Antimicrobial Peptides in Farm animals: An updated review on its diversity, function, mode of action and therapeutic prospects

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Abstract

Antimicrobial peptides (AMPs) are the arsenals of the innate host defense system exhibiting the ancient evolutionarily conserved characteristics that is present in practically all forms of life. Recent years have witnessed emergence of antibiotic resistant bacteria compounded with a slow discovery rate for new antibiotics that has necessitated scientific efforts to search for alternatives to antibiotics. Research on the identification of AMPs has generated very encouraging evidences that they curb infectious pathologies and are also useful as novel biologics to function as immunotherapeutic agents. Being innate, they exhibit least toxicity to the host and exert wide spectrum of biological activity including low resistance among microbes, and increased wound healing actions. Notably, in veterinary science, the constant practice of massive doses of antibiotics with inappropriate withdrawal programs led to the high risk of livestock-associated antimicrobial resistance. Therefore, the world faces tremendous pressure for designing and devising strategies to mitigate the use of antibiotics in animals and keep it safe for the posterity. In this review, we illustrate the diversity of farm animals specific AMPs, their biochemical foundations, mode of action and prospective application in clinics. Subsequently, we present the data for their systematic classification by the major and minor groups, antipathogenic action, and allied bioactivities in the host. Finally, we address the limitations to their clinical implementation and envision areas for further advancement.

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Antimicrobial Peptides in Farm animals: An updated review on its diversity, function, mode of action and therapeutic prospects

SummaryAntimicrobial peptides (AMPs) are the arsenals of the innate host defense system exhibiting the ancient evolutionarily conserved characteristics that is present in practically all forms of life. Recent years have witnessed emergence of antibiotic resistant bacteria compounded with a slow discovery rate for new antibiotics that has necessitated scientific efforts to search for alternatives to antibiotics. Research on the identification of AMPs has generated very encouraging evidences that they curb infectious pathologies and are also useful as novel biologics to function as immunotherapeutic agents. Being innate, they exhibit least toxicity to the host and exert wide spectrum of biological activity including low resistance among microbes, and increased wound healing actions. Notably, in veterinary science, the constant practice of massive doses of

antibiotics with inappropriate withdrawal programs led to the high risk of livestock-associated antimicrobial resistance. Therefore, the world faces tremendous pressure for designing and devising strategies to mitigate the use of antibiotics in animals and keep it safe for the posterity. In this review, we illustrate the diversity of farm animals specific AMPs, their biochemical foundations, mode of action and prospective application in clinics. Subsequently, we present the data for their systematic classification by the major and minor groups, antipathogenic action, and allied bioactivities in the host. Finally, we address the limitations to their clinical implementation and envision areas for further advancement. Keywords: Anti microbial peptide, AMP, farm animals, cow, pig, horse

Introduction

Globally, intensive livestock farming has led to a rise in the consumption of antibiotics. Imprudent and excessive use of antibiotics in livestock has resulted in an increase in the incidences of antibiotic resistance in several pathogenic bacterial strains and contamination of dairy and meat products with higher levels of antibiotic residues, posing a very severe threat to human health. In 2010, China, the United States, Brazil, India, and Germany were the five top countries in terms of antimicrobial consumption in food animals with 23%, 13%, 9%, 3%, and 3% shares, respectively. The expected rise in this figure by 2030 may be like China (30%), the United States (10%), Brazil (8%), India (4%), and Mexico (2%) (Van Boeckel et al., 2015).

The multi-cellular organism has the natural capacity to derive and devise defense strategies against pathogenic attack. AMPs are part of such an inbuilt defense system, which majorly comes from the cleavage of critical proteins related to innate and adaptive immunity. AMPs are evolutionarily conserved weapons against pathogens in almost every organism. These are also known as "host defense peptides" because of their involvement in conferring non-specific innate immunity to the host. Systematic screening for AMPs in various species has allowed the discovery of many AMPs, some of which have proven very effective as broad-spectrum. Gordon research conference 2019 on Mechanisms and Application: Realizing the Potential of Antimicrobial Host Defense Peptides for Human and Veterinary Medicine has highlighted its importance in the current and post-antibiotic era.

AMPs have been found in almost every stratum of life, ranging from amphibians, reptiles to mammals (van Hoek, 2014; Dutta and Das, 2015; Patocka et al., 2018). It is not surprising that, to date, more than 5000 AMPs have been documented so far either discovered as *denovo*or synthesized in lab (X. Zhao et al., 2013). Farm animals have constantly been exposed to a heavy dose of antibiotics with improper withdrawal programs. The danger of livestock-associated antimicrobial resistance in humans is on the rise. Though there are studies on understanding the innate immunity in farm animals, dedicated and systematic studies on AMPs derived from farm animals and their potential as a substitute to antibiotics are not available. In this review, we comprehensively updated the information about AMPs in farm animals, their diversity, chemical characteristics, mode of action, and challenges in their clinical applications.

Classification of AMPs

Nuclear magnetic resonance (NMR) has played significant role in determining the structural details of AMPs. Analysis of the peptides' three-dimensional structure has offered a deeper insights to their functions. Two dimensional NMR methods are mainly used for obtaining three dimensional structures for small sized peptides AMPs can be divided into five classes according to their secondary structure (Pasupuleti et al., 2012). Though these peptides remain unstructured in solution, they may adopt specific structural characteristics after coming in contact with the membrane. Four such structural characteristics have been observed, namely alpha-helical, beta stranded, Beta hairpin or loop, and extended conformation.

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Most of the AMPs belongs to the well investigated group of α-helical conformations. Various sequences have been identified from and natural sources in addition to the synthetic sequences. In aqueous solution, α-helical AMPs have a linear structure, and upon contact with bacterial membrane or organic solvent, it forms amphipathic helical structure (Mahlapuu et al., 2016). Some of these members are free of cysteine residues

and contain about 50 percent of hydrophobic residues facilitating amphiphilic conformation on membrane interaction. Various AMPs from animal origin fall into this category for example Cecropin P1, BMAP-34, SMAP-29, PMAP, eCATH1, eCATH2 and eCATH3 (Renato Gennaro et al., 1998; Barbara Skerlavaj et al., 1999; B. Skerlavaj et al., 2001; K. Park et al., 2002; Baek et al., 2016).

β-Sheet AMPs

In general, β -sheet AMPs are cyclic molecules composed of at least two antiparallel β -sheets stabilized by intramolecular disulfide bonds. The β-sheet peptides are more ordered in aqueous solution due to their rigid structure and do not undergo a drastic conformation shift like helical peptides upon membrane interaction. (Yeaman and Yount, 2003). The well-researched β-sheet peptides are the defensins, a broad group of AMPs that are formed in neutrophils, macrophages, and epithelial cells as inactive precursors (Lai and Gallo, 2009; Pasupuleti et al., 2012). The β-sheet peptides contain primarily plant defensins, mammal α defensins and β defensins (BNBDs, Bovine β defensin-1, porcine β-defensin), insect defensins, proline-rich antibacterial peptides, protegrin, and tachyplins.

Loop AMPs

Loop AMPs have a loop structure stabilized by amide, disulfide, and isopeptides bonds. A prominent member of this group is isolated from the spinning soldier bug by thanatin. A single disulphide bond between residue 11 and 18 stabilized the structure of thanatin (Powers and Hancock, 2003).

Extended helix AMPs

The regular secondary structures usually lack extended helix structure (Powers and Hancock, 2003). Often they are rich in certain amino acids, including residues of glycine, arginine, tryptophan, proline, and histidine (Nguyen et al., 2011; Angélique et al., 2015). The structure is only stabilized by interactions with membrane lipids by hydrogen and Vander Walls. Circular dichroism (CD) spectroscopy shows that indolicidin has a poly-L-proline II extended helix structure (Falla et al., 1996).

Other AMPs

Several AMPs do not belong to any of these classes, and some appear only in aggregated form or when communicating with the membrane(Bahar and Ren, 2013). The plant-derived circulin A is a clear example of this, consisting of a combined α-helix and β-sheet structure that forms the cyclic cysteine knot (Angélique et al., 2015). Proline-rich Bactenecins like Bac-5 and Bac-7, identical to cathelicidins and arginine-rich PR-39, fall under this category (Anbanandam et al., 2008).

Databases

AMP databases are the platform that combines stored knowledgebase with predictive algorithms, BLAST, Physicochemical property prediction of the input sequences, identification of hidden markov model and various other tools. These types of platforms facilitate the discovery and generation of new sequences with bioactive properties. Steady research in the field of bioactive has led to the discovery and generation of peptides with diverse activities. Hence, a database is required to store the endogenous or chemically synthesized sequences that have been *in vitro* functionally characterized to assist in further research and development and designing of peptides with therapeutical importance. This section briefly talks about such knowledgebase and their salient features.

APD3

The AMPs database (http://aps. unmc.edu/AP/) was originally established in 2003 and with regular update it has been upgraded to APD3. The prime focus of the database is the natural antimicrobial and currently it stores 3198 AMPs from six different kingdoms (357 bacteriocin from bacteria, 5 from archaea, 8 from protists, 20 from fungi, 352 from plants and 2347 from animals). These peptides are functionally annotated and are categorized accordingly. APD3 has functionally categorized these sequences into diverse categories on the basis of their properties such as antibacterial peptides, antifungal peptides, anti-TB peptides, anti-diabetic

peptides, anti-inflammatory, protease inhibitors, antioxidant peptides, wound healing peptides, spermicidal peptides chemotactic peptides, anticancer peptides, antiviral peptides, anti- toxin peptides, ion channel inhibitors anti-parasitic and antimalarial peptides. The prediction feature of platform helps in prediction if the sequence in query possess antimicrobial activity with its physico-chemical properties and other parameters viz, Grand Average hydropathy value (GRAVY) of the peptide, Boman index, Wimley-White whole-residue hydrophobicity of the peptide etc (G. Wang et al., 2016).

ANTIMIC

ANTIMIC database (http://research.i2r.a-star.edu.sg/Templar/DB/ANTIMIC/) hosts 1700 sequence created on BioWare data-warehousing platform which was used for retrieving Antimicrobial sequences from NCBI's GenBank and Swiss-Prot databases. The database features integrated tools like BLAST for sequence similarity search, HMMER for identifying hidden Markov model (Brahmachary, 2004). This database has been updated to and replaced by Dragon AMPs Database (DAMPD, (http://apps.sanbi.ac.za/dampd) containing 1232 experimentally validated AMPs sequences (Sundararajan et al., 2012).

CAMPR3

CAMPR3 (www.camp3.bicnirrh.res.in), a database of sequences, structures and family is an update over pre-existing database CAMP and currently holds 10247 sequences, 114 signatures from 45 AMP families and 757 structures. It provides platform for prediction of antimicrobial activity which uses algorithms like Suport Vector Machine (SVM), Random Forest (RF), Discriminant Analysis (DA) and Artificial Neural Network (ANN). Besides this, it provides BLAST tool for similarity search, alignment tool Clustal Omega, PRATT for AMP specific pattern generation (Waghu et al., 2016).

BACTIBASE

BACTIBASE (http://bactibase.pfba-lab-tun.org) is a dedicated database on bacteriocin, containing 123 bacteriocins from Gram positive and negative bacteria (Hammami et al., 2007). The database has been updated to contain 177 sequences, out of which 156 are derived from Gram positive bacteria and 18 from Gram negative bacteria. The updated version incorporate BLAST, CLUSTALW, MUSCLE , T-COFFEE for multiple sequence alignment and HMMER program for Hidden Markov Model (Hammami et al., 2010).

PhytAMP

PhytAMP (http://phytamp.pfba-lab.org) is the only dedicated database on AMP sequences of plant origin. It currently hosts 271 sequences functionally categorized into antibacterial, antiviral, antifungal, insecticidal, antiyeast and sodium channel blocker. Like other databases it has tools for homology search, multiple sequence alignment and Hidden Markov Model (Hammami et al., 2009).

Biological function and mode of action:

Depending on the nature of AMP, the targets can be one or more bacteria (Gram-positive or G negative), fungi or viruses (Bai et al., 2007; Chang and Yang, 2013; Hein et al., 2015; H. Zhao et al., 2016; Sala et al., 2019). The propensity to bind membrane is a conclusive feature of AMPs, which may or may not be associated with membrane permeabilization (Varkey et al., 2006). These AMPs employ several mechanisms to wipe out pathogens; most of them rely on the disruption of cell membrane or alteration in cell membrane permeability which ultimately relies on various physicochemical parameters possessed by the sequence. While most of the AMPs mainly adopt this approach for killing, others interfere with intracellular functions like DNA and protein synthesis, protein folding, or cell wall synthesis. Physicochemical parameters like size, charge, hydrophobicity, amino acid composition directly affects the ability of the AMPs to insert in the membrane, pore formation ultimately allowing them to disrupt membrane by widely accepted mechanism of barrel stave, carpet and toroidal pore (Fig.1). Slightest tweak in these parameters might augment the activity of AMPs. Alternately, they may enter the cell and interact with intracellular molecules, including enzymes, to disrupt vital cellular functions. Polarized ATR-FTIR study showed that AMP Cecropin P1, preferentially adopts a parallel orientation when reconstituted into phosphotidyletahnolamine/phosphotidylglycerol lipid

membrane, suggesting that the AMP uses "carpet" model disrupting the lipid bilayer (Gazit et al., 1995, 1996). SMAP-29 from the cathelicidin family also use the same carpet model as a mode of action as confirmed by fluorescence and molecular dynamics simulations (Orioni et al., 2009). Conclusively, most of the AMPs exhibit their lethal effects by the formation of pores in the bacterial membrane, just like the complement system, resulting in increased permeability followed by membrane disruption. The fundamental basis for antimicrobial action is the difference between bacterial and mammalian cell composition. The outer leaflets of bacterial membranes are populated mainly by negatively charged phospholipids, whereas mammalian cells are mostly composed of neutral zwitterionic phospholipids such as phosphatidylcholine and sphingomyelin (J.M. Graham, 1997). Shai Matsuzaki Huang model is the proposed model to study the interaction of α helical AMPs targeting bacterial membranes. It shows the stepwise accumulation of AMPs followed by either integration into the membrane resulting in membrane disruption or diffusion from membrane onto intracellular targets (Matsuzaki, 1999; Shai, 1999; L. Yang et al., 2000). PR 39, a porcine AMP, exerts its lethal effect by halting the process of translation and DNA synthesis (Boman et al., 1993). Indolicidin also exerts its antimicrobial activity by halting DNA synthesis (Chilukuri Subbalakshmi and Sitaram, 1998; Hsu et al., 2005). March and co-workers reported that it inhibits DNA synthesis as well as topoisomerase I, thus employing multiple mechanisms at the DNA level for its antimicrobial activity. It exerts antimicrobial activity in gram-negative bacteria by causing disruption of cytoplasmic membrane by forming channels and membrane thinning (Falla et al., 1996; Neale et al., 2014). A similar mechanism for the peptide was reported for a pathogenic fungus *Trichosporon beigelii* (D. G. Lee et al., 2003). Nevertheless, a different study suggested that even though the peptide caused permeabilization of the bacterial membrane, it was inefficient in lysing bacterial cells (Chilukuri Subbalakshmi and Sitaram, 1998).

More recently, Indolicidin has been shown to exert its effect by translocation mechanism where it induces the release of negatively charged fluorescent dyes such as carboxyfluorescein (CF), calcein and sulforhodamine B by forming a peptide-dye complex and not by the formation of pores (Rokitskaya et al., 2011). Some AMPs tend to work by sequestration of ions, which are necessary for bacterial survival. Hepcidin and Psoriasin reduce the concentration of iron and zinc ions, respectively, and thus restrict the growth of pathogens (K. C. Lee and Eckert, 2007; Oliveira-Filho et al., 2014).

It is spectacular that unlike antibiotics, many AMPs are more bactericidal than being bacteriostatic (Reddy et al., 2004). There are several unique features in the sequence of AMPs like net charge, hydrophobicity, secondary structure etc. which stimulates additional anti-infective support in the host to fight against infection (Fig.1). In addition to having a direct effect on the target organism, AMPs can also act as a potent immunomodulator (Fig.2). They act as effector molecules of the innate immune system and influence diverse processes such as phagocytosis, cytokine release, cell apoptosis (Zuyderduyn et al., 2006; Paredes-Gamero et al., 2012). They also act as chemotactic factor, assisting in recruitment and aggregation of immune cells at the site of inflammation (D. Yang et al., 2002; Elsbach, 2003), promote angiogenesis (Kanazawa et al., 2016; Torres et al., 2017), and induce wound healing (Mu et al., 2014) by cytokine release and cell proliferation (W. Wang et al., 2016, 2017).

Farm animals harbour a variety of AMPs that help them to counter invading pathogens. Some of these AMPs have been utilized in studies where they were characterized, and tons of experimental pieces of evidence, discussed in later sections, suggest that these AMPs have activity against a wide range of pathogens. Some AMPs of animal origin, such as Indolicidin from bovine, have reached clinical II/ III phase trials against several conditions.

AMPs in farm animals can be grouped under two leading families, namely Cathelicidins and Defensins. The cathelicidin family comprises of small cationic AMPs that are stored in neutrophils and macrophages. They are part of the innate immune system and are generally proteolytically active proteins. Members of this family have a conserved cathelicidin domain. Defensin is a family of AMPs consisting of 6 cysteine residues that form 3 disulfide bonds. It includes three subfamilies, namely α-defensins, β-defensins, and ϑ-defensins, and these differ from each other in the arrangement of disulfide bonds. These are found in tissues that are involved in host immune response against microbial infections and are abundant in leukocyte granules. The

following sections describe the AMPs in these classes in different farm animals comprehensively which are summarised in tabular form (Table1).

Bovine cathelicidins

Indolicidin

Indolicidinbelongs to the family of cathelicidin AMPs. It was first discovered in bovine as tridecapeptide (13 amino acid long) amide from neutrophils showing bactericidal effect (Selsted et al., 1992) including its action on *Staphylococcus aureus* (ABEL et al., 1995). So far, seven protein coding cathelicidin gene have been identified which are traced to single cluster on chromosome 22 (Tomasinsig and Zanetti, 2005). Indolicidins are rich in tryptophan residues and cause cytoplasmic disruption in bacteria by forming channels. When compared to its phenylalanine analog, it was found that the presence of a tryptophan residue gives hemolytic activity to the peptide (C. Subbalakshmi et al., 1996). Treatment of pathogenic *C. albicans* with Indolicidin coated gold nanoparticles (Indolicidin –AuNP) inhibits its ability to produce biofilm by reducing the expression of biofilm-related genes and increasing the rate of efflux of Rhodamine 6G (de Alteriis et al., 2018). Another *in vitro* study reported that Indolicidin–AuNP increases Reactive Oxygen Species (ROS) which results in DNA damage in*S. cerevisiae* (Ghosh et al., 2014).

Bovine Myeloid Antimicrobial Peptide (BMAPs)

Bovine Myeloid AMPs are a series of variable length peptides that belong to the cathelicidin family and show potent antimicrobial activity against a wide range of pathogens. BMAP-18, BMAP-27, and BMAP-28 are the main AMPs belonging to this group. BMAP-27 and BMAP-28 are encoded by CATHL6 and CATHL5 genes respectively. It kills the bacteria by forming small channels in the membrane resulting in the release of small ions rather than by membrane disruption (Boman et al., 1993).

BMAP-27

BMAP-27 is bovine cathelicidin, which exhibits broad-spectrum antimicrobial activity against Gram -negative and Gram-positive bacteria, viruses, and fungi but may also induce some toxicity to erythrocytes (Benincasa et al., 2003, 2006). in addition to its strong LPS neutralizing activity, the peptide also inhibited the formation of biofilm of multidrug resistant bacterial strains collected from Cystic fibrosis patients.(Pompilio et al., 2011). These roles of BMAP-27 were considered useful for the treatment of Gram -negative sepsis (Ciornei et al., 2005; Mookherjee et al., 2006). Thus, BMAP-27 has a significant role as a potential therapeutic agent against antibiotic-resistant bacteria. The peptide was also found to exert cytotoxicity against human tumor cells by disrupting membrane and Ca2+ influx into the cytosol, ultimately leading to apoptosis (Risso et al., 1998).

The structural analysis of BMAP-27 using 2D NMR spectroscopy revealed that the peptide acquires α-helix with aromatic residues clustered at N-terminal and a short hydrophobic C-terminal helix. (S. Yang et al., 2019).

BMAP-28

BMAP-28 was effective in inhibiting the growth of pan-drug-resistant*Acinetobacter baumannii* (PDRAB) by damaging the cell surface and rapid killing ability (Y. Guo et al., 2018). In another study, Lynn and coworkers found that the two stereoisomers of BMAP-28, D-BMAP-28 and the retro-inverso form (RI-BMAP-28), were highly effective promastigotes and the intracellular amastigotes stages of Leishmania lifecycles, being resistant to GP6 metalloprotease. They also reported that the L-form of BMAP-28 was susceptible to degradation by GP63, a metalloproteinase that covers the parasite surface. Takagi et al. (2011) observed the effect of BMAP-28 on methicillin-susceptible *S. aureus* (MSSA) and methicillin-resistant *Staphylococcus aureus* (MRSA) (Takagi et al., 2012). They concluded that for MSSA, minimal inhibitory concentration (MIC) ranged from 1.25 to 20ug/ml and for MRSA (MIC) ranged; 5–20 ug /ml. Like BMAP-27, it also exerted cytotoxicity against human tumor cells and healthy proliferating cells (Risso et al., 1998). In a cell culture experiment involving RAW 264.7 macrophages, BMAP-28 obstructed LPS induced expression

of cytokine gene (D'Este et al., 2012). Risso *et al.* (2002) showed that the cytotoxic effect of the peptide depends upon its ability to form various transition pores in mitochondria, causing depolarization of the inner mitochondrial membrane (Risso et al., 2002).

BMAP-18

BMAP-18 is derived from its parent peptide BMAP-27. It lacks the hydrophobic C-terminal sequence and showed strong inhibitory activity against several species and life cycle stages of African trypanosomes, fish trypanosomes and Leishmania with reduced cytotoxicity towards mammalian cells as compared to BMAP-27 (Haines et al., 2009).

A comparative study of BMAP-27 with that of BMAP18 showed that BMAP-18 exerts only antibacterial activity, whereas BMAP-27 showed potent antibacterial as well as anticancer activity. It was observed that BMAP-27 ultimately killed bacteria within 20 min by disrupting membrane integrity (S. Yang et al., 2019).

BMAP-34

BMAP-34, a peptide with α helical structure, is stored as proform in cytoplasmic granules of neutrophil and exerts broad-spectrum activity against Gram-positive and Gram-negative bacteria (Renato Gennaro et al., 1998). Leucine and phenylalanine zippers play an essential role in maintaining the assembly of BMAP 27 on the mammalian cell (Ahmad et al., 2009).

Βοινε β-δεφενσινς

Tracheal Antimicrobial Peptide

Tracheal AMPs (TAP) is a 38-amino acid peptide that belongs to the β-defensin family and is produced by mucosal epithelial cells of cattle (Diamond et al., 1991). It was the first defensin to be identified in cattle. One study reported its expression in bovine Mammary Epithelial Cells (bMEC), and following *Staphylococcus aureus* infection, its expression was down-regulated (López-Meza et al., 2009). Quantitative RT-PCR analysis showed that Pam3CSK4 (a TLR2/1 agonist), IL-17A, and LPS significantly induce the expression of the TAP gene in tracheal epithelial cells (Berghuis et al., 2014). Taha-Abdelaziz *et al.* (2016) reported that NF-ϰB activation is necessary for the LPS, Pam3CSK4 or IL-17A mediated induction of TAP gene expression (Taha-Abdelaziz et al., 2016).A study on lung tissue from neonatal calves with acute *Mannheimia haemolytica* pneumonia showed that there is an up-regulation of basal mRNA expression. TAP, NF-ϰB, and intercellular adhesion molecule 1 occurred after infection, among which the expression of TAP and IL-8 were highly correlated (Caverly et al., 2003).

Bovine origin TAP was expressed in transgenic mice using an expression vector under the control sequences from the murine whey acidic protein (WAP) gene. The bTAP was then later purified by acid precipitation, RP-HPLC, and ion-exchange chromatography with proposed activity against*Escherichia coli* (Yarus et al., 1996). Studies have shown the*in vitro* antibacterial activity of the peptide *against Escherichia coli, Staphylococcus aureus, Klebsiella pneumonia, Pseudomonas aeruginosa, Candida albicans Mannheimia haemolytica, Histophilussomni, Pasteurella multocida, and Mycoplasma bovis* (Diamond et al., 1991; Yarus et al., 1996; Taha-Abdelaziz et al., 2013).

Lingual Antimicrobial Peptide (LAP)

LAP, a β-defensin first isolated from squamous epithelium of the bovine tongue, has a broad spectrum of antimicrobial activities against Gram-positive and Gram-negative micro-organisms, as well as antifungal activity (Schonwetter et al., 1995). Immunolocalization studies showed the presence of LAP in stratum corneum of the stratified squamous epithelium of the tongue, esophagus, rumen reticulum, omasum, chief cells of gastric glands of the abomasum and mammary alveolar tissue of cattle (N. Isobe et al., 2009; Naoki Isobe et al., 2011). It is also constitutively expressed with other β defensins in mammary lymph nodes (Tetens et al., 2010). Its expression increased in the mammary gland in response to coagulase-positive or negative staphylococci infection (Kościuczuk et al., 2014).

Studies confirmed a positive correlation between SCC in milk and LAP expression, suggesting it as an indicator of SCC (Swanson et al., 2004; Kawai et al., 2013). In the bovine mammary epithelial (BMEC) cell line, LAP expression increased in response to the treatment of vitamin D (Téllez-Pérez et al., 2012). Long term high concentrate diet feeding in lactating cows led to an increased translocation of rumen derived LPS into the blood stream activating enhanced LAP synthesis via the NF-κB signaling pathway (Jin et al., 2016). Moreover, challenging mammary glands with LPS increased the LAP level, which exerted a synergistic effect with lactoperoxidase enzyme (Naoki Isobe et al., 2009). All these studies indicate the decisive role played by LAP in providing innate immunity against invading pathogens in the mammary gland and digestive tract.

BNBΔ (Βοινε Νευτροπηιλ β-Δεφενσινς)

A variety of β- defensins have been identified in bovine neutrophils as well. Till now, 13 bovine neutrophil β-defensins have been identified and isolated from neutrophil granules. These AMPs were active *in vitro* assay against *Staphylococcus aureus* and *Escherichia coli* (Selsted et al., 1993). BNBD3 or DEFB3 is expressed in various tissues. A study reported that on treating a bovine monocyte culture with lipopolysaccharide, the expression of BNBD3 was increased. A study showed that the co-administration of BNBD3 as a fusion with a protective agent like glycoprotein D enhanced the cell-mediated immune response (Mackenzie-Dyck et al., 2014).

BNBD4, also known as DEFB4, is expressed constitutively in bovine alveolar tissues in a large amount. However, its expression was low in the small intestine. It is derived from a prepropeptide that is 63 amino acids long and is converted to a 41 amino acid long mature peptide. A study reported that the expression of BNBD4 increased after infecting mammary glands with coagulase-positive *Staphylococci* as compared to coagulase-negative *Staphylococci* (Gurao et al., 2017). Its expression was higher in both early and late lactation stages. Another β-defensin known as BNBD5 or DEFB5 was reported, and it resembled BNBD4. It showed increased expression during intramammary infections. The expression of BNBD5 was observed to be high during late lactation stages. BNBD4 and BNBD5 are the best-known members and are expressed constitutively in the pulmonary macrophages (Ryan et al., 1998). BNBD12 and BNBD13 are derived from a common precursor of 60 amino acids, and the mature peptides are composed of 38 and 42 residues, respectively (Yount et al., 1999).

Εντερις β–δεφενσινς

Bovine enteric β defensin (EBD) has been identified in the epithelial cells of bovine small intestine and colon. Tarver et al. (1998) showed that on infecting calves with an intestinal bacteria C*ryptosporidium parvum,* the EBDs expression was highly inducible, and it increased 5 and 10 fold in the intestine of infected animals. It was observed that EBD mRNA was more abundant in colon tissues as compared to the small intestine tissues. They also used Northern blot analysis to show that the expression of other β-defensins in the enteric tissues was low as compared to EBDs. The EBD prepropeptide and mature peptide show a 72% and 67% similarity to those of TAP, respectively (Tarver et al., 1998).

Βοινε β-δεφενσιν 1

Aono et al. (2006) identified a novel β-defensin that showed more similarity to human β-defensin than to other bovine β-defensins and named it as bovine β-defensin 1gene (bBD1). It containstwo exons and one large intron, which resulted in 69 amino acids propeptide. The translated product exhibits a strong response against *Escherichia coli,* but less effective against *Staphylococcus aureus*infections. It was expressed in the urogenital tract suggesting that it might have a role in protecting the reproductive tract from invading bacteria (Aono et al., 2006).

Bovine Psoriasin

Psoriasin is an important bovine antimicrobial protein. It is homologous to the human Psoriasin, another name for calcium-binding S100A7 protein. It was first identified as a respiratory allergen in cattle but played an essential role in providing local defense in the udder. It also shows antimicrobial activity, which

is observed to be limited against*Escherichia coli* , a mastitis-causing agent. It can be used as an important agent in preventing coliform mastitis (K. C. Lee and Eckert, 2007).

Proline-rich AMPs: Bac-5 and Bac-7

Bac5 and Bac7 are two bactenecin that were isolated from bovine neutrophils. They are rich in proline and have a molecular mass of 5 and 7 kDa.Both of them are efficient in suppressing the growth and killing of Gram-negative bacteria, *Escherichia coli* , S*almonella typhimurium,* and *Klebsiella pneumoniae* . They are also responsible for arresting the growth of *Enterobacter cloacae* . It was observed that *Pseudomonas aeruginosa* and *Staphylococcus epidermis* were susceptible to Bac7 but not Bac5 (R. Gennaro et al., 1989). Instead of disrupting the bacterial membranes, the proline-rich AMPs (prAMPs) use a non-lytic mode of action. They exert their effects by entering the bacterial cytoplasm via inner membrane transporters SbmA and YjiL/MdtM and inhibit protein synthesis. It was observed that the bacteria lacking these membrane transporters were resistant to prAMPs (Mattiuzzo et al., 2007). They bind to the ribosome and prevent the process of translation from proceeding to the elongation phase by blocking the entry site of aminoacyl-tRNA and thus inhibiting protein synthesis; however, they do not affect DNA and RNA synthesis. Studies reported that Bac5 and Bac7 had little effect on eukaryotic translation as compared to bacterial translation suggesting that they cause minimal damage to eukaryotic cells(Mardirossian et al., 2018).

Another study showed that Bac-7 utilized a stereospecificity dependent mode of action at near MIC value where L-enantiomer was more actively internalized into the bacteria. However, it depends on a non-stereospecific lyticmechanismat concentrations higher than MIC value (Podda et al., 2006).

Equine Cathelicidin

Skerlavaj & co-workers reported three putative horse myeloid cathelicidin- eCATH1, eCATH2, and eCATH3 and their cDNA sequences, each peptide having α-helical conformation confirmed by circular dichroism measurement. *In vitro* studies revealed potent broad-spectrum antimicrobial activity for eCATH1; however, the activity of eCATH2 was somewhat restricted. It was observed that eCATH2 and eCATH3 were produced are propeptides in neutrophils and were cleaved to form mature proteins after neutrophil activation. The activity of eCATH3 showed a strong dependence on salt concentration as revealed by the efficient killing of bacterial and fungal species in low ionic strength media, which was inhibited in the presence of physiological salt medium (B. Skerlavaj et al., 2001).

In another study, eCATH1 showed antimicrobial activity against*Rhodococcus equi* , the causal agent of rhodococcosis in foals. They also tested eCATH1 against equine isolates of *E.coli* ,*S.enterica* , *K. pneumoniae* , *Pseudomonas spp* . and*Rhodococcus equi* with MICs ranging from 0.5-16 µg/ mL (Schlusselhuber et al., 2014). It was also reported that eCATH1 an*in vitro* IC50 of 9.5µM against T*rypanosoma brucei, Trypanosoma evansi* and *Trypanosoma equiperdum* (Cauchard et al., 2016).

Cathelicidin derived AMPs EA-CATH1 and EA-CATH2 were isolated from a constructed lung cDNA library of a donkey using nested PCR based cloning. Chemically synthesized EA-CATH1 had MIC against Gram-positive bacteria in the range of 0.3–2.4µg/mL. CD measurement studies showed that EA-CATH1 adopts an α-helical conformation in a 50% trifluoroethanol/water solution, but a random coil in aqueous solution. It also showed serum stability and had no hemolytic activity against erythrocyte. Scanning electron microscope studies showed that treatment of EA-CATH1 caused rapid disruption of the *Staphylococcus aureus*(ATCC2592) membrane (Lu et al., 2010).

Equine Neutrophil Antimicrobial Peptides

The eNAP-1 is a 7.2 KDa cysteine-rich endogenous AMPs that was purified from the extracts of cytoplasmic granules of equine neutrophils. The peptide was found to be effective against *Streptococcus zooepidemicus* , an equine uterine pathogen. However, it showed less activity against *Escherichia coli* and *Pseudomonas aeruginosa* . It was suggested that eNAP-1 might play an essential role in phagocyte mediated host defense during equine infections(Couto et al., 1992b).

Another peptide in this series is eNAP-2, a 6.5 KDa endogenous AMPs, which was also isolated from the extracts of cytoplasmic granules of equine neutrophils. It was also efficient in killing *Streptococcus zooepidemicus.* Studies showed that eNAP-2 caused bacteriostasis activity after incubating with *Klebsiella pneumoniae* . It exhibited selective activity against microbial serine protease*viz.* Subtilisin A and Proteinase K, without any inhibitory effect on mammalian serine protease(Couto et al., 1992a, 1993).

Equine Hepcidin

Hepcidin is a 25 amino acid peptide produced in the liver. In addition to its role in iron homeostasis, it also shows antifungal and antibacterial effects. Hepcidin is synthesized as 84 amino acid prepropeptide with a 24 amino acid N-terminal signal sequence, targeting the peptide to the endoplasmic reticulum. Posttranslational processing of hepcidin is carried out by proprotein convertases such as furin, PC7/LPC, PACE4, and PC5/6 (Valore and Ganz, 2008). During acute inflammation, the increase in levels of iron supports the growth of pathogens, and hepcidin reduces bacterial survival by restricting the availability of iron. In one study, healthy horses were administered with Freund's complete adjuvant by intramuscular injection at two-time point*viz.* 0h and 12h. It was observed that 6h post-infection, hepcidin mRNA increased and remained high till 18h. The plasma iron concentration decreased significantly between 16h and 72h post-infection as compared to control, suggesting the role of hepcidin in the rapid onset of hypoferremia to restrict the availability of iron for the growth of pathogens (Oliveira-Filho et al., 2014). Overexpression of hepcidin in transgenic mice resulted in decreased body iron levels and severe microcytic hypochromic anaemia, confirming its role in iron sequestration and central to its activity (Nicolas et al., 2002).

A report in donkey identified that the high expression of hepcidin in the liver was found to be similar to the reference gene expression. The study also stated that mature hepcidin sequence from donkey exhibited 100% sequence homology to mature hepcidin sequence from the horse (Oliveira-Filho et al., 2012).

Εχυινε β δεφενσιν

Equine β defensin 1 (eBD-1) expression was first reported in hepatic tissue, after database search for Expressed Sequence Tags ESTs presumed its expression to be in hepatic tissue. Another study showed its expression in respiratory epithelial tissue (Quintana et al., 2011). Equine beta-defensin peptide sequence is conserved among human and porcine orthologues, sharing the highest sequence similarity with porcine β defensin (Davis et al., 2004).

The horse is the only α-defensin expressing species in the Laurasiatheria group (Bruhn et al., 2009). DEFA1 is the first α-defensin to be characterized in equine and is expressed in the small intestine. It exhibited homology with Panethcell-specific α-defensins from primates and glares. It showed antimicrobial activity against Gram-positive, Gram-negative, and Candida albicans by employing membrane permeabilization as the mechanistic approach (Bruhn et al., 2009; Shomali, 2012).

Porcine Cathelicidins

Protegrins

Protegrins are a group of arginine and cysteine-rich cationic AMPs. They belong to the cathelicidin family and are composed of 16-18 amino acid residues (Kościuczuk et al., 2012). These peptides were first isolated from porcine leukocytes. There are five known protegrins (PG-1 to PG-5). They exhibit broad-spectrum activity against Gram-negative and Gram-positive bacteria and exerts its effect by interacting with the bacterial membranes and releasing their cellular contents. Inactive proforms of protegrins are stored in neutrophils. During Phorbol Myristate Acetate stimulated secretion from neutrophil, these inactive proforms get converted into mature protegrins via Neutrophil elastase mediated cleavage (Panyutich et al., 1997). In addition to antimicrobial activity, these mature protegrins also modulate immune activity and cell migration by activating insulin-like growth factor-1 receptor (IG1R) (Penney and Li, 2018). Its expression has been found in the lung and intestinal tissue of porcine. The infection with Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) significantly suppressed its expression in the lungs (JunBin, 2013) *In vitro* study

showed that the AMP prevents PRRSV virus attachment in Marc-145 cells and suppressed virus RNA and protein synthesis (C. Guo et al., 2015).

PG-1 is the most studied protegrin. Two dimensional NMR studies show that synthetic and natural protegrin-1 (PG-1) adopts a well-defined structure in solution, which is composed of two stranded antiparallel β sheets connected by a β turn (Fahrner et al., 1996). Alteration in the β turn region can significantly decrease the activity of PG-1 (Cho et al., 1998). High-resolution NMR studies showed that PG-2 forms a well-defined structure comprising of two stranded antiparallel β-sheets when it binds with DPC micelles. In addition to this, they also showed that PG-3 adopts an antiparallel NCCN dimer conformation in the presence of DPC micelles (Usachev, Efimov, Kolosova, Filippov et al., 2015). Moreover, PG-5 initially adopts an antiparallel dimer structure, but it undergoes further dimerizations of form an octameric pore-forming structure (Usachev, Efimov, Kolosova, Klochkova et al., 2015).

A study conducted on crossbred piglets showed that weaning reduced the expression of PG-1 significantly (Han et al., 2007) but supplementation of 20mg/Kg of copper in the diet increased the expression of PG-1 and reduced the cases of diarrhea in them (Yan et al., 2015). PG-1 was effective in inhibiting Gram-negative, facultative periodontal pathogens. Three strains, each from *Actinobacillus actinomycetemcomitans* and *Capnocytophaga* spp. were treated with L- and D-enantiomers of PG-1 and L-enantiomers of PG-2, PG-3, and PG-5. This study suggested that both D-form and the L-form of PG-1 were equally effective against *strains of Actinobacillus actinomycetemcomitans* and *Capnocytophaga* spp. with its ED99 ranging from 0.5 to 3ug/mL and 4 to 19ug/mL, respectively (Miyasaki et al., 1997). STD causing pathogens like *Neisseria gonorrhoeae* and*Chlamydia trachomatis* were also found to be susceptible to the peptides. Electron microscope observations showed damages in the cell membrane of pathogens (Qu et al., 1996; Yasin et al., 1996).*Candida albicans,* a pathogenic yeast, was also susceptible to PG-1 and other protegrins in series *viz,* PG-2, -3, and -5 were equally effective as a candidacidal (Cho et al., 1998). PG-1 also exhibits bactericidal activity against MRSA and *Pseudomonas aeruginosa* and is capable of reducing three log units of viable CFU in less than 15 minutes. *In vivo* studies showed that mice that were previously inoculated with *P. aeruginosa* or *S. aureus*showed better survival rate after receiving an intraperitoneal injection of PG-1 (0.5 mg/kg of body weight) as compared to the mice in control group exhibiting 93 to 100% mortality (Steinberg et al., 1997).

PG-1 works in a concentration-dependent manner. At concentration below 4ug/mL, it destabilizes the membrane edge resulting in a finger-like structure. The sieve like nanoporous structures is formed at the highest concentration, and at concentration [?] 20ug/mL, it exhibits the maximum degree of membrane disruption (Lam et al., 2006). Enhanced Bactericidal activity of PG-1 was observed at physiological NaCl concentration (Harwig, 1996). PG-1 exhibits hemolytic activity against phosphatidylcholine rich human erythrocytes but not against phosphatidylethanolamine rich ruminant erythrocytes suggesting that the lipid composition is an essential factor in deciding the hemolytic activity of an AMP (Ishitsuka et al., 2006). A similar observation was made in another study where PG-1 was relatively targeted, and it got readily inserted in anionic lipids as compared to the zwitterionic lipids, under similar experimental conditions (Gidalevitz et al., 2003).

Using *Pichia pastoris* as host, PG-1 was expressed by cloning its sequence fused with 6X His Tag in pPICZα-A vector, and the resulting system yielded ˜20 mg pure active PG-1 from the supernatant of 500 ml culture broth (Niu et al., 2015). In another study, a similar host was used for the expression of the peptide, and a matrix metalloproteinase cleavage site was introduced into the preform PG-1 sequence allowing its efficient release at the site of skin inflammation (Hill, 2017).

PG-1 has a complex secondary structure and antimicrobial activity, making its production difficult in the microbial system. Lee *et al.* created a chloroplast transformation vector containing GFP sequence, Factor Xa cleavage site to release the peptide from the fusion protein and His-tag to facilitate purification of the peptide by affinity chromatography. Confocal microscopy studies showed the localization of peptide in chloroplasts, and the system yielded an adequate amount of purified peptide from transplastomic plants. Transgenic mice capable of ectopically expressing PG-1 were produced; these mice exhibited enhanced resistance against

Actinobacillus suis infection when compared to their wild-type counterparts (Cheung et al., 2008).

PR39

PR39 is proline and arginine-rich, 39 amino acid residue long AMPs isolated from pig intestine. Linkage and in situ hybridization mapping studies showed PR39 localization on pig chromosome 13 (Gudmundsson et al., 1995). Immunolocalization studies showed its presence in the upper and lower respiratory tract tissue of pigs. Expression was found in type 2 pneumocytes in alveoli (Hennig-Pauka et al., 2012). Studies showed that alterations in the C terminal region of the peptide affected its antimicrobial activity, whereas no significant loss in activity was observed in the case of truncated N-terminal (Chan et al., 2001; Veldhuizen et al., 2014). However, the presence of a charged N-terminus is vital for the activity of the peptide in bacterial as well as mammalian systems (Veldhuizen et al., 2014).PR39 is a multifunctional protein. In addition to its antimicrobial activity, it also has angiogenic effects. The primary mechanism underlying its inflammation-induced angiogenic effect is the inhibition of ubiquitin–proteasome-mediated degradation of the hypoxia-inducible factor-1α protein (Li et al., 2000). PR39 exhibited an anti-apoptotic effect on macrophages in which apoptosis was induced by nutrient depletion (Ramanathan et al., 2004). The deleterious effect of the NADPH oxidase enzyme, which caused tissue injury by toxic oxidants, was inhibited by PR39. Inhibition was achieved via interactions with Src homology three domains of cytosolic component followed by blocking the assembly of the enzyme (Shi et al., 1996).

It also acts as a calcium-dependent chemoattractant for neutrophils (Huang et al., 1997). Various *in vitro* studies suggested the role of PR39 in host defense against pathogens like *Salmonella choleraesuis* and *Mycobacterium tuberculosis* (G. Zhang et al., 1997; Linde et al., 2001). Transgenic mice ubiquitously expressing PR39 showed high resistance to infection from a highly pathogenic swine isolate of *Actinobacillus*pleuropneumoniae (APP) as compared to wildtype littermates (Zeng et al., 2018).

Prophenin 1

Prophenin-1 (PF-1) from the cathelicidin family is a 79 residues long proline-rich AMPs. It was first purified and characterized from porcine leukocytes. PF-1 was more effective against Gram-negative bacteria as compared to Gram-positive bacteria (Harwig et al., 1995). One study reported that recombinant PF-2 affected the growth and integrity of*Trichomonas vaginalis* (Hernandez-Flores et al., 2015). In primary bone marrow cells, expression of the PF-2 gene is regulated by cytokines GM-CSF and IL-3 and transcription factor PU.1 (Ramanathan et al., 2005).

Cecropin P1

Cecropin P1 is a 31 residue AMP isolated from the porcine small intestine (J. Y. Lee et al., 1989). ATR-FTIR spectroscopy and NMR studies indicated that the peptide assumes an α helical structure consisting of 4-5 turns of the amphipathic region and short 1-2 turns of hydrophobic region separated by a Glu-Gly sequence, a bend forming section (SIPOS et al., 1992; Gazit et al., 1996). Also, an interaction study between CecP1 and LPS using CD and NMR showed that the peptides adopted anα-helical in a solution containing LPS (Baek et al., 2016).*In vitro* study in Marc-145 cells, it displayed antiviral activity against Porcine Reproductive and Respiratory Syndrome Virus by inhibiting viral attachment, viral particle release, and by elevating the expression of interleukin-6 (C. Guo et al., 2014). A fusion of porcine β-defensin-2 (pBD-2) and CecP1 AMPs was expressed in recombinant*Bacillus subtilis* that was capable of exhibiting antimicrobial activity against several Gram-negative *(E. coli, Salmonella typhimurium* , and *Haemophilus parasuis*) and Gram-positive bacteria (*Staphylococcus aureus*) (Xu et al., 2017). Variety of cecropin transgene constructs were transfected into the Chinook Salmon Embryo cells (CHSE-214), following its integration into the genome, cells showed antimicrobial activity against *Vibrio anguillarum, Aeromonashydrophila* and *Pseudomonas fluorescens* (Sarmasik and Chen, 2003).

Porcine Myeloid Antimicrobial Peptide

PMAP 23 was first characterized from porcine bone marrow by cDNA cloning from RNA encoding 153 residues polypeptide (Zanetti et al., 1994). The 23 residues C terminal sequence was synthesized, and it was

capable of exhibiting antibacterial activity against Gram-positive and Gram- negativebacteria with MIC in the range of 2-16 μM. It has two alpha-helices, the first one is in the N terminal region from Arg1 to Arg10, and the other one is in the C terminal region from Phe18 to Arg23. Backed by fluorescent studies, it was found that Trp21 is responsible for the antibacterial activity of PMAP23 and was found buried deep into the phospholipid membrane, signifying the role of the second alpha-helix at C terminal in antimicrobial activity (K. Park et al., 2002).

The peptide possesses antifungal activity against *Candida albicans* . The treatment of fungus with FITC labeled peptide showed its location on the plasma membrane, which tells volume about its mechanistic process (D. G. Lee et al., 2001). Following in the line of previous work, the peptide was also tested for its antinematodal activity against C. elegans, where it exerts its effect by disrupting the cell membrane structure by creating pores (Y. Park et al., 2004). Storici *et al.* (1994) identified and cloned a cDNA from pig bone marrow RNA encoding 166 residues polypeptide PMAP36, capable of showing its antibacterial activity against various Gram- positive and Gram-negative bacteria, particularly in *E. coli* where it permeabilizes bacterial membrane. Similarly, in another study, (PMAP-36)2 homodimer gene was cloned and expressed in *P. pastoris*GS115, the resultant rPMAP-36 showed *in vitro* activity against Gram-positive and Gram-negative bacteria. For examining *in vivo*activity of the peptide, 10 mg (dissolved in water)/bird/day were given to 7 days old, 120 male Arbor acres broiler,cecal, and serum samples were taken for the assay. It was observed that serum exhibited an elevated level of IgM, and cecalmicroflora showed an increase in the population of *Bifidobacterium* and a decrease in the population of*E. coli* (L. Wang et al., 2014).

Πορσινε β Δεφενσιν

Porcine β Defensin gene contains two exons separated by a 1.5 kilobases intron. The entire length of gene spans 1.9 kilobases and mapping by fluorescence in situ hybridization studies revealed the location of the gene on porcine chromosome 15q14-q15.1 (G. Zhang et al., 1999). Recombinant pBD2 with a purity of 93.7% was obtained after pBD2 cDNA was cloned and expressed in *Pichia pastoris* followed by its purification, yielding 383.7 mg/L of the peptide. It showed antimicrobial activity against a wide range of pathogenic pig bacteria*viz. Streptococcus suis, Salmonella choleraesuis, Staphylococcus aureus* with MIC ranging from 32-64 μg/mL (Peng et al., 2014). The peptide also showed broad-spectrum activity against porcine intestinal pathogenic bacteria. It was capable of neutralizing the *Salmonella typhimurium, Listeria monocytogenes,* and *Erysipelothrix* within 3 hours of treatment at 4-8μM concentration, suggesting its role in defending intestine from pathogenic invasion (Veldhuizen et al., 2008). Feed additives containing rpBD2 from the culture supernatant of pBD 2 expressing *Pichia pastoris* resulted in the reduced incidence of post-weaning diarrhea in piglets (Peng et al., 2016). Bao *et al.*(2015) raised anti-Pbd -2 polyclonal antibodies in the New Zealand white rabbit. These antibodies were used in immunohistochemistry studies, which revealed pBD2 distribution in the tongue, liver, kidney, small intestine, and large intestine of pigs (Bao et al., 2015).

Caprine AMPs

Exploration studies of AMPs in caprine are still in its early phase, although few attempts have been made to characterize and define a variety of AMPs that may be present. Mostly, these studies were focussed on Bactenecin homologous to the bovine Bac5. Proline-rich AMPChBac5 was isolated from elastase treated extracts of goat leucocytes, respectively. It homologous to Bac5 from bovine and showed broad-spectrum activity against diverse pathogens (Olga Shamova et al., 1999).

Another proline-rich AMP was isolated from leukocytes of *Capra hircus* and was designated as ChBac3. It had a 50% sequence similarity to Bac5. It shows broad-spectrum antimicrobial activity even at low salt concentrations and shows a high microbial membrane damaging ability as compared to other proline-rich AMPs. However, it showed no hemolytic activity against human erythrocytes (Olga Shamova et al., 2009). Two AMPs designated as mini bactenecin, mini-ChBac7.5Nα, and mini-ChBac7.5Nβwere isolated from neutrophils of domestic goat. These AMPs possessed antimicrobial activity against Gram-negative bacteria as wells as drug-resistant strains of bacteria *(Klebsiella spp., Pseudomonas aeruginosa, and Acinetobacter baumanii).* They also showed activity against some Gram-positive bacteria (*Listeria monocytogenes* & *Mi-*

crococcus luteus) and claimed to have no hemolytic effect on human red blood cells (O. V. Shamova et al., 2016).

OVINE AMPs

The genes coding for ovine defensins and cathelicidins were mapped to chromosome 26 and chromosome 19, respectively. Two exons on chromosome 26 code for 2 defensins SBD-1 and SBD-2 and 4 exons on ch19 code for 4 cathelicidins, OaBac5, OaBac7.5, OaBac6 and OaBac11 (Kenneth M. Huttner et al., 1998).

Ovine cathelicidins

In addition to ChBac3, Shamova and co-workersalso isolated OaBac5α from sheep leukocytes via elastase treatment. It was homologous to caprine ChBac3 and bovine Bac5 and showed broad-spectrum antimicrobial activity as well.OaBac5α was considered as a variant of OaBac5. OaBac5β was another variant isolated from sheep leukocytes and was 20-30% abundant as OaBac5α.OaBac5 and OaBac7.5 are present in truncated forms OaBac5mini and OaBac7.5mini (Olga Shamova et al., 1999). They are proline and arginine-rich peptides that were isolated from sheep neutrophils. It was observed that OaBac5mini exerted a strong response against Gram-negative bacteria but was not as effective against Gram-positive bacteria and yeast. On the other hand, OaBac7.5mini was not effective against most of them. Once inside the cell, they caused depolarization of the cytoplasmic membranes (Anderson et al., 2004).

SMAP-29, an AMP from the cathelicidin family, was derived from sheep myeloid RNA. It was observed that the N-terminal amphipathic α-helical region and the C-terminal hydrophobic regions were essential for antibacterial and hemolytic activities of SMAP-29 (Shin et al., 2001). It also possessed antibacterial activity against antibiotic-resistant clinical isolates such as MRSA and VERF isolates and was also active against *Cryptococcus neoformans* and *Pseudomonas aeruginosa*(Barbara Skerlavaj et al., 1999).

Οινε β-δεφενσινς

Two β-defensins in sheep are referred to as SBD-1 and SBD-2 and are located on chromosome-26 (K. M. Huttner et al., 1998). SBD-1 and SBD-2 are small cationic AMPs that are active against Gram-negative and Gram-positive bacteria. SBD-1 expression was found in the trachea, tongue, and gastrointestinal tract, whereas SBD-2 was present in only ileum and colon (Kenneth M. Huttner et al., 1998). It was observed that the expression of SBD-1 and SBD-2 along IL-8 occurred during *M. haemolytica* infections. However, the expression of SBD-1 was low in*M. haemolytica* infection as compared to PI-3 infections. It was also observed that SBD-1 was primarily expressed in respiratory epithelium instead of leukocytes(Ackermann et al., 2004).

Zhao et al. (2011) used the *Pichia pastoris* vector to express 6-His tagged mature SBD-1. The peptide produced had similar activity as mature SBD-1 and had inhibitory effects on *Escherichia coli, Staphylococcus aureus, Proteus valgaris, Pseudomonas aeruginosa* and*Shigella flexneri.* This approach could be used for the production of bioactive SBD-1 inefficient manner(P. Zhao and Cao, 2012).

Milk derived AMPs

Milk has always been considered as a rich source of proteins and other nutrients. The milk protein is composed of 80% casein and 20% whey protein. Several AMPs have been derived from these proteins, and they have the potential to be used in the pharmaceutical industry. Caseinomacropeptide (CMP) is a biologically active peptide that is derived from ϰ-casein. It is composed of 64 amino acids and is also known as glycomacropeptide (Thomä-Worringer et al., 2006). It has various effects, including inhibiting adhesion of bacteria and viruses and binding to endotoxins. Kappacin is the non-glycosylated and phosphorylated derivative of CMP, which show growth inhibitory effect against *Streptococcus mutans* (Malkoski et al., 2001). It exerts its effect by forming pores in the membrane. Another AMP isolated from trypsin digested ϰ-casein is ϰ-casecidin, which exerts its effects by inhibiting the growth of bacteria.The first AMP isolated from N-terminal bovine αs1-casein after chymosin cleavage is Isracidin. It has inhibitory effects on the growth of Gram-positive and Gram-negative bacteria. It is was observed that this peptide protected udders

of cattle and sheep from chronic staphylococcal mastitis, and thus it was suggested that it might have prophylactic and therapeutic effects (Haque and Chand, 2008). Hayes et al. (2006) identified and reported three AMPs namely produced by *Lactobacillus acidophilus* DPC6026 fermentation of bovine casein and were referred to as Caseicin A, Caseicin B, and Caseicin C. It was observed that Caseicin A and B show inhibitory activity against pathogenic strains such as *E. sakazakii* (ATCC 12868) which causes meningitis in neonates. Thus these peptides can be used for the production of therapeutic drugs that can protect against this pathogen (Hayes et al., 2006). A study conducted by Zucht et al. (1995) isolated and identified an antibacterial peptide- Casocidin1, which was derived from ∝s2-casein bovine milk. This peptide was composed of 39 amino acids and inhibited the growth of *Escherichia coli* , and *Staphylococcus carnosus* (Zucht et al., 1995).

Several other AMPs have been derived from whey protein of milk as well. ∝-lactoglobulinis a globular whey protein present in bovine milk. It shows bactericidal activity after digesting it with chymotrypsin and trypsin. The three fragments that were produced were more active against Gram-positive bacteria (Pellegrini et al., 1999). On the other hand, digestion with pepsin and trypsin generated fragments that inhibited metabolic activities in *E.coli* . β-Lactoglobulin is the most abundant whey protein. Several AMPs are generated after proteolytic cleavage of b- lactoglobulin. On digesting β-Lb with trypsin, four fragments were generated, and these showed antimicrobial activity towards Gram-positive bacteria only (Pellegrini et al., 2001). They also showed that after the modification of a few amino acids, the activity of these fragments could be extended to Gram-negative bacteria as well. Bovine lactoferrin is a 17-41 amino acid glycoprotein that is secreted in body secretions like saliva, tears, urine, etc. It shows antimicrobial activity against Gram-positive and Gram-negative bacteria and even against viruses and fungi. It is rich in tryptophan and arginine. It acts by disrupting the outer membrane (Tidona et al., 2009). The activity of lactoferrin was mainly due to the peptide on N1-domain called lactoferricin. Marieke et al. (2004) identified another peptide in this domain and referred to this as lactoferrampin, and interestingly this peptide showed more significant activity than lactoferrin. It showed a strong response against *Bacillus subtilis* , *Escherichia coli,* and *Pseudomonas aeruginosa* . However, it was not against fermenting bacteria like *Actinomyces naeslundii* (Van Der Kraan et al., 2004).

Therapeutic potential of farm animal-derived AMPs

AMPs derived from animals have shown their effectiveness against various conditions and have either cleared or are in advanced stages of clinical trials (Table 2). Besides their performance in the clinical trial, a particular class of AMPs from animals are evaluated against *in vitro* and *in vivo* studies and tested against other conditions like cancer. Various studies confirm the utility of animal origin AMPs. BMAP-28 incorporated with polyurethane PEGU25 showed an inhibitory effect against *Proteus mirabilis* , a common pathogen in catheter-related urinary tract infection (J. Wang et al., 2015). It was also effective in killing of Methicillin-Resistant *Staphylococcus aureus* with MIC in range of 5-20 μg/mL (Takagi et al., 2012). Besides antimicrobial study, the BMAP-28 administration also resulted in a significant reduction in tumor growth in the TT-xenograft mouse model.*In vitro* study on human thyroid cancer TT cell line showed that the peptide could induce apoptotic effects in the TT cell line which were confirmed by Annexin V-fluorescein isothiocyanate/propidium iodide and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay (D. Zhang et al., 2015).

So far, we discussed the peptides which are still in preclinical studies and being evaluated for their antimicrobial properties, but some of them have also made their way to the clinical studies where they are being assessed for their safety and efficacy. Administration of MBI-226 (omignan) as 1% topical gel, an indolicidin derivative from bovine, proved effective against catheter-related infections in the Phase 3 clinical trial. The study design will be directed toward the assessment of skin irritation, erythema, edema, purulence, moisture, ecchymosis, abnormally warm tissue temperature, and/or site pain/tenderness after changing the dressing on every third day. Cutanea Life Sciences is currently conducting a phase 2 trial to explore the efficacy of 1.75% omignan topical gel in the treatment of Seborrheic dermatitis (NCT03688971).

Use of SGX942 with dusquetide as active ingredient for the treatment of oral mucositis in patients receiving

chemoradiation therapy for the treatment of head and neck cancer by intravenous infusion is in the clinical phase-3 trial (NCT03237325). Dusquetide is a synthetic 5 residue sequence derived from Indolicidin endowed with broad spectrum activity against Gram-positive and Gram-negative bacteria and also augment the activity of standard antibiotics (North et al., 2016). The study has been planned to administer 1.5 mg/mL of the drug as a 4 minute IV infusion (twice/week), within three days after initiating radiation therapy and will be continued for two weeks after the end of radiation therapy. IB367 Isegnan, derived from protegrin-1, is a porcine AMP being tested for its safety and efficacy for treating oral mucositis in patients receiving radiation therapy for head and neck cancer (NCT00022373). The study is sponsored by National Cancer Institute and will be conducted on a total of 504 patients (252 for arm I, 168 for arm II, and 84 for arm III). Arm I patient will receive oral rinse with isegnan HCL solution six times daily, and treatment will be continued for the scheduled duration of radiotherapy. Arm II patients will receive oral rinse with oral placebo six times daily for the scheduled duration of radiotherapy while Arm III patients receive standard-of-care supportive treatment. Study plans to assess oral cavity pain, ability to swallow, and weight loss twice weekly and on follow up days 28 and 56. Phase 2 study for POL7080, a protegrin-1 analog, is being conducted by Polyphor Limited, and it aims to assess pharmacokinetics safety and efficacy of the drug in the patients suffering from Ventilator-Associated Pneumonia (VAP) caused by Pseudomonas aeruginosa infection (NCT02096328). Findings are not posted yet for the proposed study.

Conclusion

Farm animals harbor a wide range of AMPs. Although it is difficult to confine all of them in the set definition of AMP most of them belong to cathelicidins and β- defensin family. There are similarities and overlap about the AMPs in various livestock species which is not surprising as they are evolutionarily conserved. Nevertheless, species specific uniqueness and preference for certain AMPs over other in a particular species encourages us to explore the sea of AMPs and exploit them as alternative line of treatment in parallel with antibiotics. Despite a large number of such peptides in various livestock species, it is still waiting for its real application in clinical indications. It is felt in this review that more clinical trials are needed to capitalize the virtues of AMPs. Another mystery about such AMPs is their mode of action. Apart from the widely accepted mode of action as cell membrane disruption still for many of these truly effective AMPs the finer details about the mechanism of action is only poorly understood. The ability of farms animals to thrive under harsher conditions than that of human beings, can be seen to be translated in the extraordinary power of AMPs. No wonder, Livestock associated AMPs can prove to be of immense application in human diseases. Besides their antimicrobial activity, these molecules also serve as immunomodulator, angiogenic factor, vasodilator, chemoattractant etc. The presence of AMPs in almost every stratum of life and its contribution in innate immunity makes it a suitable candidate for therapeutics against wide range of pathogens. Some of the peptide of animal origin have shown promising results and have made their advances to clinical trials. To mitigate the emergence of antibiotic resistant pathogen in future, AMPs can be seen as best possible alternative.

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Consent for publication

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Authors' contributions

R.K.: Designed the frame work of review and helped in the execution of the process of writing and submission. S.A.A.: Provided the illustrations, intellectual inputs for the review and helped in its compilation. S.K.S.: provided his insights on structural classification of AMPs and their therapeutic applications. V.B.: assisted in the collection of research articles relevant to the topic and authored on section "databases" and "biological function and mode of action". M.M.: authored on section "milk derived AMPs" and placed the information in review into concise tabular form. A.K.M.: Provided his intellectual inputs and critically reviewed the article. J.K.K.: scrutinized the manuscript and suggested the rectifications. S.K.: Supervised the whole process, suggested critical changes in the manuscript and provided final version of the manuscript for publication.

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