REVIEW

Piggybacking on nature: exploring the multifaceted world of porcine β-defensins

Arthur Nery Finatto[1], François Meurens[2,3] and Matheus de Oliveira Costa[1,4*]

Abstract

Porcine β-defensins (pBDs) are cationic peptides that are classically associated with the innate immune system. These molecules yield both antimicrobial and immunomodulatory properties, as evidenced by various in vitro and animal trials. Researchers have revealed that enhancing pBD expression can be achieved through dietary components and gene editing techniques in pigs and porcine cell models. This state-of-the-art review aims to encapsulate the pivotal findings and progress made in the field of pBD over recent decades, with a specific emphasis on the biological role of pBD in infection control and its usage in clinical trials, thereby offering a new landscape of opportunities for research aimed at identifying prophylactic and therapeutic alternatives for both swine medicine and translational purposes.

Keywords Antimicrobial resistance, infection control, bactericidal activity, immunomodulatory features, swine, sustainability

Table of Contents

*Correspondence:
Matheus de Oliveira Costa
matheus.costa@usask.ca
[1] Large Animal Clinical Sciences, Western College of Veterinary Medicine, University of Saskatchewan, Saskatoon, SK S7N 5E2, Canada
[2] Swine and Poultry Infectious Diseases Research Center, Faculty of Veterinary Medicine, University of Montreal, St. Hyacinthe, QC J2S 2M2, Canada
[3] Department of Veterinary Microbiology and Immunology, Western College of Veterinary Medicine, University of Saskatchewan, Saskatoon, SK S7N 5E2, Canada
[4] Department of Population Health, Faculty of Veterinary Medicine, Utrecht University, Utrecht, The Netherlands

Finatto *et al. Veterinary Research*   (2025) 56:47

Page 2 of 13

1 Introduction

Antimicrobial resistance and infection control are global public concerns [1–4]. The effectiveness of antibiotic drugs, which were once widely employed in human and veterinary medicine, has significantly declined over time such that their ability to mitigate microbial infections is no longer as potent as it was observed when they were first introduced in 1928 with the discovery of penicillin [5, 6]. Antibiotic-resistant bacteria are causing concern among healthcare professionals and emotional distress within communities [7, 8]. Hospitalized patients are experiencing infections that no longer respond to existing antibiotics, resulting in untreatable infections and high mortality [8–10]. The interplay between the indiscriminate use of antibiotic drugs and antimicrobial resistance is a cause for concern [1, 3, 4, 11–13], particularly due to the potential for horizontal gene transfer [14, 15]. Horizontal gene transfer allows antibiotic resistance genes to move from one bacterium to another, even between different bacterial species [16, 17]. Such transfer may occur at the farm, wildlife, and urban levels, potentially leading to the acquisition and exchange of resistance genes by human pathogens [16, 18]. The exploration of alternatives to traditional antibiotics, which are less likely to cause resistance, is imperative. The large-scale production of animal protein continues to depend on the use of antibiotic drugs to address animal health issues and promote herd welfare [2, 19]. Furthermore, the environmentally friendly and resource-intensive nature of meat production poses significant challenges for sustainability and food security in the future [20, 21].

Host defence peptides (HDPs) have gained increased attention because of their combined antimicrobial and immunomodulatory properties [22, 23] (Figure 1). HDPs are expressed by the majority of eukaryotic organisms [24], ranging from arthropods to higher mammals [25–30], either in response to microbial stimuli or
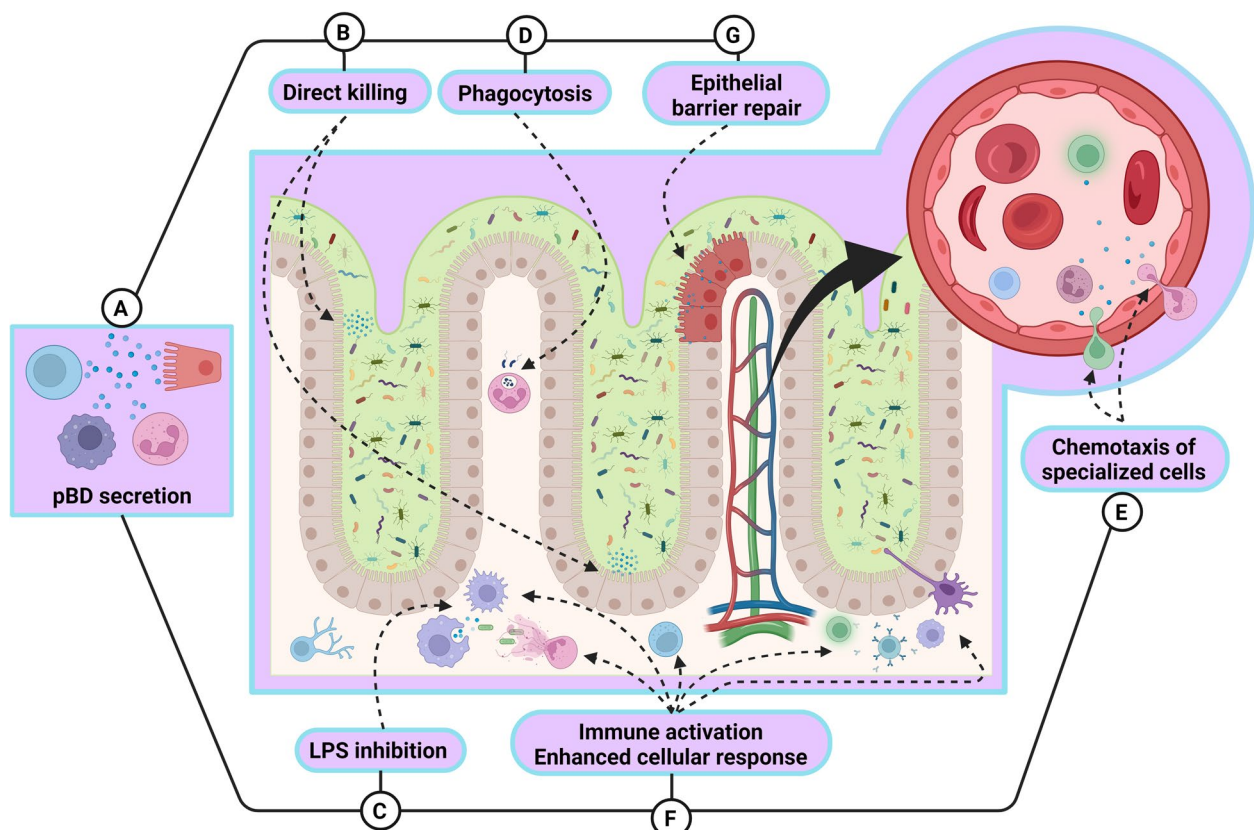
Figure 1 Multifunctional roles of pBD. Porcine β-defensins (pBDs) are secreted by a variety of specialized cells ("A"), including B and T lymphocytes, macrophages, neutrophils, and epithelial cells. The roles of pBDs extend beyond direct antimicrobial activity against bacteria, viruses, and fungi ("B"); pBDs also possess the ability to neutralize lipopolysaccharides (LPS) ("C"). These findings suggest that pBDs play a pivotal role in immunomodulating host cells, triggering immune activation and boosting cellular immune responses, thereby enhancing the ability of the host to combat target pathogens. Some of these mechanisms include enhanced phagocytosis ("D"), chemotaxis of specialized cells to sites of infection ("E"), fine-tuned secretion of inflammatory cytokines ("F"), and the strengthening and maintenance of epithelial barrier integrity through the induction of tight junctions ("G").

Finatto *et al. Veterinary Research*   (2025) 56:47

Page 3 of 13

continuously [31–35], and are secreted at specific sites in the body [36, 37]. These peptides modulate the microbiome and protect the host against infections by inhibiting the growth of microbial cells and orchestrating the host immune response [22, 36, 38–40]. There are few bacterial mechanisms of resistance against these HDPs [41, 42], which demonstrates another advantage over the antibiotics currently used [35]. Biotechnological approaches for the discovery and chemical synthesis of HDPs constitute a new scientific frontier to explore, with the ultimate goal of overcoming the "silent pandemic" of antimicrobial resistance [43].

This literature review focused on porcine β-defensins (pBDs) is timely as it compiles numerous studies into a single review focused on pigs, which are among the most important species for translational health studies, and pork is one of the most consumed animal proteins by humans worldwide [44–47]. Additionally, amid frequent accusations that swine production contributes to the emergence of drug-resistant microbes and is negligent in addressing this issue [15, 48–50], our review highlights the extensive efforts undertaken to address this challenge.

Here, we provide a summary of the structural, biochemical, and biological characteristics of pBDs, as well as recent advances in the use of these peptides to treat and prevent infections. pBDs exhibit swift and wide-ranging antimicrobial capabilities [51], possess immunomodulatory functions [52], show minimal toxicity to host cells [53, 54], maintain stability across diverse environments [55], have a low likelihood of promoting resistance [42], and hold promise for a range of clinical and preventive applications [23, 51, 56–59]. Our aim is to describe the mode of action of β-defensins (BDs), highlight recent progress in this field, and encourage further research. We have structured this review to highlight the innate and acquired immunity mechanisms by which pBDs are involved at both the mucosal and systemic levels, their unique biological properties, in vitro attempts to induce their expression, applications in clinical trials to address infectious diseases, and how new BDs can promote sustainability and help fight antimicrobial resistance.

2 Porcine innate immunity and host defence peptides

The porcine innate immune system is similar to that of most mammals and differs in only a few mechanisms or characteristics [46, 60–62]. One example, among others, is the expression of toll-like receptor (TLR) 7 and TLR9, which are restricted to plasmacytoid dendritic cells (pDCs), unlike in mice, where they are also expressed on conventional dendritic cells (cDCs) [60, 63]. Unlike mice [64, 65], pigs have a functional *toll-like receptor 8* (*TLR8*)

gene. This receptor can help the immune system of pigs recognize whether pathogens are alive or dead [66]. This ability leads to improved responses from follicular helper T (T_{FH}) cells and antibody production when animals are challenged with live bacterial vaccines [66]. Another difference in vaccine development is that, unlike mice and humans, swine lack interleukin (IL)-4, which directs the development of the T_H2 phenotype [67]. Instead, research has demonstrated that IL-4 inhibits antibody and IL-6 secretion and suppresses the antigen-stimulated proliferation of B cells [67]. In swine, IL-13 affects the role of IL-4 in the development of monocyte-derived dendritic cells (MoDCs) [68]. Pigs have increased diversity of type I interferons, with 39 genes compared with 19 in humans [69, 70]. Additionally, pigs have a duplication of the *IL-1β* gene (*IL-1β* and *IL-1βL*), which does not induce nitric oxide synthase 2 (NOS2) in response to lipopolysaccharides (LPS), as in mice [60, 71, 72]. Pigs also have more cathelicidin genes (11 genes) than humans do, with only one reported gene [70, 73], and thus far, no α-defensins have been identified in swine [60, 74].

Host defence peptides are components of the innate immune system that assume the role of frontline defenders in the early stages of infection. Through direct antimicrobial activity [51], immunomodulation [52], or wound healing [75], HDPs efficiently modulate the commensal microbiome and protect the surrounding host cells [37]. This versatile set of attributes enables the innate immune system to adapt its defence mechanisms precisely to a specific pathogen type or infection stage.

Swine produce a variety of HDPs, including cathelicidin, natural killer (NK)-lysin, hepcidin, liver expressed antimicrobial peptide-2 (LEAP-2), peptidoglycan recognition proteins (PGRPs) and BDs [38]. Cathelicidins typically have an N-terminal signal peptide, a cathelic domain, and a C-terminal antimicrobial domain. They are known for their strong antibacterial activity [76, 77], immunomodulatory properties [78], low haemolytic activity [79], high cytotoxicity towards drug-resistant bacteria [80, 81], and limited antiviral activity against porcine epidemic diarrhoea virus (PEDV) [82]. To date, 11 porcine cathelicidin isoforms have been identified, which are divided into 4 categories on the basis of the biochemical and conformational characteristics of the molecules: PR-39, PG1-5 (protegrins), PF1-2 (prophenins), and porcine myeloid antimicrobial peptides (PMAPs) [77]. All four groups have distinct cellular origins, structural peculiarities, and unique biological features. Shi et al. [77] provided a detailed examination of the characteristics and applications of these peptides in swine. Currently, with advances in molecular engineering techniques, new cathelicidins are being discovered, and modifications of known cathelicidin sequences are being

Finatto *et al. Veterinary Research*　　(2025) 56:47

Page 4 of 13

conducted to increase their performance and reduce the risk of microbial resistance [83, 84].

Porcine NK-Lysine presents a linear, amphipathic structure. It has broad-spectrum antimicrobial activity [85–89], has little haemolytic activity against host cells [85], can increase the abundance and distribution of intestinal tight junctions [85], and can modulate the expression of inflammatory cytokines such as IL-6, tumour necrosis factor (TNF)-α, nuclear factor-κB (NF-κB), Caspase 3, and Caspase 9 [85].

Hepcidin contains a conserved disulfide-bonded structure and is cysteine-rich. It is synthesized by liver cells and possesses antimicrobial activity, as shown by in vitro studies [90–92]. Although its primary function is to regulate iron activity [90], the release of hepcidin is an immune strategy to deprive microbes of iron, thus reducing the growth rate of iron-dependent microbes [90]. This iron-linked host defence phenomenon is characterized by an acute decrease in the plasma iron concentration at the onset of inflammatory responses to infection or other proinflammatory stimuli [90]. Initially, Ganz [90] proposed that the mechanism of action of hepcidin involves iron retention in macrophages and a reduction in iron absorption in the intestine.

Liver-expressed antimicrobial peptide 2 (LEAP-2), a typically cationic and amphipathic peptide, was first characterized for its antimicrobial activity [93, 94]. Research has demonstrated that swine infected with *Salmonella enterica* serovar Typhimurium exhibit inducible expression of LEAP-2 in bone marrow and intestinal tissues [94]. Recent research has indicated that these peptides may act as endogenous blockers of growth hormone secretagogue receptor 1a (GHS-R1a), acting both as endogenous competitive antagonists of ghrelin and inverse agonists of constitutive GHS-R1a activity [93]. This dual activity affects food intake and hormonal secretion, which makes LEAP-2 a promising target for the development of obesity drugs [93]. Thus, like other HDPs, LEAP-2 has a dual biological role.

Peptidoglycan recognition proteins (PGRPs) are produced by many species of animals, including swine [38, 95–98]. These proteins are involved in the activation of toll-like receptors (TLRs) [97, 99], the induction of proteolytic cascades with the goal of producing antimicrobial products (e.g., β-defensins) [95], and direct bactericidal activity [100]. Sang et al. [95] reported that porcine PGRP-L1 and PGRP-L2 play important roles in the onset of antimicrobial peptide β-defensin-1 (pBD-1) expression under experimental conditions. These findings demonstrated that the silencing of the *pPGRP* gene downregulates pBD-1 [95]. In addition to revealing the intracellular pathways involved in the production of pBD-1, these findings shed light on the complexity

and interdependence of the pathways involved in HDP production.

Currently, 28 pBDs have been described on swine chromosomes 7, 14, 15 and 17 [74] (Table 1). These molecules are cysteine-rich peptides that are less than 100 amino acids long and contain disulfide bridges and common end-to-end macrocyclization in their structures [38]. These biochemical properties confer hyperstability at high temperatures and salt concentrations, the presence of serum and the degradation of proteases [55]. There is evidence that the genetic codons responsible for encoding BDs in swine exhibit the highest level of sequence conservation compared with codons encoding other host defence peptides [38, 101, 102]. This finding is consistent with the hypothesis that the production of BDs has offered adaptive benefits throughout evolution and may have contributed to the persistence of these genetic traits to the present day [103, 104].

β-defensins are produced by epithelial cells on mucosal surfaces and by granule-containing leukocytes, including neutrophils, natural killer (NK) cells, and cytotoxic T lymphocytes [36]. There is increasing evidence that different cell types express different pBD genes [105], each expressing pBDs at different levels [37, 105]. Veldhuizen et al. [37] reported that pBD-1 gene expression increases from the proximal to the distal part of the small intestine. This may constitute a strategy to modulate the microbiome topographically, providing specific homeostasis for each mucosal niche [37].

The regulation of pBD expression is believed to involve a complex interplay of numerous factors, including cytokine signalling [106–108], hormonal mediation [109], dietary factors [110–112], and the fine-tuning of gene expression through epigenetic modifications [113]. To date, most of the knowledge concerning the regulation of pBD expression comes from in vitro or comparative studies involving animal species other than swine.

2.1 Antimicrobial role of β-defensins
2.1.1 Antimicrobial spectrum
pBDs have broad-spectrum antimicrobial activity, inhibiting bacterial, viral and fungal growth. The inhibition of both Gram-positive and Gram-negative bacteria has been described [51]. In bacteria, the inhibitory capacity of pBDs may be influenced by the presence or absence of a cell wall, as well as by the presence, absence, and amount of lipopolysaccharide and peptidoglycan layers. One example of this is the first discovered pBD, pBD-1. It more efficiently inhibits the growth of Gram-negative bacteria than Gram-positive, as evidenced by in vitro trials [38, 114]. pBD-2, on the other hand, has broad activity against both targets [51]. Assays involving the inhibition of viral growth show modest activity against viral

Finatto *et al. Veterinary Research*   (2025) 56:47

Page 5 of 13

Table 1 Genomic locations of porcine β-defensin (pBD) genes in the pig genome (*Sscrofa 11.1*)

pBD	Gene symbol	Chr	Location
pBD-1	DEFB1	15	NC_010457.5 (38076057..38077899)
pBD-2	Unknown	Unknown	Unknown
pBD-110	DEFB110	7	NC_010449.5 (44019705..44024322, complement)
pBD-112	DEFB112	7	NC_010449.5 (44031495..44041549, complement)
pBD-113	DEFB113	7	NC_010449.5 (43999119..44000402, complement)
pBD-114	DEFB114	7	NC_010449.5 (43982219..43993156, complement)
pBD-115	DEFB115	17	NC_010459.5 (35029031..35034148)
pBD-116	DEFB116	17	NC_010459.5 (35063977..35067003, complement)
pBD-121	DEFB121	17	NC_010459.5 (35154177..35155772, complement)
pBD-123	DEFB123	17	NC_010459.5 (35185257..35190686)
pBD-124	DEFB124	17	NC_010459.5 (35200560..35205507, complement)
pBD-125	DEFB125	17	NC_010459.5 (35003914..35010677, complement)
pBD-127	DEF127	17	NC_010459.5 (34973682..34975738, complement)
pBD-128	DEFB128	17	NC_010459.5 (34948185..34965455)
pBD-129	DEFB129	17	NC_010459.5 (34939180..34942357, complement)
pBD-133	DEFB133	7	NC_010449.5 (43970402..43975033, complement)
pBD-134	DEFB134	14	NC_010456.5 (15171675..15176787, complement)
pBD-135	DEFB135	14	NC_010456.5 (15143118..15145096)
pBD-136	DEFB136	14	NC_010456.5 (15109961..15110778, complement)
pBD-119	LOC100157086	17	NC_010459.5 (35131724..35142093, complement)
pBD-104A-like	LOC106504227	15	NC_010457.5 (37960250..38006690)
pBD-105-like	LOC106506206	15	NC_010457.5 (37982133..37989094, complement)
pBD-108B-like	LOC110256909	15	NC_010457.5 (38135983..38150772)
pBD-130-like	LOC110257049	15	NC_010457.5 (38189899..38195975)
pBD-15-like	LOC110257188	15	NC_010457.5 (37989086..37996091)
pBD-110-like	LOC110261742	7	NC_010449.5 (44008804..44009329, complement)
prepro-pBD-3	LOC404703	15	NC_010457.5 (37960248..38065632)
prepro-pBD-108-like	LOC692190	17	NC_010459.5 (35043632..35044109, complement)

This table provides detailed genomic information on pBD genes, including their gene symbols, chromosome numbers, and specific chromosomal locations within the pig genome assembly Sscrofa 11.1. The prefix "*prepro*" denotes that the protein is synthesized as a precursor molecule requiring processing for activation. The suffix "*like*" indicates that the defensin's sequence or structure resembles another defensin, on the basis of NCBI annotations. The specific evolutionary relationships of "*like*" genes (e.g., paralogue or orthologue status) could not be determined from the available data.

replication [51]. Notably, pBD-3 suppressed between 50 and 80% of porcine reproductive and respiratory syndrome virus (PRRSV) infections in an in vitro study involving porcine lung cells [115]. Intriguingly, pBD-1 and pBD-2 do not suppress viral activity at the same rate as pBD-3 does [115]. These findings demonstrate how the chemical structure of pBDs significantly impacts their activity spectrum. Finally, pBD-1 was the only molecule shown to have fungicidal activity against *Candida albicans* [114].

2.1.2 Antimicrobial mechanisms

Although the majority of the knowledge regarding antimicrobial mechanisms involving antimicrobial peptides (AMPs) is derived from human molecules, it is worth considering these mechanisms in swine, given the considerable similarities between these two species [46, 69]. Currently, three membrane disruption mechanisms have been proposed, namely, the barrel-stave, toroidal, and carpet models [116–120] (Figure 2). The barrel-stave pore model postulates that defence peptides assemble into dimers or multimers after binding to the negatively charged bacterial membrane [121]. This peptide array then traverses the cell membrane and reaches the interior of the bacterial cell. The assembly of peptides is a critical step in the formation of pores, and assembled peptides create barrel-like channels reminiscent of staves. In the toroidal pore model, peptides create a monolayer that bridges the outer and inner lipid layers of the membrane [122]. Conversely, the carpet model explores the possibility that defence peptides may act as surfactants, covering the surface of the bacterial cell and disrupting
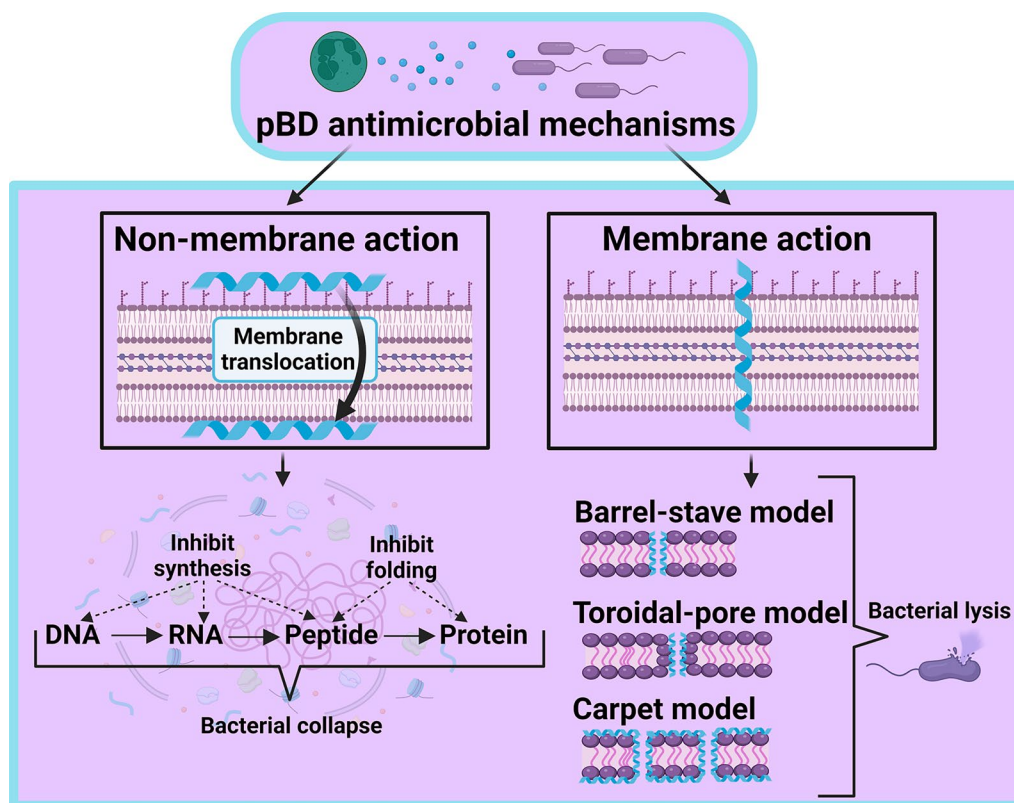
Finatto *et al. Veterinary Research*   (2025) 56:47

Page 6 of 13

Figure 2 Antimicrobial mechanisms of pBD. This figure illustrates the membrane and non-membrane action mechanisms of porcine β-defensins (pBDs) in the inhibition of microbial growth. The barrel-stave model shows that pBDs form barrel-like channels in the bacterial membrane. The toroidal pore model depicts pBDs connecting the outer and inner lipid layers, disrupting the membrane structure. The carpet model demonstrates that pBDs coat the bacterial surface, disrupting the bilayer structure. The non-membrane action model illustrates that pBDs impair DNA transcription, translation, and protein folding through intracellular interactions.

the membrane bilayer [123]. Additionally, the existence of a threshold concentration for optimal efficiency is suggested.

In addition to the previously mentioned mechanisms, recent research involving electron microscopy and gene expression analysis revealed that *E. coli* membranes were broken, holed, and wrinkled after treatment with pBD-2 [124]. This study also suggested that pBD-2 interferes with *E. coli* DNA transcription and translation [124] (Figure 2).

3 Immunomodulatory features of β-defensins

Immunomodulatory features have also been extensively associated with BDs across many species. These peptides play a role in reducing exacerbated inflammatory responses [125, 126], wound healing [127], chemokine production and chemotaxis of specialized cells [128, 129], and interactions with effector cells of the innate and adaptive immune systems [39, 125, 126, 128, 129].

Transgenic founder pBD-2-expressing mice are relatively resilient to *Salmonella enterica* subsp. *enterica*

serovar Typhimurium infection as a consequence of the direct bactericidal effect of pBD-2 [23]. Compared with control mice, animals that expressed pBD-2 presented with reduced epithelial damage, intestinal submucosal oedema and less neutrophil accumulation in the caecum (unable to express pBD-2). Systemic effects of pBD-2 in mice include reduced levels of circulating IL-6, TNF-α, IL-1β, and IL-12 following inoculation with *S.* Typhimurium or LPS [23]. Genetically edited mouse macrophages that produce pBD-2 suppress cytokine expression by inhibiting the LPS-activated NF-κB signalling pathway [23]. Zhang et al. [130] used IPEC-J2 cells to demonstrate that pBD-2 reduces cell apoptosis; downregulates the expression of the apoptosis-related genes *Cox-2* and *Caspase-3*; decreases the expression of *IL-6*, *IL-8*, *IL-1α*, and *C-X-C motif chemokine ligand 2* (*CXCL2*) and *C–C motif chemokine ligand 20* (*CCL20*); reduces the expression of *transforming growth factor-β-activated kinase 1* (*TAK-1*); and inhibits *NF-κB p65*. The authors suggested that alleviation of inflammation involves cytoplasmic interference with the TAK1-NF-κB signalling pathway. Moreover, the

findings of Zhang et al. [130] corroborate Huang et al. [23], suggesting that pBD-2 inhibits the NF-κB inflammatory pathway.

To address the intracellular mechanisms involving pBD-2 and the mitigation of inflammation, Huang et al. [23] conducted a study using fluorescently labelled pBD-2. The authors demonstrated that pBD-2 inhibits *vasohibin-1* (*VASH1*), a known angiogenesis inhibitor [131]. They also demonstrated how LPS could induce inflammation through the AKT/NF-κB signalling pathway, while pBD-2 could disrupt this mechanism, suppressing the inflammatory response.

Su et al. [132] have provided further evidence of the critical role played by the NF-κB signalling pathway in the biological function of pBD-114. pBD-114 activation through the TLR-activated NF-κB signalling pathway suppressed inflammation and reduced epithelial cell apoptosis in both mice and IPEC-J2 cells following challenge with enterotoxigenic *E. coli* K88 CVCC 225 [132].

A study in mice demonstrated the potential prophylactic effects of murine β-defensin 2 (mBD-2) during *Mycobacterium tuberculosis* infection [58]. Researchers have reported that mBD-2 functions as a liaison between the innate and adaptive immune systems, inducing a Th$_1$ response through the upregulation of interferon γ (IFN-γ), IL-12, and IL-6 in dendritic cells [58]. Another study explored gene therapy with a recombinant adenovirus vector to induce the overexpression of human β-defensin 3 (hBD-3), human cathelicidin, and mouse TNF-α. This strategy was used for the prevention of experimental latent tuberculosis reactivation in a murine model [133]. Gene therapy with hBD-3 reduces pneumonia, possibly through increased levels of IFN-γ and inducible nitric oxide synthase (iNOS) expression and the chemoattraction of lymphocytes and macrophages [133]. The role of pBDs in the chemotaxis of leukocytes, activation of immune cells, and regulation of adaptive immunity in swine has remained underexplored. This gap has the potential to reveal new strategies for prophylaxis and treatment (e.g., enhancing the antibody response to vaccines) [36, 58, 134].

4 Attempts to induce pBD expression in porcine epithelial cells

Zeng et al. [112] demonstrated that the expression of pBD-2 and pBD-3 mRNAs can be increased through exposure of IPEC-J2 cells, 3D4/31 macrophages and primary monocytes to butyrate. Similarly, butyrate analogues such as glyceryl tributyrate, benzyl butyrate, and 4-phenylbutyrate could also induce the expression of pBD-2 and pBD-3. The expression of pBD-1 remained largely unaltered in IPEC-J2 cells, 3D4/31 macrophages and primary monocytes.

Using IPEC-J2 cells, Mao et al. [111] measured the mRNA expression of pBD-1, -2 and -3 after 12 h of exposure to either ʟ-isoleucine or zinc sulphate. The expression of all pBDs increased following treatment. Interestingly, high doses (≥ 250 μg/mL of ʟ-isoleucine or ≥ 250 μmol/mL of zinc sulphate) resulted in an abrupt decrease in gene expression for the three target pBD genes. These findings suggest that the increase in pBD expression is not dose dependent. This statement is especially pertinent in swine medicine, as zinc oxide supplementation is a widespread practice used to control postweaning diarrhoea (PWD) [135]. While not yet fully explored and validated in vivo [111], this insight may have practical applications in the field. Wang et al. [110] demonstrated that IPEC-J2 cell expression of pBD-1, pBD-2 and pBD-3 is dose- and time-dependent following exposure to L-threonine at concentrations up to 1 mM. At this concentration, the expression levels of the pBDs were reduced. The same study also revealed that pBD expression inversely correlated with the gene expression of *IL-6*, *IL-8*, *IL-1β* and *TNF-α*, emphasizing the regulatory role of pBD.

Xue et al. [136] reported that recombinant mature porcine IL-22 (mpIL-22) inhibited the infection of porcine epithelial cells with porcine rotavirus (PorRV) and enteric coronaviruses (PEDV and transmissible gastroenteritis virus (TGEV)) through the overexpression of pBD-2, IL-18 and IFN-λ. According to the authors, the mechanism of pBD-2 overexpression was mediated by STAT3, as suggested by the phosphorylation of *signal transducer and activator of transcription 3* (STAT3) at phosphorylation sites Ser727 and Tyr705 in IPEC-J2 cells. To verify this outcome, the authors further used S3I-201, a STAT3-specific inhibitor, to verify how mpIL-22 behaves in the absence of STAT3. By doing so, they confirmed that the presence of S3I-201 can abolish the antiviral activity of mpIL-22, inhibiting the production of pBD-2 and promoting viral growth. These findings shed light on the transcription mechanism of pBD-2. DNA phosphorylation is possibly involved in the regulation of pBD-2 expression, affecting the accessibility of the DNA for the transcriptional machinery to bind and initiate transcription.

5 In vivo advances in the use of pBD overexpression to mitigate disease progression

One of the first clinical trials shedding light on the role of pBD-1 as a potential disease mitigator in pigs was conducted by Elahi et al. [59]. The authors reported that pBD-1 expression was significantly associated with protection against *Bordetella pertussis* in 4- to 5-week-old pigs but not in newborns, independent of whether they received colostrum. The authors validated their findings

in vitro, verifying that pBD-1 could inhibit the growth of *B. pertussis*.

Tang et al. [137] exposed 30 animals to the enterotoxigenic *E. coli* strain 0149:K88 (ETEC) and reported that pBD-2-treated animals presented growth performance comparable to that of neomycin sulphate-treated animals. pBD-2 treatment also induced similar histological, immune, and intestinal microbiome characteristics to those of the antibiotic-treated group.

Huang et al. [57] conducted a clinical trial in which they exposed genetically edited pigs that overexpressed pBD-2 and conventional animals to *Glaesserella* (*Haemophilus*) *parasuis*. Compared with control pigs, pBD-2 pigs presented significantly milder clinical signs and less severe gross pathological changes. Notably, the necroscopic and histological findings were accompanied by reduced loads of *G. parasuis* in tissues, which corroborates the hypothesis that pBD-2 may have mitigated the clinical signs associated with Glässer's disease [57].

Huang et al. [56] verified that pigs overexpressing pBD-2 had alleviated influenza-A virus-associated clinical signs. In vitro models confirmed that pBD-2 penetrates epithelial cells to interact with the mitochondrial solute carrier family 25 member 4 (SLC25A4) through hydrogen bonding, alleviating cell apoptosis induced by the virus, and that pBD-2 interferes with virus adsorption and post-entry stages in the cell. Although more studies are needed to clarify the adsorption mechanisms by which pBD-2 is involved, overexpressing pBD-2 in pigs led to reduced shedding and transmission between pigs, which may represent an applicable solution for pig farmers.

Yang et al. [138] reported that overexpressing pBD-2 in pigs has an effect on the organ distribution of *Actinobacillus pleuropneumoniae* in a cohabitation clinical trial. They demonstrated that, compared with normal-producing pBD-2 pigs, pBD-2-overexpressing pigs presented reduced bacterial loads in the lungs, although no differences were observed in terms of survival rate. The authors reported that the control pigs had severe congestion, haemorrhages, and oedema in the lungs, whereas only mild congestion and oedema were observed in those of pBD-2-overexpressing pigs.

6 Future perspectives

The possibilities surrounding pBDs are vast and largely untapped. Various pBDs have been identified [74], their bactericidal properties against different microorganisms have been established [23, 53, 57, 59, 137], and their potential for immunomodulation has been noted [23, 110–112, 136, 139]. While these advancements are significant for the development of applicable solutions, there is still much to be understood to fully harness the potential of these innate host peptides. Their immunomodulatory capacity remains modestly explored but holds immense long-term benefits for the livestock industry.

To the best of our knowledge, the use of pBDs in combination with a vaccination protocol has not yet been employed in swine. By incorporating a diet that enhances the expression of pBDs and closely monitoring cell dynamics, cytokines, and vaccine antibody responses, researchers can take a major step forward in understanding the role of innate peptides in disease prevention. Such a clinical trial could have far-reaching implications, not only by offering valuable insights into the optimization of existing vaccines in the swine industry but also by providing a potential solution for the prophylaxis of diseases that require specific cellular responses but are not functional via currently available strategies.

The potential for pigs genetically modified to produce pBDs to contribute to scientific breakthroughs is undoubtedly appealing [23, 56, 57, 138]. However, it remains uncertain whether this technology will be embraced by the pork industry [140–142]. While producers and packagers may be inclined to use those animals, consumer acceptance of these products is not always as expected. The market and legislation for genetically modified crops have already been established, but the challenges faced by the market for genetically edited animals appear to be much greater. A survey conducted by the Pew Research Centre revealed that while 50% of Americans said they would be willing to eat genetically modified foods, only 29% said they would be willing to eat meat from genetically edited animals [143]. This attitude is even more pronounced among European citizens, where it was shown that meat consumers support technologies that improve animal welfare standards but dislike excessive manipulation and lack the naturalness of their meat products, such as those represented by gene-editing technologies [144, 145].

The study of editing pBD sequences for chemical synthesis appears to yield promising results and is likely to receive increased attention in the near future [130]. In our opinion, the biochemical engineering of biologically derived molecules presents vast opportunities for enhancing their biological potential in controlling bacterial growth and offers intriguing prospects for reducing the likelihood of developing antimicrobial resistance [146–149].

A critical aspect that must be considered when discussing genetic selection and breeding, gene editing, or simply the administration of pBDs to pigs is the risk of selecting pathogens resistant to pBDs produced by the animals themselves. Although current evidence suggests that pathogens rarely develop resistance mechanisms to

Finatto *et al. Veterinary Research*   (2025) 56:47

Page 9 of 13

pBD in their wild form because of their multiple modes of action [124], this assumption may be overly optimistic. Vigilance is essential, as the potential for resistance development cannot be dismissed [150–152]. Gene editing and selection programs for pigs with high pBD production or clinical administration of synthetic pBDs must rigorously assess the potential risks of fostering antimicrobial resistance, balancing these risks with any health or production benefits. Pathogens may eventually develop resistance to pBDs [41, 42, 59], but we hypothesize that modulating innate and acquired immunity through these compounds as a prophylactic measure is much more difficult to circumvent and represents the most promising area to explore.

Finally, it is crucial to identify the actors who drive efforts to transform these ideas into applicable products for pig farmers and ultimately benefit society as a whole. The development of pBDs requires the collaboration of scientists from various disciplines, including biology, veterinary science, chemistry, and biotechnology. Additionally, legal support, such as patent protection and copyright defense, should be prioritized. To simplify the complex process and streamline marketing strategies, academic research centers should consider partnering with the industry. Properly funded and coordinated efforts to produce pBDs have the potential to increase sustainability in the pork industry and contribute to a more prosperous world.

7 Conclusions

Over the past two decades, interest in pBDs has increased due to their potent antimicrobial properties. The results of research in this area have been highly encouraging, demonstrating the ability of pBDs to mitigate numerous production-limiting diseases in the swine industry. This has far-reaching consequences, not only in terms of animal welfare but also in terms of the economic viability and profitability of the productive sector. Moreover, there is abundant evidence to support the immunomodulatory role of pBDs, albeit with less emphasis placed on this aspect than on their antimicrobial activity. Nonetheless, our hypothesis is that the field of immunomodulation holds enormous potential for the development of high-value, applicable technologies.

Authors' contributions
Conceptualization, ANF; writing—original draft preparation, ANF; visualization, ANF; writing—review and editing, ANF, FM, and MOC; and supervision and resources, MOC. All the authors read and approved the final manuscript.

Availability of data and materials
Data sharing does not apply to this article as no new data were created or analyzed in this study.

Declarations

Competing interests
The authors declare that they have no competing interests.

References
1. Waluszewski A, Cinti A, Perna A (2021) Antibiotics in pig meat production: restrictions as the odd case and overuse as normality? Experiences from Sweden and Italy. Humanit Soc Sci Commun 8:172
2. Albernaz-Gonçalves R, Antillón GO, Hötzel MJ (2022) Linking animal welfare and antibiotic use in pig farming—a review. Animals 12:216
3. Cuong NV, Padungtod P, Thwaites G, Carrique-Mas JJ (2018) Antimicrobial usage in animal production: a review of the literature with a focus on low-and middle-income countries. Antibiotics 7:75
4. Tang KL, Caffrey NP, Nóbrega DB, Cork SC, Ronksley PE, Barkema HW, Polachek AJ, Ganshorn H, Sharma N, Kellner JD, Ghali WA (2017) Restricting the use of antibiotics in food-producing animals and its associations with antibiotic resistance in food-producing animals and human beings: a systematic review and meta-analysis. Lancet Planet Heal 1:e316–e327
5. Aminov RI (2010) A brief history of the antibiotic era: lessons learned and challenges for the future. Front Microbiol 1:134
6. Wainwright M (1990) Miracle cure: the story of penicillin and the golden age of antibiotics. Basil Blackwell, Oxford
7. Jindal BAK, Pandya MK, Khan MID (2015) Antimicrobial resistance: a public health challenge. Med J Armed Forces India 71:178–181
8. Neto RM, Ansaldi MA, da Costa MESM, da Silva SO, Luz VHF (2012) A case report of a multi-drug resistant bacterial infection in a diabetic patient treated in northeast Brazil. Diabet Foot Ankle 3:18656
9. Aghapour Z, Gholizadeh P, Ganbarov K, Bialvaei AZ, Mahmood SS, Tanomand A, Yousefi M, Asgharzadeh M, Yousefi B, Kafil HS (2019) Molecular mechanisms related to colistin resistance in enterobacteriaceae. Infect Drug Resist 12:965–975
10. Dhingra S, Rahman NAA, Peile E, Rahman M, Sartelli M, Hassali MA, Islam T, Islam S, Haque M (2020) Microbial resistance movements: an overview of global public health threats posed by antimicrobial resistance, and how best to counter. Front Public Heal 8:535668
11. Myers J, Hennessey M, Arnold JC, McCubbin KD, Lembo T, Mateus A, Kitutu FE, Samanta I, Hutchinson E, Davis A, Mmbaga BT, Nasuwa F, Gautham M, Clarke SE (2022) Crossover-use of human antibiotics in livestock in agricultural communities: a qualitative cross-country comparison between Uganda, Tanzania and India. Antibiotics 11:1342
12. Manyi-Loh C, Mamphweli S, Meyer E, Okoh A (2018) Antibiotic use in agriculture and its consequential resistance in environmental sources: potential public health implications. Molecules 23:795
13. Ludden C, Raven KE, Jamrozy D, Gouliouris T, Blane B, Coll F, de Goffau M, Naydenova P, Horner C, Hernandez-Garcia J, Wood P, Hadjirin N, Radakovic M, Brown NM, Holmes M, Parkhill J, Peacock SJ (2019) One Health genomic surveillance of *Escherichia coli* demonstrates distinct lineages and mobile genetic elements in isolates from humans versus livestock. MBio 10:e02693–18
14. Rodríguez-Beltrán J, DelaFuente J, León-Sampedro R, MacLean RC, San Millán Á (2021) Beyond horizontal gene transfer: the role of plasmids in bacterial evolution. Nat Rev Microbiol 19:347–359

Finatto *et al. Veterinary Research*   (2025) 56:47

Page 10 of 13

15. Murray GGR, Hossain ASMM, Miller EL, Bruchmann S, Balmer AJ, Matuszewska M, Herbert J, Hadjirin NF, Mugabi R, Li G, Ferrando ML, Fernandes de Oliveira IM, Nguyen T, Yen PLK, Phuc HD, Zaw Moe A, Su Wai T, Gottschalk M, Aragon V, Valentin-Weigand P, Heegaard PMH, Vrieling M, Thein Maw M, Thidar Myint H, Tun Win Y, Thi Hoa N, Bentley SD, Clavijo MJ, Wells JM, Tucker AW, Weinert LA (2023) The emergence and diversification of a zoonotic pathogen from within the microbiota of intensively farmed pigs. Proc Natl Acad Sci 120:e2307773120

16. Redondo-Salvo S, Fernández-López R, Ruiz R, Vielva L, de Toro M, Rocha EPC, Garcillán-Barcia MP, de la Cruz F (2020) Pathways for horizontal gene transfer in bacteria revealed by a global map of their plasmids. Nat Commun 11:3602

17. Acman M, van Dorp L, Santini JM, Balloux F (2020) Large-scale network analysis captures biological features of bacterial plasmids. Nat Commun 11:2452

18. de Been M, Lanza VF, de Toro M, Scharringa J, Dohmen W, Du Y, Hu J, Lei Y, Li N, Tooming-Klunderud A, Heederik DJJ, Fluit AC, Bonten MJM, Willems RJL, de la Cruz F, van Schaik W (2014) Dissemination of cephalosporin resistance genes between *Escherichia coli* strains from farm animals and humans by specific plasmid lineages. PLoS Genet 10:e1004776

19. Karavolias J, Salois MJ, Baker KT, Watkins K (2018) Raised without antibiotics: impact on animal welfare and implications for food policy. Transl Anim Sci 2:337–348

20. Parlasca MC, Qaim M (2022) Meat consumption and sustainability. Annu Rev Resour Econ 14:17–41

21. Bartlett H, Zanella M, Kaori B, Sabei L, Araujo MS, de Paula TM, Zanella AJ, Holmes MA, Wood JLN, Balmford A (2024) Trade-offs in the externalities of pig production are not inevitable. Nat Food 5:312–322

22. Zharkova MS, Orlov DS, Golubeva OY, Chakchir OB, Eliseev IE, Grinchuk TM, Shamova OV (2019) Application of antimicrobial peptides of the innate immune system in combination with conventional antibiotics-a novel way to combat antibiotic resistance? Front Cell Infect Microbiol 9:128

23. Huang C, Yang X, Huang J, Liu X, Yang X, Jin H, Huang Q, Li L, Zhou R (2019) Porcine beta-defensin 2 provides protection against bacterial infection by a direct bactericidal activity and alleviates inflammation via interference with the TLR4/NF-κB pathway. Front Immunol 10:1673

24. Shafee TMA, Lay FT, Hulett MD, Anderson MA (2016) The defensins consist of two independent, convergent protein superfamilies. Mol Biol Evol 33:2345–2356

25. Hughes AL (1999) Evolutionary diversification of the mammalian defensins. Cell Mol Life Sci 56:94–103

26. Froy O, Gurevitz M (2003) Arthropod and mollusk defensins—evolution by exon-shuffling. Trends Genet 19:684–687

27. van der Weerden NL, Anderson MA (2013) Plant defensins: common fold, multiple functions. Fungal Biol Rev 26:121–131

28. Wu J, Gao B, Zhu S (2014) The fungal defensin family enlarged. Pharmaceuticals 7:866–880

29. Ganz T, Selsted ME, Szklarek D, Harwig SS, Daher K, Bainton DF, Lehrer RI (1985) Defensins. Natural peptide antibiotics of human neutrophils. J Clin Invest 76:1427–1435

30. Selsted ME, Harwig SSL, Ganz T, Schilling JW, Lehrer RI (1985) Primary structures of three human neutrophil defensins. J Clin Invest 76:1436–1439

31. Harder J, Schröder JM, Gläser R (2013) The skin surface as antimicrobial barrier: Present concepts and future outlooks. Exp Dermatol 22:1–5

32. Voss E, Wehkamp J, Wehkamp K, Stange EF, Schröder JM, Harder J (2006) NOD2/CARD15 mediates induction of the antimicrobial peptide human beta-defensin-2. J Biol Chem 281:2005–2011

33. Lai Y, Cogen AL, Radek KA, Park HJ, MacLeod DT, Leichtle A, Ryan AF, Di Nardo A, Gallo RL (2010) Activation of TLR2 by a small molecule produced by staphylococcus epidermidis increases antimicrobial defense against bacterial skin infections. J Invest Dermatol 130:2211–2021

34. de Koning HD, Simon A, Zeeuwen PLJM, Schalkwijk J (2012) Pattern recognition receptors in infectious skin diseases. Microbes Infect 14:881–893

35. Mwangi J, Hao X, Lai R, Zhang ZY (2019) Antimicrobial peptides: new hope in the war against multidrug resistance. Zool Res 40:488–505

36. Meade KG, O'Farrelly C (2019) β-defensins: farming the microbiome for homeostasis and health. Front Immunol 10:3072

37. Veldhuizen EJA, van Dijk A, Tersteeg MHG, Kalkhove SIC, van der Meulen J, Niewold TA, Haagsman HP (2007) Expression of β-defensins pBD-1 and pBD-2 along the small intestinal tract of the pig: Lack of upregulation in vivo upon *Salmonella typhimurium* infection. Mol Immunol 44:276–283

38. Sang Y, Blecha F (2009) Porcine host defense peptides: expanding repertoire and functions. Dev Comp Immunol 33:334–343

39. Machado LR, Ottolini B (2015) An evolutionary history of defensins: a role for copy number variation in maximizing host innate and adaptive immune responses. Front Immunol 6:115

40. Urmi UL, Vijay AK, Kuppusamy R, Islam S, Willcox MDP (2023) A review of the antiviral activity of cationic antimicrobial peptides. Peptides 166:171024

41. Cole JN, Nizet V (2016) Bacterial evasion of host antimicrobial peptide defenses. Microbiol Spectr 4:10.1128/microbiolspec.VMBF-0006-2015

42. Browne K, Chakraborty S, Chen R, Willcox MDP, Black DS, Walsh WR, Kumar N (2020) A new era of antibiotics: the clinical potential of antimicrobial peptides. Int J Mol Sci 21:7047

43. Laxminarayan R (2022) The overlooked pandemic of antimicrobial resistance. Lancet 399:606–607

44. Godfray HCJ, Aveyard P, Garnett T, Hall JW, Key TJ, Lorimer J, Pierrehumbert RT, Scarborough P, Springmann M, Jebb SA (2018) Meat consumption, health, and the environment. Science 361:eaam5324

45. Godfray HCJ, Beddington JR, Crute IR, Haddad L, Lawrence D, Muir JF, Pretty J, Robinson S, Thomas SM, Toulmin C (2010) Food security: the challenge of feeding 9 billion people. Science 327:812–818

46. Lunney JK, Van Goor A, Walker KE, Hailstock T, Franklin J, Dai C (2021) Importance of the pig as a human biomedical model. Sci Transl Med 13:1–20

47. Meurens F, Summerfield A, Nauwynck H, Saif L, Gerdts V (2012) The pig: a model for human infectious diseases. Trends Microbiol 20:50–57

48. Aarestrup FM, Oliver Duran C, Burch DGS (2008) Antimicrobial resistance in swine production. Anim Health Res Rev 9:135–148

49. Jones BA, Grace D, Kock R, Alonso S, Rushton J, Said MY, McKeever D, Mutua F, Young J, McDermott J, Pfeiffer DU (2013) Zoonosis emergence linked to agricultural intensification and environmental change. Proc Natl Acad Sci U S A 110:8399–8404

50. Gilbert W, Thomas LF, Coyne L, Rushton J (2021) Review: mitigating the risks posed by intensification in livestock production: the examples of antimicrobial resistance and zoonoses. Animal 15:100123

51. Veldhuizen EJA, Rijnders M, Claassen EA, van Dijk A, Haagsman HP (2008) Porcine β-defensin 2 displays broad antimicrobial activity against pathogenic intestinal bacteria. Mol Immunol 45:386–394

52. Han F, Zhang H, Xia X, Xiong H, Song D, Zong X, Wang Y (2015) Porcine β-defensin 2 attenuates inflammation and mucosal lesions in dextran sodium sulfate-induced colitis. J Immunol 194:1882–1893

53. Huang J, Qi Y, Wang A, Huang C, Liu X, Yang X, Li L, Zhou R (2020) Porcine β-defensin 2 inhibits proliferation of pseudorabies virus in vitro and in transgenic mice. Virol J 17:18

54. Su G, Xie K, Chen D, Yu B, Huang Z, Luo Y, Mao X, Zheng P, Yu J, Luo J, He J (2019) Differential expression, molecular cloning, and characterization of porcine beta defensin 114. J Anim Sci Biotechnol 10:60

55. González-Castro R, Gómez-Lim MA, Plisson F (2021) Cysteine-rich peptides: hyperstable scaffolds for protein engineering. ChemBioChem 22:961–973

56. Huang J, Liu X, Sun Y, Huang C, Wang A, Xu J, Zhou H, Li L, Zhou R (2022) Porcine β-defensin 2 confers enhanced resistance to swine flu infection in transgenic pigs and alleviates swine influenza virus-induced apoptosis possibly through interacting with host SLC25A4. Antiviral Res 201:105292

57. Huang J, Yang X, Wang A, Huang C, Tang H, Zhang Q, Fang Q, Yu Z, Liu X, Huang Q, Zhou R, Li L (2020) Pigs overexpressing porcine β-defensin 2 display increased resilience to *Glaesserella parasuis* infection. Antibiotics 9:903

58. Rivas-Santiago B, Cervantes-Villagrana A, Sada E, Hernández-Pando R (2012) Expression of beta defensin 2 in experimental pulmonary tuberculosis: tentative approach for vaccine development. Arch Med Res 43:324–328

59. Elahi S, Buchanan RM, Attah-Poku S, Townsend HGG, Babiuk LA, Gerdts V (2006) The host defense peptide beta-defensin 1 confers protection against *Bordetella pertussis* in newborn piglets. Infect Immun 74:2338–2352

60. Mair KH, Sedlak C, Käser T, Pasternak A, Levast B, Gerner W, Saalmüller A, Summerfield A, Gerdts V, Wilson HL, Meurens F (2014) The porcine innate immune system: an update. Dev Comp Immunol 45:321–343

61. Käser T (2021) Swine as biomedical animal model for T-cell research—success and potential for transmittable and non-transmittable human diseases. Mol Immunol 135:95–115

62. Rothkötter HJ (2009) Anatomical particularities of the porcine immune system-a physician's view. Dev Comp Immunol 33:267–272

63. Miller JC, Brown BD, Shay T, Gautier EL, Jojic V, Cohain A, Pandey G, Leboeuf M, Elpek KG, Helft J, Hashimoto D, Chow A, Price J, Greter M, Bogunovic M, Bellemare-Pelletier A, Frenette PS, Randolph GJ, Turley SJ, Merad M, Jakubzick C, Best AJ, Knell J, Goldrath A, Koller D, Cohen N, Brennan P, Brenner M, Regev A, Fletcher A, et al. (2012) Deciphering the transcriptional network of the dendritic cell lineage. Nat Immunol 13:888–899

64. Cervantes JL, Weinerman B, Basole C, Salazar JC (2012) TLR8: the forgotten relative revindicated. Cell Mol Immunol 9:434–438

65. Guiducci C, Gong M, Cepika AM, Xu Z, Tripodo C, Bennett L, Crain C, Quartier P, Cush JJ, Pascual V, Coffman RL, Barrat FJ (2013) RNA recognition by human TLR8 can lead to autoimmune inflammation. J Exp Med 210:2903–2919

66. Ugolini M, Gerhard J, Burkert S, Jensen KJ, Georg P, Ebner F, Volkers SM, Thada S, Dietert K, Bauer L, Schäfer A, Helbig ET, Opitz B, Kurth F, Sur S, Dittrich N, Gaddam S, Conrad ML, Benn CS, Blohm U, Gruber AD, Hutloff A, Hartmann S, Boekschoten MV, Müller M, Jungersen G, Schumann RR, Suttorp N, Sander LE (2018) Recognition of microbial viability via TLR8 drives T FH cell differentiation and vaccine responses. Nat Immunol 19:386–396

67. Murtaugh MP, Johnson CR, Xiao Z, Scamurra RW, Zhou Y (2009) Species specialization in cytokine biology: is interleukin-4 central to the TH1-TH2 paradigm in swine? Dev Comp Immunol 33:344–352

68. Bautista EM, Nfon C, Ferman GS, Golde WT (2007) IL-13 replaces IL-4 in development of monocyte derived dendritic cells (MoDC) of swine. Vet Immunol Immunopathol 115:56–67

69. Groenen MAM, Archibald AL, Uenishi H, Tuggle CK, Takeuchi Y, Rothschild MF, Rogel-Gaillard C, Park C, Milan D, Megens H-J, Li S, Larkin DM, Kim H, Frantz LAF, Caccamo M, Ahn H, Aken BL, Anselmo A, Anthon C, Auvil L, Badaoui B, Beattie CW, Bendixen C, Berman D, Blecha F, Blomberg J, Bolund L, Bosse M, Botti S, Bujie Z, Bystrom M et al (2012) Analyses of pig genomes provide insight into porcine demography and evolution. Nature 491:393–398

70. Dawson HD, Loveland JE, Pascal G, Gilbert JGR, Uenishi H, Mann KM, Sang Y, Zhang J, Carvalho-Silva D, Hunt T, Hardy M, Hu Z, Zhao S-H, Anselmo A, Shinkai H, Chen C, Badaoui B, Berman D, Amid C, Kay M, Lloyd D, Snow C, Morozumi T, Cheng RP-Y, Bystrom M, Kapetanovic R, Schwartz JC, Kataria R, Astley M, Fritz E et al (2013) Structural and functional annotation of the porcine immunome. BMC Genomics 14:332

71. Jungi TW, Adler H, Adler B, Thöny M, Krampe M, Peterhans E (1996) Inducible nitric oxide synthase of macrophages. Present knowledge and evidence for species-specific regulation. Vet Immunol Immunopathol 54:323–330

72. Zelnickova P, Matiasovic J, Pavlova B, Kudlackova H, Kovaru F, Faldyna M (2008) Quantitative nitric oxide production by rat, bovine and porcine macrophages. Nitric Oxide 19:36–41

73. van Harten RM, Veldhuizen EJA, Haagsman HP, Scheenstra MR (2022) The cathelicidin CATH-2 efficiently neutralizes LPS- and *E.* coli-induced activation of porcine bone marrow derived macrophages. Vet Immunol Immunopathol 244:110369

74. Choi MK, Le MT, Nguyen DT, Choi H, Kim W, Kim JH, Chun J, Hyeon J, Seo K, Park C (2012) Genome-level identification, gene expression, and comparative analysis of porcine ß-defensin genes. BMC Genet 13:98

75. Takahashi M, Umehara Y, Yue H, Trujillo-Paez JV, Peng G, Nguyen HLT, Ikutama R, Okumura K, Ogawa H, Ikeda S, Niyonsaba F (2021) The antimicrobial peptide human β-defensin-3 accelerates wound healing by promoting angiogenesis, cell migration, and proliferation through the FGFR/JAK2/STAT3 signaling pathway. Front Immunol 12:712781

76. Bals R, Wilson JM (2003) Cathelicidins—a family of multifunctional antimicrobial peptides. Cell Mol Life Sci 60:711–720

77. Shi S, Shen T, Liu Y, Chen L, Wang C, Liao C (2021) Porcine myeloid antimicrobial peptides: a review of the activity and latest advances. Front Vet Sci 8:664139

78. van Harten RM, van Woudenbergh E, van Dijk A, Haagsman HP (2018) Cathelicidins: immunomodulatory antimicrobials. Vaccines 6:63

79. Yu K, Lai BFL, Gani J, Mikut R, Hilpert K, Kizhakkedathu JN (2015) Interaction of blood components with cathelicidins and their modified versions. Biomaterials 69:201–211

80. Wang Y, Wang M, Shan A, Feng X (2020) Avian host defense cathelicidins: structure, expression, biological functions, and potential therapeutic applications. Poult Sci 99:6434–6445

81. Yu H, Wang C, Feng L, Cai S, Liu X, Qiao X, Shi N, Wang H, Wang Y (2017) Cathelicidin-Trypsin inhibitor loop conjugate represents a promising antibiotic candidate with protease stability. Sci Rep 7:1–18

82. Pashaie F, Hoornweg TE, Bikker FJ, Veenendaal T, Broere F, Veldhuizen EJA (2024) Antiviral activity of cathelicidins against porcine epidemic diarrhea virus (PEDV): mechanisms, and efficacy. Virus Res 350:199496

83. Kulkarni NN, O'Neill AM, Dokoshi T, Luo EWC, Wong GCL, Gallo RL (2021) Sequence determinants in the cathelicidin LL-37 that promote inflammation via presentation of RNA to scavenger receptors. J Biol Chem 297:100828

84. Cirone KM, Lahiri P, Holani R, Tan YL, Arrazuria R, De Buck J, Barkema HW, Cobo ER (2020) Synthetic cathelicidin LL-37 reduces *Mycobacterium avium* subsp. paratuberculosis internalization and pro-inflammatory cytokines in macrophages. Cell Tissue Res 379:207–217

85. Lin Q, Fu Q, Chen D, Yu B, Luo Y, Huang Z, Zheng P, Mao X, Yu J, Luo J, Yan H, He J (2021) Functional characterization of porcine nk-lysin: a novel immunomodulator that regulates intestinal inflammatory response. Molecules 26:4242

86. Andrä J, Koch MHJ, Bartels R, Brandenburg K (2004) Biophysical characterization of endotoxin inactivation by NK-2, an antimicrobial peptide derived from mammalian NK-Lysin. Antimicrob Agents Chemother 48:1593–1599

87. Andrä J, Monreal D, De Tejada GM, Olak C, Brezesinski G, Gomez SS, Goldmann T, Bartels R, Brandenburg K, Moriyon I (2007) Rationale for the design of shortened derivatives of the NK-lysin-derived antimicrobial peptide NK-2 with improved activity against gram-negative pathogens. J Biol Chem 282:14719–14728

88. Andersson M, Gunne H, Agerberth B, Boman A, Bergman T, Sillard R, Jörnvall H, Mutt V, Olsson B, Wigzell H, Dagerlind Å, Boman HG, Gudmundsson GH (1995) NK-lysin, a novel effector peptide of cytotoxic T and NK cells. Structure and cDNA cloning of the porcine form, induction by interleukin 2, antibacterial and antitumour activity. EMBO J 14:1615–1625

89. Andersson M, Gunne H, Agerberth B, Boman A, Bergman T, Olsson B, Dagerlind Å, Wigzell H, Boman HG, Gudmundsson GH (1996) NK-lysin, structure and function of a novel effector molecule of porcine T and NK cells. Vet Immunol Immunopathol 54:123–126

90. Ganz T (1966) Hepcidin—a peptide hormone at the interface of innate immunity and iron metabolism. Antimicrobial peptides and human disease, vol 72. Springer, Berlin, pp 183–198

91. Park CH, Valore EV, Waring AJ, Ganz T (2001) Hepcidin, a urinary antimicrobial peptide synthesized in the liver. J Biol Chem 276:7806–7810

92. Krause A, Neitz S, Mägert HJ, Schulz A, Forssmann WG, Schulz-Knappe P, Adermann K (2000) LEAP-1, a novel highly disulfide-bonded human peptide, exhibits antimicrobial activity. FEBS Lett 480:147–150

93. Lu X, Huang L, Huang Z, Feng D, Clark RJ, Chen C (2021) LEAP-2: an emerging endogenous ghrelin receptor antagonist in the pathophysiology of obesity. Front Endocrinol 12:717544

94. Sang Y, Ramanathan B, Minton JE, Ross CR, Blecha F (2006) Porcine liver-expressed antimicrobial peptides, hepcidin and LEAP-2: cloning and induction by bacterial infection. Dev Comp Immunol 30:357–366

95. Sang Y, Ramanathan B, Ross CR, Blecha F (2005) Gene silencing and overexpression of porcine peptidoglycan recognition protein long isoforms: involvement in β-defensin-1 expression. Infect Immun 73:7133–7141

96. Liu C, Xu Z, Gupta D, Dziarski R (2001) Peptidoglycan recognition proteins: a novel family of four human innate immunity pattern recognition molecules. J Biol Chem 276:34686–34694

Finatto *et al. Veterinary Research*   (2025) 56:47

Page 12 of 13

97. Dziarski R, Gupta D (2006) Mammalian PGRPs: novel antibacterial proteins. Cell Microbiol 8:1059–1069

98. Guan R, Wang Q, Sundberg EJ, Mariuzza RA (2005) Crystal structure of human peptidoglycan recognition protein S (PGRP-S) at 1.70 Å resolution. J Mol Biol 347:683–691

99. Garver LS, Wu J, Wu LP (2006) The peptidoglycan recognition protein PGRP-SC1a is essential for Toll signaling and phagocytosis of *Staphylococcus aureus* in Drosophila. Proc Natl Acad Sci U S A 103:660–665

100. Lu X, Wang M, Qi J, Wang H, Li X, Gupta D, Dziarski R (2006) Peptidoglycan recognition proteins are a new class of human bactericidal proteins. J Biol Chem 281:5895–5907

101. Patil AA, Cai Y, Sang Y, Blecha F, Zhang G (2005) Cross-species analysis of the mammalian β-defensin gene family: presence of syntenic gene clusters and preferential expression in the male reproductive tract. Physiol Genomics 23:5–17

102. Yeaman MR, Yount NY (2007) Unifying themes in host defence effector polypeptides. Nat Rev Microbiol 5:727–740

103. Loth K, Vergnes A, Barreto C, Voisin SN, Meudal H, Da Silva J, Bressan A, Belmadi N, Bachère E, Aucagne V, Cazevielle C, Marchandin H, Rosa RD, Bulet P, Touqui L, Delmas AF, Destoumieux-Garzón D (2019) The ancestral N-terminal domain of big defensins drives bacterially triggered assembly into antimicrobial nanonets. MBio 10:e01821-19

104. Bulet P, Stocklin R, Menin L (2004) Anti-microbial peptides: from invertebrates to vertebrates. Immunol Rev 198:169–184

105. Nakamura K, Yokoi Y, Fukaya R, Ohira S, Shinozaki R, Nishida T, Kikuchi M, Ayabe T (2020) Expression and localization of paneth cells and their α-defensins in the small intestine of adult mouse. Front Immunol 11:570296

106. O'Neil DA (2003) Regulation of expression of β-defensins: endogenous enteric peptide antibiotics. Mol Immunol 40:445–450

107. Abubakar S, Isa M, Omar N, Tan S (2020) Relative quantification of human β-defensins gene expression in pterygium and normal conjunctiva samples. Mol Med Rep 22:4931–4937

108. Shelley JR, Davidson DJ, Dorin JR (2020) The dichotomous responses driven by β-defensins. Front Immunol 11:1176

109. Fasina YO, Obanla T, Dosu G, Muzquiz S (2021) Significance of endogenous antimicrobial peptides on the health of food animals. Front Vet Sci 8:585266

110. Wang C, Yang Y, Gao N, Lan J, Dou X, Li J, Shan A (2021) L-Threonine upregulates the expression of β-defensins by activating the NF-κB signaling pathway and suppressing SIRT1 expression in porcine intestinal epithelial cells. Food Funct 12:5821–5836

111. Mao X, Qi S, Yu B, He J, Yu J, Chen D (2013) Zn2+ and l-isoleucine induce the expressions of porcine β-defensins in IPEC-J2 cells. Mol Biol Rep 40:1547–1552

112. Zeng X, Sunkara LT, Jiang W, Bible M, Carter S, Ma X, Qiao S, Zhang G (2013) Induction of porcine host defense peptide gene expression by short-chain fatty acids and their analogs. PLoS One 8:e72922

113. Dou X, Han J, Ma Q, Cheng B, Shan A, Gao N, Yang Y (2018) TLR2/4-mediated NF-κb pathway combined with the histone modification regulates β-defensins and interleukins expression by sodium phenyl butyrate in porcine intestinal epithelial cells. Food Nutr Res 62:1493

114. Shi J, Zhang G, Wu H, Ross C, Blecha F, Ganz T (1999) Porcine epithelial β-defensin 1 is expressed in the dorsal tongue at antimicrobial concentrations. Infect Immun 67:3121–3127

115. Sang Y, Ruchala P, Lehrer RI, Ross CR, Rowland RRR, Blecha F (2009) Antimicrobial host defense peptides in an arteriviral infection: differential peptide expression and virus inactivation. Viral Immunol 22:235–242

116. Shai Y (2002) Active antimicrobial peptides. Biopolymers 66:236–248

117. Brogden KA (2005) Antimicrobial peptides: pore formers or metabolic inhibitors in bacteria? Nat Rev Microbiol 3:238–250

118. Cuperus T, Coorens M, van Dijk A, Haagsman HP (2013) Avian host defense peptides. Dev Comp Immunol 41:352–369

119. Jenssen H, Hamill P, Hancock REW (2006) Peptide antimicrobial agents. Clin Microbiol Rev 19:491–511

120. Pálffy R, Gardlík R, Behuliak M, Kadasi L, Turna J, Celec P (2009) On the physiology and pathophysiology of antimicrobial peptides. Mol Med 15:51–59

121. Vogel H, Jähnig F (1986) The structure of melittin in membranes. Biophys J 50:573–582

122. Sengupta D, Leontiadou H, Mark AE, Marrink SJ (2008) Toroidal pores formed by antimicrobial peptides show significant disorder. Biochim Biophys Acta - Biomembr 1778:2308–2317

123. Pouny Y, Shai Y (1992) Interaction of D-amino acid incorporated analogs of pardaxin with membranes. Biochemistry 31:9482–9490

124. Chen RB, Zhang K, Zhang H, Gao CY, Li CL (2018) Analysis of the antimicrobial mechanism of porcine beta defensin 2 against *E. coli* by electron microscopy and differentially expressed genes. Sci Rep 8:14711

125. Cieślik M, Bagińska N, Górski A, Jończyk-Matysiak E (2021) Human β-defensin 2 and its postulated role in modulation of the immune response. Cells 10:2991

126. Semple F, Dorin JR (2012) β-defensins: multifunctional modulators of infection, inflammation and more? J Innate Immun 4:337–348

127. Petkovic M, Vangmouritzen M, Mojsoska B, Jenssen H (2021) Immunomodulatory properties of host defence peptides in skin wound healing. Biomolecules 11:952

128. Weinberg A, Jin G, Sieg S, McCormick TS (2012) The Yin and Yang of human β-defensins in health and disease. Front Immunol 3:294

129. Yang D, Chertov O, Bykovskaia SN, Chen Q, Buffo MJ, Shogan J, Anderson M, Schröder JM, Wang JM, Howard OMZ, Oppenheim JJ (1999) β-defensins: linking innate and adaptive immunity through dendritic and T cell CCR6. Science 286:525–528

130. Zhang K, Lian S, Shen X, Zhao X, Zhao W, Li C (2022) Recombinant porcine beta defensin 2 alleviates inflammatory responses induced by *Escherichia coli* in IPEC-J2 cells. Int J Biol Macromol 208:890–900

131. Zhao G, Na R, Li L, Xiao H, Ding N, Sun Y, Han R (2017) Vasohibin-1 inhibits angiogenesis and suppresses tumor growth in renal cell carcinoma. Oncol Rep 38:1021–1028

132. Su G, Luo Y, Chen D, Yu B, He J (2021) NF-κB-dependent induction of porcine β-defensin 114 regulates intestinal epithelium homeostasis. Int J Biol Macromol 192:241–249

133. Ramos-Espinosa O, Hernández-Bazán S, Francisco-Cruz A, Mata-Espinosa D, Barrios-Payán J, Marquina-Castillo B, López-Casillas F, Carretero M, del Río M, Hernández-Pando R (2016) Gene therapy based in antimicrobial peptides and proinflammatory cytokine prevents reactivation of experimental latent tuberculosis. Pathog Dis 74:075

134. Adams LG, Khare S, Lawhon SD, Rossetti CA, Lewin HA, Lipton MS, Turse JE, Wylie DC, Bai Y, Drake KL (2011) Enhancing the role of veterinary vaccines reducing zoonotic diseases of humans: linking systems biology with vaccine development. Vaccine 29:7197–7206

135. Schokker D, Kar SK, Willems E, Bossers A, Dekker RA, Jansman AJM (2023) Dietary supplementation of zinc oxide modulates intestinal functionality during the post-weaning period in clinically healthy piglets. J Anim Sci Biotechnol 14:122

136. Xue M, Zhao J, Ying L, Fu F, Li L, Ma Y, Shi H, Zhang J, Feng L, Liu P (2017) IL-22 suppresses the infection of porcine enteric coronaviruses and rotavirus by activating STAT3 signal pathway. Antiviral Res 142:68–75

137. Tang Z, Xu L, Shi B, Deng H, Lai X, Liu J, Sun Z (2016) Oral administration of synthetic porcine beta-defensin-2 improves growth performance and cecal microbial flora and down-regulates the expression of intestinal toll-like receptor-4 and inflammatory cytokines in weaned piglets challenged with enterotoxigeni. Anim Sci J 87:1258–1266

138. Yang X, Cheng YT, Tan MF, Zhang HW, Liu WQ, Zou G, Zhang LS, Zhang CY, Deng SM, Yu L, Hu XY, Li L, Zhou R (2015) Overexpression of porcine beta-defensin 2 enhances resistance to *Actinobacillus pleuropneumoniae* infection in pigs. Infect Immun 83:2836–2843

139. Huang C, Sun Y, Qiu X, Huang J, Wang A, Zhang Q, Pang S, Huang Q, Zhou R, Li L (2022) The intracellular interaction of porcine β-defensin 2 with VASH1 alleviates inflammation via Akt signaling pathway. J Immunol 208:2795–2805

140. Zhang M, Chen C, Hu W, Chen L, Zhan J (2016) Influence of source credibility on consumer acceptance of genetically modified foods in China. Sustain 8:1–16

141. Fozouni Ardekani Z, Akbari M, Pino G, Zúñiga MÁ, Azadi H (2021) Consumers' willingness to adopt genetically modified foods. Br Food J 123:1042–1059

142. Wunderlich S, Gatto KA (2015) Consumer perception of genetically modified organisms and sources of information. Adv Nutr 6:842–851

143. Funk C, Kennedy B (2016) The new food fights: U.S. public divides over food science. Pew Research Center, pp 43–58

144. Verbeke W, Pérez-Cueto FJA, de Barcellos MD, Krystallis A, Grunert KG (2010) European citizen and consumer attitudes and preferences regarding beef and pork. Meat Sci 84:284–292
145. Martin-Collado D, Byrne TJ, Crowley JJ, Kirk T, Ripoll G, Whitelaw CBA (2022) Gene-edited meat: disentangling consumers' attitudes and potential purchase behavior. Front Nutr 9:856491
146. Hansen PR, Oddo A (2015) Fmoc solid-phase peptide synthesis. Methods Mol Biol 1348:33–50
147. Schulz A, Klüver E, Schulz-Maronde S, Adermann K (2005) Engineering disulfide bonds of the novel human β-defensins hBD-27 and hBD-28: differences in disulfide formation and biological activity among human β-defensins. Pept Sci 80:34–49
148. Klüver E, Adermann K, Schulz A (2006) Synthesis and structure-activity relationships of β-defensins, multi-functional peptides of the immune system. J Pept Sci 12:243–257
149. Walewska A, Kosikowska-Adamus P, Tomczykowska M, Jaroszewski B, Prahl A, Bulaj G (2022) Improving Fmoc solid phase synthesis of human beta defensin 3. Int J Mol Sci 23:12562
150. El Shazely B, Yu G, Johnston PR, Rolff J (2020) Resistance evolution against antimicrobial peptides in *Staphylococcus aureus* alters pharmacodynamics beyond the MIC. Front Microbiol 11:103
151. Assoni L, Milani B, Carvalho MR, Nepomuceno LN, Waz NT, Guerra MES, Converso TR, Darrieux M (2020) Resistance mechanisms to antimicrobial peptides in gram-positive bacteria. Front Microbiol 11:593215
152. Shireen T, Singh M, Das T, Mukhopadhyay K (2013) Differential adaptive responses of *Staphylococcus aureus* to in vitro selection with different antimicrobial peptides. Antimicrob Agents Chemother 57:5134–5137

Publisher's Note