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FISH MUCUS: A NEGLECTED RESERVOIR FOR ANTIMICROBIAL PEPTIDES

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ABSTRACT:-

Antimicrobial resistance has posed a great global burden, with the fear that by 2050 it would have killed more people than cancer if nothing much is done about it. Alongside several attempts in place, zoo-therapy is becoming one of important remedies in the modern society, with hope for solution believed to be hidden in nature. In this study, the authors present a review of journal articles and reports obtained through key word search of several literature databases on recent developments in the battle against the antimicrobial resistance using fish derived antimicrobial peptides. The findings indicate despite some limitations of these antimicrobial peptides, their very broad spectrum activity against pathogens keeps them among promising antibioticsas far as the battle against multidrug resistance is concerned. Much as various methods to study antimicrobial peptides do exist, fish mucus remainsless explored. The study recommends aquatic habitat exploration in search for novel bacterial antimicrobial peptides.

Key words: *mucus, peptides, antimicrobial, fish, drug*

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INTRODUCTION

The contemporary increase in mortality due to bacterial infections has solely been attributed to antimicrobial resistance [1]. The potentials of antibiotics to combat bacterial infections have greatly decreased due to the ever increasing multidrug resistance[2,3], that claims over 700,000 lives every year[4], with a projected rise to 10 million by 2050 if alternative solutions are not found[5]. Mortality due to bacterial infections will thus be far worse than malaria which claimed 445,000 lives in 2016 [6], HIV which claims about a million lives annually [7] and cancers which accounted for 8.8 million deaths in 2015 [8]

At the beginning of 2017, the World Health Organization (WHO) made a desperate global call for new class of antibiotics[4]. Seven month later (September, 2017), WHO confirms the world is running out of antibiotics' and declared a global health emergency that calls for an urgent need for more investment in research and development for antibiotic-resistant infections especially for the ranked 'priority pathogens' that if not watched may within this century, return the world back to the "pre-antibiotic era"[9]. This point out the fact that, the search for novel antimicrobials is cardinal as far as combating the antimicrobial resistance is concerned.

In light of the above, four new strategies of overcoming resistance by microbes, are being explored: modifying old antibiotics into entirely new classes,combining antibiotics,supplementing antibiotics with adjuvants or searching nature for novel antibiotics [10]. This is in line with the 2010 Infectious Diseases Society of America (IDSA)'s proposed *10x'20 Global*

Initiative aimed at developing 10 new, safe, and effective antibiotics by 2020 [11]. Regrettably, the rate at which these microorganisms develop resistance has outpaced the rate of discovery/development of new class of antibiotics as scientists dig into various ecological niches: marine, plants, animals, soil in search for novel alternatives [12]. This study believes, the antimicrobial peptides (AMPs) from the fish mucus could be one of such novel antimicrobials nature can offer, however clear information on their source, recent extraction technologies and utilizability need to be brought into limelight. .

Fish mucus as source of antimicrobial peptides

Fish mucus, a fish by-product, is a cardinal component of fish innate immunity. This mucus constitutes an innate defense barrier of fish skin that continuously prevent stable colonization of majority of infectious microbes such as bacteria, fungus into the fish body[13]. Fish mucus is secreted by epidermal goblet cells and comprises of mucins and other substances such as inorganic salts, immunoglobulin, proteins and lipids suspended in water giving it characteristic lubricating properties[14]. The mucus of some fish species like cat fish of *Claris* spp. have for centuries been used in traditional medicine to heal wounds[15-16], burns[17-18] is concerned.

tumors [18]. Their mucus is known for their activity as antibacterial and antifungal agents[14-15,19-20]

In the Indian traditional medicine, *Anguiilabengalensis* (eel fish) has for long been used in treatments of anaemia, burn injury, piles, weakness among other diseases(16) and C*hannastriatus*is well known for itswound healing, anti-inflammation, immune-modulatory as well as mild antifungal and antibacterial roles[21-22]. These properties have mostly been attributed to the presence of antimicrobial peptides (AMPs), polyunsaturated fatty acids (PUFA), mycosporine-like amino acids (MAAs), organic acids among others[18-21]. Common antimicrobial peptides (Table 1) are secreted both by the fish goblet cells and the skin microbes they harbor and are known for their broad spectrum activity against parasitic microorganisms at a very low minimum inhibitory concentration (MIC), Epand and Vogel [22] emphasized the fact that AMPs are classically known for damaging the cell membrane. Much as most of them interacts electrostatically with the surface of negatively charged cell membrane, some interacts with the membrane molecules, as others target intracellular molecules[23-24]. Since they rarely interacts with specific receptors, their microbial targets rarely develops resistant phenotypes[24]. This has kept a number of peptide antibiotics still standing as far as fighting AMR

Table 1: Common Antimicrobial peptides (AMP) produced by bacteria[+] and Fish[*]

Organism	AMP	Sequence	Activity		Reference
			G+	G-	
*M. saxatilis**	Piscidin 1	FFHHIFRGIVHVGKTIHRLVTG	+	+	(25)
*M. chrysops**	Piscidin 3	FIHHIFRGIVHAGRSIGRFLTG	+	+	(25)
P. americanus	Pleurocidin	GWGSFFKKAAHVGKHVGKAALTHYL	+	+	(26)
*O. mykiss**	*rt*CATH_1(R146 -P181)	RRSKVRICSRGKNCVSRPGVGSIIGRPGGGSLIGRP		+	(27)
*G. morhua**	*cod*Cath	SRSGRGSGKGGRGGSRGSSGSRGSKGPSGSRGSSGSRGSKGSRGGRSGRGSTIAGN GNRNNGGTRTA		+	(28)
	Cod β-defensin	WSCPTLSGVCRKVCLPTEMFFGPLGCGKEFQCCVSHFF		+	(29)
*C. semilaevis**	CsHEP	LPLDQVQETEGVGMVRGAGMSDTPAAANEETSVDQWITPYHARVKR		+	(30)
L. lactis[†]	Nisin A	IDhb*cyclo*(AIDhaLA)*cyclo*(AbuPGA)K*cyclo*(AbuGALMGA)NMK*cyclo*(AbuAAbuAHA) SIHVDhaK	+	+	(31)
P. polymyxa[†]	Polymyxin B	(S)-6-Methyloctanoyl-DabTDab-*cyclo*(DabDab$_D$FLDabDabT)		+	(32)
	Polymyxin E	(S)-6-Methyloctanoyl-BTB-*cyclo*(BB D LLBBT)		+	(24)
B. subtilis[†]	Bacitracin A	1-(N-((2-(1-amino-2-methylbutyl)-4,5-dihydro-4-thiazolyl)carbonyl)LDEI-*cyclo*(KDOrnIDFHDDN)	+	+	(33)
B. brevis[†]	Gramicidin A	CHOVGADLADVVDVWDLWDLWLWNHCH2CH2OH	+	+	(34)
	Gramicidin S	*cyclo*(VOrnLDFP)2	+	+	(35)
	Tyrocidines	*cyclo*(VOrnLDFPX3/Orn3;K1,2/F1,2L);X(W3NQYVX2DX1)	+	+	(36)

acid; Dha: didehydroalanine; A:animobutidic acid; Dab: diaminobutyric acid.

*Pisces; †Bacteria G+: Gram+ bacteria; G -: Gram – bacteri; Orn: ornithine; Dhb: didehydroaminobutyric

Methods used in the study of antimicrobial peptides

Different approaches have been employed in the isolation, purification and characterization of AMPs. This has been summarized in the Table 2. As far as production is concerned, most studies encountered have utilized batch fermentation. This is justified by the easy of manipulation and the cost involved as such can easily be performed in the laboratory without having any effect on the result quality[37]. A number of the studies on the AMPs are utilized in their crude form[38-40]. This is justified by the cost and tediousness of the purification process. However, it limits the applicability and potency of the AMPs due to high level of contaminants, leaving RP-HPLC as an extremely valuable tool as AMPs are generally resistant to different organic solvents used in mobile phase and to the pressure employed through the chromatographic process [41].

Table 2: Methods commonly used in the study of AMPs

AMPs source (skin mucus and symbiotants)	**Purification**	**Characterization**	**Reference**
Channapunctatus	Precipitation	Disc diffusion assay	(19)
Clariasbatrachus and *Tilapia mossambicus*	Precipitation	SDS-PAGE	(42)
Frog	Precipitation RP-HPLC	SDS-PAGE MALDI-TOF	(43)
Spiny eel	Precipitation	UV Spectrophotometer SDS-PAGE GC/MS	(44)
Hag fish	Precipitation Solid-phase RP-HPLC	protein–dye binding Electro blotting Edman degradation SDS-PAGE nanoelectrospray MS BLAST FPLC	(45)
Bacillus sp.	RPC RP-HPLC C_{18}-LC	SDS-PAGE LC-MS/MS SEM	(2)
Hag fish	RPC	SDS-PAGE	(46)
Aerobacillus species SAT4	Precipitation Gel permeation RP-HPLC	UV Spectrophotometer SDS-PAGE FTIR NMR XRD	(47)
Gadusmorhua	Precipitation HPLC	SDS-PAGE MALDI MS BLAST	(48)
Lactobacillus plantrum	Precipitation Ion exchange RP-HPLC AA analyzer	SDS-PAGE IR UV MS	(41)
Aeribacillus sp.	Precipitation GPC RP-HPLC	SDS-PAGE FTIR XRD	(47)
Cynoglossusareland Arius caelatus	Precipitation	FTIR Immunomodulation	(49)

U.V-Ultraviolet, GC- Gas chromatography, SEM- Scanning Electron Microscopy, IR- Infrared, MS- Mass spectrometry, SDS-PAGE- sodium dodecyl sulphate polyacrylamide gel electrophoresis, FTIR- Fourier Transform Infrared, NMR- Nuclear Magnetic Resonance, MALDI-TOF- Matrix assisted laser desorption ionization Time-of-flight, LC- Liquid chromatography, XRD- X-ray diffraction

Elucidation of the structure of AMPs is vital as far as understanding their utilization potentials is concerned. To elucidate the structure, the knowledge about the primary amino acid sequence is paramount. Amino acid analyzer connected to a RP-HPLC will reveal this information [41]. However, this can only reveal the percentage composition of the amino acids in a protein not the sequence[2], hence a matrix assisted laser desorption ionization time of flight mass spectroscopy (MALDI-TOF/MS), is commonly used for protein sequence revelation due to its speed, sensitivity and specificity [43]. Although NMR and X-Ray crystallography are the most important tools in determining high-resolution structural information, protein interactions together with size and conformational flexibility limit their use [50]. Therefore, when studying AMPs, mostly 2dimension structures are determined through a combination of FTIR and NMR spectroscopy and bioinformatics tools employed to predict the three dimension structure[47].

Limitations in utilization of antimicrobial peptides

AMPs are known for their high sensitivity to pH, temperature and enzymes, this hinders their production and utilization because of their they are protein in nature and can easily be denatured during the process [37]. Further, their small size and limited number of amino acids limits the structure elucidation of the AMPs. This limited information on the structure of AMPs based on the primary amino acid sequences has jeopardized functional pharmacological approach to dose-response assessments of AMPs and their susceptibility to target cell membrane[51]. Conversely, with the contemporary advancement in bioinformatics, this hold-back of the AMPs have been extinguished(24). AMPs with limited number of rare and D-amino acids are highly vulnerable to protease activity thereby reducing their bioavailability[52] as well as alternative routes of administration[53] and has enabled *S. aureus* gain a substantial resistance against dermicidin[54] yet some have poor penetration of AMPs through the intestinal mucosa when taken orally and implicated toxic.

Summary of the review findings

In this review, the following facts were established about the antimicrobial peptides.

Broad spectrum activity

The review has reveals the vast activity of the antimicrobial peptides against both the gram positive and gram negative bacteria, an activity not common to the conventional standard antibiotics like penicillin and Chloramphenicol. This ability to overcome resistance as highlighted earlier due to the AMPs multiple target sides and non-specific mode of action.

Diversity of study approaches

Several methods to study the AMPs do exist. However, the small size of AMPs together with protein interactions and conformational flexibility limit the study of AMPs. To this effect, combinations of recent technologically advanced approaches have been engaged so as to explore various AMPs from different habitats.

Paying AMPs limitation debt

Low stability of AMPs during production into pharmaceutical forms, toxicity and poor pharmacokinetics jeopardize their utilization. However, structurally altering AMPs [55] and functional pharmacological approach to dose-response assessments of AMPs prior to production[24] are some of the suggested ways to reduce toxicity.Further, the poor pharmacokinetics, especially poor oral bioavailability; short plasma half-lives among others may be overcomeby increasing the number of rare amino acids during AMPs synthesis and production alongside analogue synthesis [52]. Lastly, incorporation of D-amino acids that are stable in the presence of protease [56] and synergism of AMPs with standard antibiotics [57] can increase their stability.

Conclusion and future perspective

Much as AMPs show broad antimicrobial activity especially those isolated from marine habitat using recent technologies, other habitats such as fresh water fish mucus remain less explored.

Conflict of interests

The authors have do declare no conflict of interests.

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