). A feasible production method

461   is required to develop AMPs as drugs. Further studies need to be conducted in this regard, and

462   such efforts will require a lot of investments. For instance, the production of heterologous AMPs

463   within prokaryotic systems is considered to be extremely difficult, as AMPs are associated with

464   the poisoning risk in prokaryotic cells (Aoki & Ueda, 2013).

465 **Conclusion**

466 Physicians and scientists describe the antibiotic resistance crisis as increasingly threatening. This

467 problem may worsen unless the public takes precautionary measures. This issue has prompted

468 research to look for new antimicrobials, leading to ongoing research on the AMPs. However, the

469 development has been slow due to several challenges such as high cost of salvage, potential

470 toxicity and lack of solid guideline for rational design. In addition, it might be possible to modify

471 the characteristics of naturally occurring AMPs to produce synthetic AMPs through certain

472 available methods, but to predict the impact of these modifications in clinical usage is still

473 challenging. Thus, there is a need to observe the effects of structural modifications on their

474 function, activity, and target spectrum. On the other hand, the mechanisms of action of AMPs

475 targeting the cell membrane, make them as a good potential approach in controlling the antibiotic

476 resistant pathogen. A close collaboration between different disciplines and the development of

477 new tools that can decipher the structure-function relationship, as well as efficiently synthesize

478 and modify AMPs molecules will be the key to AMPs related research in the future.

479 **List of abbreviations**

480 AMPs (Antimicrobial peptides); GRE (Gentamicin-Resistance *Enterecoccus*)*;* MRSA

481 (Methicillin-Resistant *Staphylococcus Aureus*); MR-CoNS (Methicillin resistant coagulase-

482 negative *Staphylococci*); HGT (horizontal gene transfer); CAMA (Cecropin A-Magainin 2); DNA

483 (Deoxyribonucleic acid); RNA (Ribonucleic acid); U87 MG (Uppsala 87 Malignant Glioma); LL-

484 37 (form of LL-37); *X. laevis (Xenopus laevis)*; solid phase peptide synthesis (SPSS); native

485 chemical ligation (NCL); expressed protein ligation (EPL).

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861   **Table legends**

862   **Table 1: The analogies between Naturally Occurring AMPs and Synthetic AMPs**

863 **Figure's legends**

864 **Figure 1: Numbers of diversity of AMPs from the data repository of antimicrobial peptides**
865 **(DRAMP). Data obtained from http://dramp.cpu-bioinfor.org/browse/**

866 **Figure 2: Structure of AMPs. The PBD IDs for these structures are: (a) 2K6O for LL-37**
867 **(10.2210/pdb2K6O/pdb) (b) 6MY3 for Gromesin (10.2210/pdb6MY3/pdb) (c) 1FD4 for**
868 **Human β defensin 2 (10.2210/pdb1fd4/pdb) (d) 1G89 for Indolicin (10.2210/pdb1G89/pdb)**
869 **and (e) 5T56 for Microcin J25 (10.2210/pdb5t56/pdb). Structural coordinates were obtained**
870 **from the RCSB Protein Data Bank (PDB) (https://www.rcsb.org/)**

871 **Figure 3: Bacterial resistance mechanisms to antibiotics and the mechanisms of AMPs in**
872 **bacteria.**

Table 1 : The analogy between Naturally Occurring AMPs and Synthetic AMPs

Table 1: The analogies between Naturally Occurring AMPs and Synthetic AMPs

	Naturally Occurring AMPs	Synthetic AMPs	References
Sources/Origin	-Found in many tissues of many different species -Found in nearly all forms of life, and mostly reported to be isolated from eukaryotes, such as animals, plants and fungi -Found in prokaryotic cells	-Non-natural sources -Often created by mimicking natural sequences	(Jiang et al., 2021; Kumar, Kizhakkedathu, & Straus, 2018; Nakatsuji & Gallo 2012)
Content	-Comprised of L-amino acids recognizable by proteases	-The rational design of sequences comprising analogous D-amino acids substituted for L-amino acids	(da Cunha et al., 2017; Zhao et al., 2016)
Discovery methods	-Using classic purification and *in vitro* and *in vivo* techniques	-Combination of trial-and-error experimentation, screening, or computer aided design (increasing the peptide post-translational stability without altering biological function).	(da Cunha et al., 2017; Jiang et al., 2021)
Characteristics	-Frequently susceptible to protease degradation -Low bioavailability (i.e. presence of bioactive molecules at usually low levels). -Low resistance to proteolytic degradation resulting in shorter half-lives	-High bioavailability -Longer half-lives *in vivo*, while maintaining a similar activity and selectivity. -Designed to improve their potential without side effects -Incorporation of multiple functions in the same peptide sequence	(Azmi, Skwarczynski & Toth, 2016; da Cunha et al., 2017; Jiang et al., 2021; Lei et al., 2019; Lu et al., 2020; Mahlapuu et al., 2016; Wimley, 2019)
Examples	-Protegerin -Indolicin -Magainin 2 -*Moringa oleifera* chitin-binding protein (Mo-CBP)	-Iseganan (protegerin as template) -Omiganan (developed from indolicin) -Pexiganan (developed from magainin 2) -Mo-CBP$_3$-PepIII (developed from Mo-CBP)	(Ge et al., 1999; Gottler & Ramamoorthy, 2009; Oliveira et al., 2019; Sader et al., 2004; Trotti et al., 2004)

Figure 1

Numbers of diversity of AMPs from the data repository of antimicrobial peptides (DRAMP).

Data obtained from http://dramp.cpu-bioinfor.org/ browse/
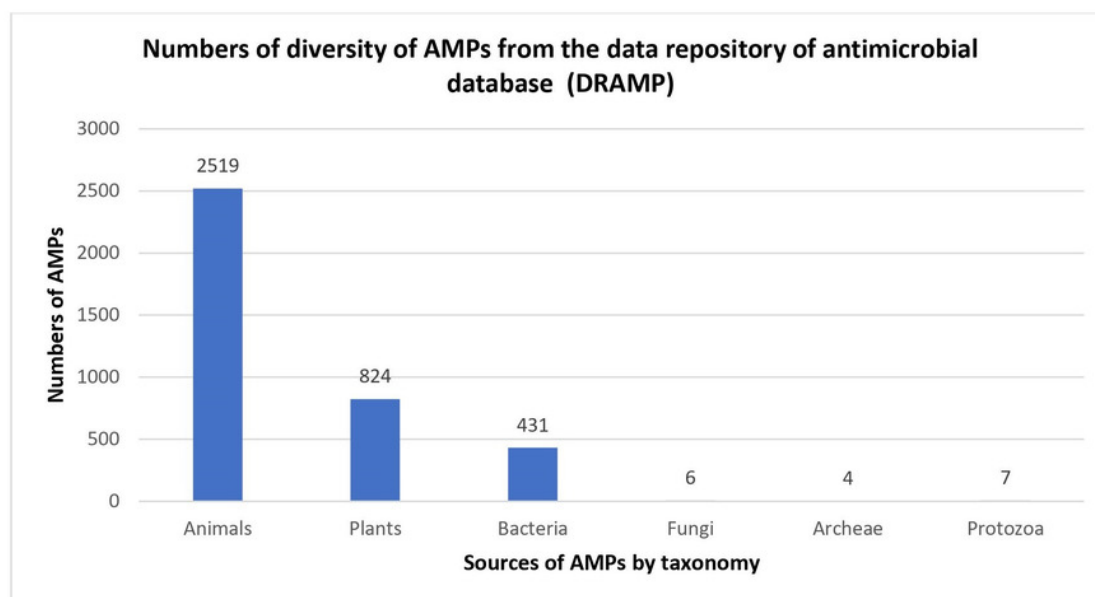
Figure 2

Structure of AMPs.

(a) LL-37 with α-helices structure

(b) Gomesin with β-sheets structure

(c) Human β-defensin 2 with mixed structure
(αβ)

(d) Indolicin with extended structure
(non-αβ)

(e) Microcin J25 with cyclic peptide structure
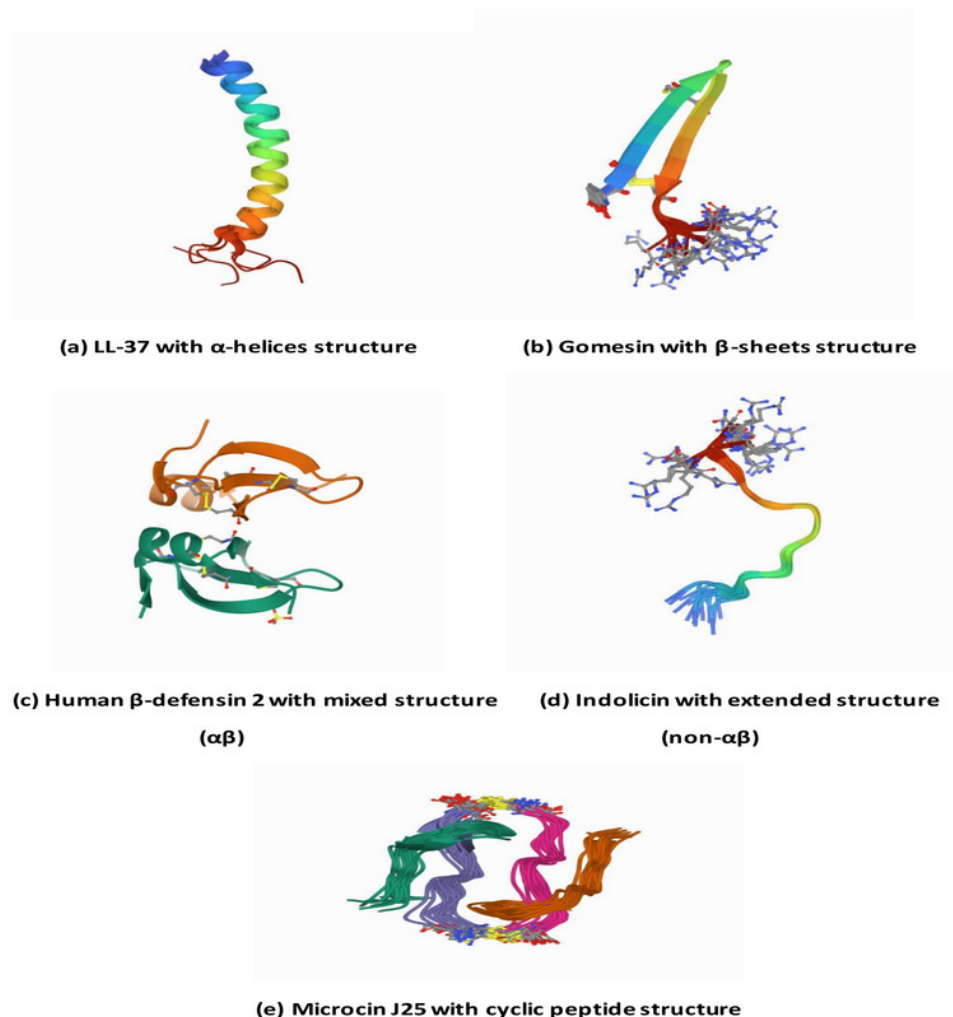
Figure 2: Structure of AMPs. The PBD IDs for these structures are: (a) 2K6O for LL-37 (b) 6MY3 for Gromesin (c) 1FD4 for Human β-defensin 2 (d) 1G89 for Indolicin and (e) 5T56 for Microcin J25. Structural coordinates were obtained from the RCSB Protein Data Bank (PDB) (https://www.rcsb.org/).

Figure 3

Bacterial resistance mechanisms to antibiotics and the mechanisms of AMPs in bacteria.