Intervention Strategies Using Antimicrobial Peptides

Subjects: Medicine, General & Internal
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The emergence of multidrug-resistant H. pylori poses a public healthcare threat, particularly in low- and middle-income countries. Recently, the World Health Organization has classified clarithromycin-resistant H. pylori as high priority in the research and discovery of novel antibiotics. This study was aimed to systematically review the prevalence of primary antibiotic resistance in H. pylori in Southeast Asian countries (SEAC) and to review current studies of antimicrobial peptides against H. pylori. We systematically searched through electronic databases of studies conducted on antimicrobial resistance of H. pylori in SEA countries. Furthermore, we searched articles that conducted studies on antimicrobial peptides, naturally occurring host's defense molecules, against H. pylori. After a series of screening processes, 15 studies were included in our systematic review. Our analysis revealed that primary resistance of H. pylori to metronidazole, clarithromycin, and levofloxacin were high in SEAC, although the primary resistance to amoxicillin and tetracycline remains low. Multidrug-resistant H. pylori are emerging in SE Asian countries. The antimicrobial peptides show promising antibacterial and antibiofilm activity against drug-resistant H. pylori. The research and discovery of antimicrobial peptides against H. pylori in SEAC will help in limiting the spread of antimicrobial resistance of H. pylori.

Keywords: Helicobacter pylori ; antimicrobial resistance ; antimicrobial peptides

1. Introduction

Helicobacter pylori is one of the most common infectious disease agents worldwide, with more than 50% of the world's population being infected with this pathogen, mostly in developing countries [1]. Transmission of *H. pylori* is still uncertain, but several studies have shown that close contact of a mother with a child during childhood is the main transmission route, apart from drinking contaminated water [2]. Since its discovery by Warren and Marshall almost three decades ago, it is now established that this bacterium orchestrates gastric carcinogenesis by producing multiple virulence factors that lead to peptic ulcer and gastric cancer [3]. Gastric cancer is still one of the most common cancers globally, with more than 1 million new cases reported yearly, leading to 768,793 deaths in 2020 alone [4]. Furthermore, gastric cancer patients are mainly detected at the advanced stage of cancer, where cancer prognosis is worse than that of detection at an early stage [5]. A high economic and mortality burden is associated with gastric cancer, with most cases occurring in developing countries where medical resources for early screening and patient management are limited [4]. Therefore, early screening of gastric cancer for detecting precancerous lesions for better prognosis and eradicating *H. pylori* are two essential strategies to prevent gastric cancer development.

Treatment of *H. pylori* includes administering proton pump inhibitors (PPI) coupled with multiple antibiotics through several treatment regimens. In the first-line triple therapy, PPI is administered with amoxicillin and clarithromycin. However, in areas where the clarithromycin resistance rate is higher than 15%, triple therapy containing metronidazole or bismuth-containing quadruple therapy is recommended [6]. Nevertheless, the increasing rate of multiple-drug-resistant *H. pylori* has implications for the eradication of bacteria to prevent gastric diseases, including gastric cancer and ulcers [7]. The World Health Organization (WHO) has declared clarithromycin-resistant *H. pylori* a high priority in the research and development of novel antimicrobial discovery [8]. As such, an alarming concern of the increasing rate of antibiotic resistance presents urgency for the discovery of novel or alternative therapies for *H. pylori*. SEAC are home to more than 650 million people with diverse ethnicities and cultures. Most of these countries are mainly low-and middle-income countries, where medical resources are limited [9]. However, it is also home to diverse natural flora and fauna, with more than 20% of global plant and animal species, and where four out of twenty-five global biodiversity hotspots are found [10]. Antimicrobial peptides (AMP) are a small class of peptides that are part of an organism's innate immunity with an inhibitory effect against pathogens such as the inhibition of nucleotide and protein synthesis of pathogens, formation of the pore at the cell membrane, and the host's immunomodulation [11]. AMPs are good candidates for alternative therapies for the treatment of antibiotic-resistant infections and are broad-spectrum antimicrobial agents to which most bacteria evolution is slow. AMP has been isolated from multiple flora and fauna such as amphibians, fish, and plants [12]. Given that SEA has one of the

world's greatest biodiversity hotspots, this region can be one in which AMP research and discovery can be conducted rapidly.

2. A Review on Intervention Strategy Using AMP

Given the high resistance rates of *H. pylori* to critically important antibiotics in this region, we searched the database on intervention strategies of *H. pylori* treatment using AMP. AMPs are small peptides that have been demonstrated to possess broad-spectrum activity against bacteria. They are usually isolated from natural sources such as the skin of amphibians and venoms. The SEACs are a region with high biological diversity and lush tropical forests where the sources for discovery and research on novel antimicrobial drugs against *H. pylori* can be further explored. As such, we aimed to summarize the studies conducted on AMPs against *H. pylori* for understanding the current progress in research. **Table 1** summarizes the studies conducted on AMPs against *H. pylori*. We found that all the studies conducted demonstrated antibacterial activity of AMPs against *H. pylori* and all studies employed synthetically synthesized AMPs (**Table 1**). Five AMPs, namely cathelicidin [13], bicarinalin [14], odorranain-HP [15], tilapia Piscidin 4 [16], and pleurain-A [17], were initially identified from natural sources and then synthesized synthetically in the laboratory. Notably, some AMPs, including cathelicidin-like AMP [18], bicarinalin [14], fusion human neutrophil peptide 1 [19], and epinecidin 1 [20], showed bactericidal activity against drug-resistant *H. pylori*. In addition, cathelicidin from humans and mice exhibited antibiofilm activity against *H. pylori* and protected the animal model from inflammation induced by *H. pylori* [13]. A study conducted by Jiang et al. [18] found that a cathelicidin-like peptide, namely Cbf-K_{16}, reduced intercellular and intracellular activity of *H. pylori* and decreased *H. pylori* colonization in animal model. Furthermore, helix-coil conformation transitional antimicrobial polypeptides demonstrated bactericidal activity against *H. pylori* at low pH, suggesting its potential for use in human. Besides that, the peptide demonstrated low toxicity in animal study and an examined stomach, suggesting that the peptide is worthy to be evaluated in clinical trials [21].

Table 1. Summary of studies conducted on antimicrobial peptides against *H. pylori*.

Author	Year	Name of AMP	Source	Finding on Antibacterial Activity against *H. pylori*	Reference
Zhang et al.	2016	Cathelicidin	Mouse and human	Bactericidal activity against clarithromycin-resistant *H. pylori*; anti-biofilm activity against *H. pylori* SS1 strain; protected mouse from *H. pylori* orchestrated inflammation and reduced *H. pylori* colonization	[13]
Guzman et al.	2018	Bicarinalin	Synthetically synthesized in lab (anti venom *Tetramorium bicarinatum*)	Perturbation of membrane permeability against drug-resistant *H. pylori*	[14]
Chen et al.	2007	Odorranain-HP	Synthetically synthesized in lab (Diskless odorous frog, *Odorrana graham*)	Showed antimicrobial activity against *H. pylori* (MIC of 20 µg/mL)	[15]
Narayana et al.	2015	Tilapia Piscidin 4 (TP4)	Synthetically synthesized in lab (Nile tilapia, *Oreochromis niloticus*)	Demonstrated potential lytic activity against *H. pylori* surface membrane; disrupted the bacterial cell membrane	[16]
Wang et al.	2007	Pleurain-A	Synthetically synthesized in lab (Yunnan frog, *Rana pleuraden*)	Inhibited growth of *H. pylori* in vitro (30 µg/mL)	[17]
Jiang et al.	2020	Cbf-K_{16}(cathelicidin-like AMP)	Synthetically synthesized	Demonstrated bactericidal activity against clarithromycin- and amoxicillin-resistant *H. pylori*; reduced intercellular and intracellular drug-resistant *H. pylori* in cell culture; showed increased membrane permeation in drug-resistant *H. pylori*	[18]
Zhang et al.	2018	Fusion human neutrophil peptide 1	Expression system in yeast	Eradication of wild type and drug-resistant *H. pylori* in animal model	[19]

Author	Year	Name of AMP	Source	Finding on Antibacterial Activity against *H. pylori*	Reference
Narayana et al.	2015	Epinecidin-1	Synthetically synthesized in lab	Showed bactericidal activity against drug-resistant *H. pylori* and modulated immune response in mouse-infected *H. pylori* for bacterial clearance	[20]
Xiong et al.	2017	Helix–coil conformation transitional antimicrobial polypeptides	Synthetically synthesized in lab	Displayed bactericidal activity against *H. pylori* at low pH, both in vitro and in vivo	[21]
Zhang et al.	2015	Pexiganan	Synthetically synthesized in lab	Inhibited the growth of *H. pylori* (MIC = 4 µg/mL); decreased *H. pylori* colonization in animal model	[22]
Zhang et al.	2017	Fusion PGLa-AM1	Synthetically synthesized in lab	Showed bactericidal activity against *H. pylori* in vitro and clearance of the bacteria in vivo	[23]
Makobongo et al.	2012	$C_{12}K\text{-}2\beta_{12}$	Synthetically synthesized in lab	Ruptured *H. pylori* surface membrane for bactericidal effect; reduced colonization of *H. pylori* in gerbil model	[24]
Rigano et al.	2012	Tomato defensin	Synthetically synthesized in lab	Showed antibacterial activity against *H. pylori* at MIC: 15 µg/mL	[25]
Iwahori et al.	1997	Magainin 2 analog	Synthetically synthesized in lab	Inhibited growth of *H. pylori* in vitro	[26]

Abbreviations: AMP: Antimicrobial peptide; MIC: Minimum inhibitory concentration.

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