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Alternatives to antibiotics in a One Health context and the role genomics can play in reducing antimicrobial use

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Abstract

Background

This review follows on from the International Conference on One Health Antimicrobial Resistance (ICOHAR 2019), where strategies to improve the fundamental understanding and management of antimicrobial resistance at the interface between humans, animals and the environment were discussed.

Objective

This review identifies alternatives to antimicrobials in a One Health context, noting how advances in genomic technologies are assisting their development and enabling more targeted use of antimicrobials.

Sources

Key articles on the use of microbiota modulation, livestock breeding and gene editing, vaccination, anti-virulence strategies and bacteriophage therapy are discussed.

Content

Antimicrobials are central for disease control, but reducing their use is paramount due to the rise of transmissible antimicrobial resistance. This review discusses antimicrobial alternatives in the context of improved understanding of fundamental host-pathogen and microbiota interactions using genomic tools.

Implications

Host and microbial genomics and other novel technologies have an important role to play in devising disease control strategies for healthier animals and humans that in turn reduce our reliance on antimicrobials.

Introduction

Over the past half century, global livestock production has dramatically increased [1], which has been made possible by intensification of livestock systems and genetic selection for improved feed conversion efficiency. However, this has come at a cost, with strong evidence that high stocking densities increases the risk of infectious disease [2–5]. Disease eradication, vaccination and antimicrobial use have been instrumental in mitigating the impacts of infectious disease on animal health, welfare and production. Whilst there are regional examples of successful disease eradication [6–10], rinderpest is the only animal disease to have been eradicated globally [11,12]. Many production limiting diseases in livestock are not satisfactorily controlled by vaccination, particularly in highly productive individuals, where metabolic resources are potentially allocated to production at the expense of immune function [13]. As such, some highly productive livestock systems rely on antimicrobials to control production limiting disease complexes, evidenced in part by predictions of an increase in global antimicrobial use in livestock production from 63,151 tons in 2010 to 105,596 tons by 2030 [14]. In some countries, sub-therapeutic doses of antimicrobials are used as dietary additives to promote the growth of farmed animals, however this is now prohibited in the European Union due to fears that this practice results in the transmission of antimicrobial resistance to clinically-relevant human pathogens.

Whilst there is ongoing debate relating to the magnitude of the impact of antimicrobial use in livestock on human health [15,16] and the environment [17], there is sufficient evidence to support the adoption of a precautionary principle with respect to antimicrobial use in many animal production systems [18]. Livestock owners are therefore faced with the challenge of how to reduce antimicrobial use, whilst improving the health, welfare and productivity of their

72 animals. Of most concern is that many production systems have become reliant on
73 antimicrobial use and that if use is reduced, without other changes, there is a significant risk of
74 increased levels of disease and decreased animal welfare and production.
75
76 Fundamental research aiding our understanding of host-pathogen interactions, and gene
77 exchange [19], is key to developing alternatives. Such research has improved our
78 understanding of the complexity of interactions between eukaryotes, prokaryotes, viruses and
79 their environments, which has been accelerated by sequencing technologies. This short review
80 will highlight some key alternative approaches to reduce infections in livestock species that in
81 turn could ease the reliance on antimicrobials, and therefore reduce the emergence and
82 maintenance of AMR alleles in these populations and the potential impact on humans. These
83 are summarized in Figure 1.
84
85 **Microbiota modulation**
86 Microbiota modulation, using probiotics, altered nutrition or faecal transplantation, has been
87 intensively studied in the context of human, livestock and plant health (as previously reviewed
88 [20–22]). Improved understanding of these techniques requires further study of host-
89 microbiota dynamics, which is reliant on genomic tools to define the microbial populations
90 present.
91
92 One of the clearest examples of the importance of the host microbiota is the protective role
93 against enteric pathogens (as previously reviewed [23–26]). Gut microbiome studies usually
94 target faeces, but unique taxonomic profiles have been revealed in discrete gut sections [27–
95 30]. For example, bacterial populations in the porcine small intestine have been shown to
96 change in response to an increase in dietary protein and exposure to enterotoxigenic

97 *Escherichia coli* [29], in the absence of changes in the faecal microbiome [29,31]. Probiotics
98 have also shown targeted effects, with *Bacillus pumilus* supplementation being shown to
99 decrease *Lawsonia intracellularis* shedding and numbers of small intestinal lesions in pigs
100 [32]. Supplementation with other *Bacillus* probiotic strains showed a reduction of intestinal
101 *Clostridium perfringens* counts and a reduction of *Salmonella* colonisation in broiler chickens
102 [33,34]. Such work highlights the importance of targeted microbiome studies, at both key
103 digestion and colonisation sites, whilst also demonstrating the potential of microbiota
104 modulation in reducing bacterial colonisation without antibiotic use.
105
106 Additionally, a higher resolution understanding of microbiota dynamics is required. For
107 example, the use of large-scale cross sectional [35] and longitudinal [36] microbiome data has
108 linked the effects of antibiotic administration with AMR gene abundance and diversity in pig
109 production systems. Specifically, metagenomic sequencing allows a comprehensive analysis
110 of both microbial taxonomy and identification of genes encoding for antimicrobial resistance,
111 toxin production and the presence of mobile genetic elements. Such an approach facilitates an
112 understanding of the maintenance of AMR gene and virulence determinants within the
113 microbiome, whilst also demonstrating the impact of management and disease (including viral
114 and parasitic infections) on microbiota composition and function.
115
116 In the context of microbiota modulation, genomic tools will be instrumental in identifying both
117 the reservoirs and mechanisms of AMR, as well as the development and testing of novel
118 interventions to reduce AMR gene carriage and colonisation by production limiting and
119 zoonotic infections. Additionally, a better functional understanding of metabolic pathways
120 within the microbiota provides the opportunity for modulation to achieve more efficient ration
121 utilisation, and this area aligns with research to understand how antibiotics can be successful

122 growth promoters. This raises the question whether this is due to reducing pathogen loads
123 and/or initiating more general changes in microbiota composition that favour nutrient
124 acquisition by the animal.
125
126 **Livestock breeding and gene editing**
127 Increased productivity has been selected for since the domestication of livestock and has been
128 accelerated in recent decades through the use of breeding indices, based on production data
129 collected from well-defined pedigrees. Initially, these indices were almost exclusively focused
130 on feed conversion efficiency and total yield, and their implementation has negated the benefits
131 achieved via use of antimicrobial growth promoters over time [37]. However, the importance
132 of including more balanced traits, including disease susceptibility, has come to the fore.
133
134 Genetic gains in livestock traits have been most obvious in developed countries, but now there
135 is an emphasis to improve productivity by dissecting the basis of livestock traits associated
136 with resilience to climate, disease and nutritional stressors. Genomic selection using breeding
137 values based on genome-wide markers [38], has the potential to further accelerate rates of
138 genetic improvement in livestock, whilst also ensuring more balanced selection for traits
139 important to health and disease. A key challenge is to balance sustainable genetic gains against
140 narrowing of genetic diversity, particularly with respect to Major Histocompatibility Complex
141 haplotypes that are vital for presentation of diverse antigens.
142
143 Transgenic and gene editing technologies open up further possibilities for disease control (as
144 previously reviewed [39]). Early applications of this technology included engineering of
145 lysozyme and lysostaphin into milk-producing species to reduce susceptibility to bacterial
146 pathogens that cause mastitis. which are significant drivers of antibiotic use in dairy cattle [40].

As new technologies have become available, more targeted approaches have been demonstrated, such as gene editing in pigs for resistance to porcine reproductive & respiratory syndrome virus (PRRSV). PRRSV is an immunomodulatory pathogen that has significant impacts on pig production globally and is a major driver of antimicrobial use due to secondary bacterial infections. A detailed understanding of host-pathogen interactions identified CD163 as an important internal receptor for the virus [41]. This was combined with knowledge of the specific domain bound by the virus, as well as splicing data, to precisely delete only the virus binding domain, leaving other important functions of CD163 intact. When homozygous, this edit results in complete resistance to both main types of the virus in macrophages and to PRRSV-1 in edited animals [42,43].

Such methods still need to pass regulatory and public acceptance [44], but they clearly have the potential to offer a step-change for resistance to certain diseases, especially where integrated supply chains exist from nucleus genetic stocks that could be modified and rapidly distributed. Genome-wide mutagenesis to identify host genes that influence viral replication [45,46] is being used to define new targets for gene editing. Whether bacterial infections offer such opportunity for targeted editing remains unclear.

Vaccination

Vaccines have a vital role in the control of antimicrobial resistance as they can reduce the number of cases of disease requiring therapy, both directly by protecting recipients and indirectly via herd immunity [47]. Moreover, they can reduce the number of pathogens associated with clinical syndromes permitting narrow-spectrum therapies, and can be used to combat the transmission of pathogens and their antimicrobial resistances from animals to humans.

Priority pathogens for veterinary vaccines remain ill-defined, compounded by the fact that some diseases are polymicrobial in origin (e.g. ovine foot rot, necrotic enteritis in poultry), or may be caused by different pathogens for which specific diagnoses are not necessarily sought (e.g. mastitis). Even for diseases associated with a single pathogen, genetic diversity and rapid evolution can impede the design of cross-protective vaccines. Priorities and novel approaches for animal vaccines were recently reviewed [48,49], and include viral diseases that attract significant antibiotic use for secondary bacterial infections owing to immunosuppression. Where effective vaccines have been implemented, reduced antimicrobial use can be documented, as with vaccines for *Lawsonia intracellularis*, *Actinobacillus pleuropneuomia*, PRRSV and porcine circovirus-2 in pigs [48].

Comparative and functional genomic analysis have powerful roles to play in vaccine development. Sequence analysis can predict pathogen-specific factors that are conserved and accessible to antibody by virtue of secretion signals, which can then be evaluated as subunit vaccines, a concept termed reverse vaccinology [50]. This was first applied to develop vaccines for group B meningococci [51] and extended to animal diseases, including brucellosis, mastitis and systemic *E. coli* infections. Analysis of pathogen gene expression within the host can further narrow the selection of targets. A major advance in recent years has been the advent of transposon sequencing approaches that can assign phenotypes to mutants screened in complex pools with minimal animal use. These rely on massively-parallel sequencing of transposon-flanking sequences in pools of mutants, and when applied to inocula and output pools recovered from animals, can simultaneously identify transposon insertion sites and quantify the relative abundance of the cognate mutants [52]. These identify key virulence factors that could be used as subunit vaccines, vectored in carrier strains, or mutated to produce live-

attenuated vaccines. However, selection of candidates for live-attenuated vaccines that will confer lasting protection with minimal persistence and in the absence of pathology remains a key challenge. Importantly, screening of the same mutant library across multiple hosts can reveal host- or tissue-specific virulence factors, with important implications for vaccine design [53]. Whilst genomics is being increasingly applied to antigen discovery, there is a need to explore its utility to understand the host response to vaccination, particularly with respect to novel adjuvant and vaccine platform development. This is particularly important in livestock species, given the limited immunological reagents to study host immune responses.

Anti-virulence strategies

'Anti-virulence' (AV) compounds disarm pathogenic bacteria by blocking activity or expression of virulence factors which are required to colonise and persist in their host. Genomic approaches have the capacity to identify virulence factors, regulons and regulators that are important for infection. These can then be the focus of screens for inhibitors, building on the regulatory disruption shown for the first published AV inhibitor, virstatin, which inhibits the expression of virulence genes in *Vibrio cholerae* via the downregulation of the transcriptional regulator, ToxT [54]. Bacterial biofilms have been targeted for AV development [55–57] and small molecule screens are key in defining biofilm inhibitors. Genomic approaches such as RNAseq can be applied to study the pathways affected [56], potentially identifying more refined targets. An example of this is ajoene, a sulphur-rich molecule isolated from garlic, that has been shown to inhibit biofilm formation by sRNA inhibition [58].

Compounds specifically targeting toxin activity are attractive areas for development, including a hydroxamate inhibitor of lethal factor anthrax toxin [59] and binders of Shiga toxin aimed at preventing toxicity systemically [60] or by 'bacterial mops' expressing the receptor in the gut

[61]. A high-throughput screen of synthetic compounds led to the identification of compounds which reduce Shiga toxin production in a dose-dependent manner [62]. An AV strategy has been designed to neutralise the key toxins produced by virulent strains of *Clostridium difficile* toxins [63,64]. In 2017, the FDA approved the monoclonal antibody Bezlotoxumab, which targets *C. difficile* Toxin B, as a therapy for patients with recurring *C. difficile* infections [65]. As with toxins, as we define critical virulence factors, these can be specifically targeted. Many significant Gram-negative pathogens express a Type III Secretion System (T3SS) and this is a primary target for AV therapy [66–69]. Notably, synthetic salicylidene acylhydrazide derivatives have been used to inhibit T3S effectively [70,71]. One promising T3S inhibitor is Aurodox which has the benefit of not inducing Shiga-toxin production [72] [73] [74].

Bacteriophage therapy

Bacteriophages (phages) are viruses that specifically infect and kill bacteria as part of their natural life cycle. The success of antibiotics has restricted the wider development of phage therapy, but it has been and continues to be applied to treat a variety of infections [75–79]. Phages can be utilised in livestock production to improve animal health or to reduce bacterial load before slaughter, or further upstream in food processing and packing. Examples of this include adding phages to aquaculture systems to improve fish health [80] and to remove *Escherichia coli* O157:H7 from cattle before entering the food chain [81,82].

There are increasing numbers of publications reporting the potential of phage therapy, but there are hurdles to overcome before phages can be used therapeutically. Firstly, phages active on the proposed target in clinically-relevant environments must be identified. Traditional methods of manually screening a phage library are labour intensive and low throughput. By contrast, a computational approach can be taken to predict phage-host bacterium interactions *in silico*

[83,84]. Our own work, based on machine learning of genotype to phenotype relationships [85], uses phage-bacterium interaction data along with the bacterial genome sequence to predict which phages will be active on a clinical isolate. As routine diagnostics implement more whole-genome sequence data, effective phage treatments could be predicted from the genome sequence as currently being applied for antibiotic sensitivity [86]. The genome sequence of the pathogen therefore will allow selection of tailored therapies including antibiotics and phages.

In addition to guiding the selection of correct phages for treatment, genomic approaches can also be used to identify bacterial resistance mechanisms. The capacity to select effective phages will be greatly improved if we can identify resistance determinants. For example, in the competitive environment of a mammalian host, it is likely that bacteria will employ CRISPR-based resistance mechanisms rather than surface factor resistance which can have a fitness cost for the bacteria [87]. Bringing phage therapy into routine clinical use will also require safety issues to be resolved and practical issues to be tackled, such as the stability of therapeutic phages. There is concern that phages could introduce AMR or virulence alleles into a pathogen and so phages used should be sequenced. A new gene caller called PHANOTATE [88] has been designed to specifically identify phage genes and phage genomes can be analysed for the presence of antibiotic/virulence factors using gene databases [89]. PHACTS software [90] can also be applied which allows identification of lytic rather than lysogenic phages.

Summary

Antimicrobial use is under scrutiny, especially in production animals which utilise over half of the world's manufactured antibiotics. The challenge is to control the infectious diseases that compromise animal welfare and production, whilst using less of these remarkable compounds. This can be achieved in part by better system management and 'high herd health' approaches,

however disease complexes and specific pathogens represent an ongoing threat to animal welfare and productivity. Genomic technologies are key to understanding both host and pathogen to drive the development of better diagnostics and more directed treatments. Rapid genomic-level diagnostics in particular are essential to delivery of bespoke treatments, not just with traditional antibiotics; but with microbiota and immune modulators, vaccines and phages. High-throughput screens are powerful approaches for the identification of key pathogen and host determinants to develop vaccines and inhibitors, as well as markers to accelerate breeding programs or as targets to engineer resistance. This work goes hand-in-hand with developments in animal genomics, computational tools and assessment of impact in One Health systems.

Transparency declaration

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Figure legend

Figure 1. Summary of alternatives to antibiotics in a one health context that are covered in this review.

298

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