Phages for Africa

Subjects: Virology

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Livestock farming is vital to Sub Saharan Africa for food supply, source of employment, and income. However, antibiotic use in livestock farming is rampant leading to the rise of antibiotic resistant bacteria. Because of this rise in antibiotic resistance, there is a growing need to find alternatives to antibiotics in the preventio, treatment and control of bacterial infections in livestock.An alternative that is going through a renaissance is the use of bacteriophages (phages), viruses that infect and kill bacteria, which have been used and administered as pharmaceutical agents even before the discovery of antibiotics. Phages are the most abundant and ubiquitous organisms on earth, and can be found in natural and man-made environments, especially those in which their bacterial host thrives. Phage therapy has therefore been proposed as one of the most promising alternatives for the treatment of infections in livestock contributing towards mitigation of antimicrobial resistance.

Keywords: antimicrobial resistance (AMR) ; multi-drug resistance (MDR) ; Sub-Saharan Africa (SSA) ; bacteriophage therapy ; regulations of phage products

1. Introduction

In Africa, a majority of the population, in a range of 250–300 million, depend on livestock for their income and livelihood, with livestock representing an average of 30% of the agricultural gross domestic product (GDP) and roughly 10% of the total GDP [1]. Animal diseases, including zoonoses, are crucial constraints in the enhancement of livestock-production systems [2] and compromise food-producing animals' nutritional and economic potential [3]. Facing its own challenges, Africa has been reported to be one of the continents with the highest number of foodborne diseases, with approximately 91 million related diseases and 137,000 death per annum [4]. Unfortunately, on a global scale, the use of antibiotics is largely unregulated, and this is worse in developing countries where the use of antibiotics for food and animal productions to accelerate the growth of animals is rampant. Compared to other continents, Africa produces fewer antibiotics, but unregulated access and inappropriate use worsens antibiotic resistance [4][5]. Other factors, such as the poor regulation on the use of antimicrobials in both human and animals, inaccessibility to appropriate therapy, weak surveillance systems, and a lack of updated use and treatment guidelines of antimicrobials, play a role in the spread of antibiotics resistance [6]. Farmers also play a massive role in the misuse of antibiotics whereby there is a tendency to store drugs and treat animals based on symptoms they are familiar with from past infections, engaging unskilled people to treat animals, and unregulated disposal of waste in dumps. Counterfeit medicines are an additional issue that could jeopardize the fight against antimicrobial resistance [7]. Due to this constant application of antibiotics, whether for prevention, treatment, or growth promotion, this creates a selective pressure on resistant bacteria. Due to this exposure, bacteria have also developed bet-hedging strategies to resist these harsh antibiotics over time; although this comes at a survival cost for the bacteria, a subpopulation of these resistant bacteria are propelled towards survival[7][8]. Among the strategies' that bacteria use to acquire resistance, include the transfer of resistant genes through horizontal gene transfer, mobile genetic elements, and the bacterial toxin–antitoxin system [9].

2. Using phages as an alternative to antibiotics in Livestock Farming

An alternative that is going through a renaissance is the use of bacteriophages (phages), viruses that infect and kill bacteria, which have been used and administered as pharmaceutical agents even before the discovery of antibiotics [10]. Phages are the most abundant and ubiquitous organisms on earth, and can be found in natural and man-made environments, especially those in which their bacterial host thrives [11][12]. After the discovery of antibiotics by Alexander Fleming in 1928, phage therapy was rapidly abandoned in the West. However, in countries that had witnessed the birth of phage therapy, such as Georgia and Poland, this therapy continued to flourish until modern days [13]. Phages are viruses that have the ability to infect bacteria, replicate within them, and eventually kill their susceptible host releasing progeny virions [14]. Phages use two primary life cycles to replicate, the lytic cycle and the lysogenic cycle, each having significant implications for their therapeutic application [14]. In the lytic cycle, the phage attaches itself to the bacterial cell, allowing

the penetration of phage nucleic acid, transcription, translation, assembly, and exit. This exit involves killing the bacteria through the expression of endolysins and releasing multiple, as low as 20 and up to hundreds or thousands of progeny phages, which can infect other bacterial cells, thereby repeating the cycle [15]. The duration from the attachment of a phage particle to a bacterial cell and its subsequent release of new phage particles usually happens within 20–40 min but can take up to 1–2 h [16]. Due to this short life cycle, phages could potentially be used for different applications such as prior slaughter, to treat or control bacteria that may pose harm to the farmer or end user [16]. The lysogenic cycle begins with inclusion its genetic material into the chromosome of the bacterial cell, after which, replication of the phage nucleic acid together with the host genes occurs for numerous generations without major metabolic consequences for the bacterial cell, thus allowing co-existence between the phage and bacteria [17]. This also facilitates the exchange of genetic material between the phage and bacteria. However, the phage may occasionally return to the lytic cycle, leading to the release of phage particles and, in some scenarios, spreading acquired bacterial DNA [18]. Temperate phages are usually not recommended for phage therapy, as during replication they can randomly pick up a wide range of segments of bacterial DNA and transfer them to a new host. This quality makes them undesirable for therapeutic applications since virulence-associated genes, or antibiotic-resistance genes, amongst other examples, could be transferred by this route [19] [20]. In some scenarios, when a suitable lytic phage cannot be isolated, it may be necessary to exclude such harmful genes, usually by synthetic biology, to circumvent or eliminate these unfavorable features. Apart from reducing undesirable qualities, other potential benefits of using synthetic biology to alter phage function include modulating the phage host range, reducing phage toxicity and immunogenicity, enhancing phage survival after administration, improving phage activity against biofilms, and enhancing bacterial killing when combined with antibiotics [19]. On the contrary to most antibiotics, phages are highly specific antibacterial agents that have the advantage of causing minor damage to the healthy microbial flora of the treated animal [21]. With the increasing cases of antimicrobial resistance worldwide, phage therapy can be used as an alternative to antibiotics and in the treatment of several bacterial infections [22]. Moreover, phages have been used to combat bacterial infections in animals with the goal of reducing the bacterial load [23].

The efficacy of bacteriophages as antimicrobials has fostered the approval and commercialization of several products intended for the reduction of different pathogenic bacterial species [24]. Examples of some phage-related products include SalmoFree and SalmoFresh™, both containing Salmonella enterica phages [25][26], ListShield™ designed with Listeria monocytogenes phages [27], as well as phage-derived enzymes such as Lysins, integrases, and excisionases, have received considerable attention as potential antibacterial agents [28]. Phage and phage related products have advantages over antibiotics in many ways; e.g., some applications may require only a single dose since phages can self-amplify. Moreover, because phages are easy to isolate from the environment, meaning short product development time frames and reduced production costs compared to antibiotics [29]. Other beneficial properties of phages include a decreased probability of resistance development if a single phage with a wide host range or a cocktail of phages is used. Additionally, phages are safe (non-toxic) for eukaryotic cells and act as a bactericidal by hijacking many essential cellular processes required by the bacteria [30]

Another advantage is that phage discovery is relatively easy because they exixt natural entities that are easier to isolate, purify, and characterize within a short time and at a lower cost as compared to antibiotics, which require several years of discovery and clinical trials [31]. The four methods of phages isolation—spot lysis, plaque testing, culture lysis, and routine test dilution (RTD)—have been shown to require only 24 h [32]. Likewise, the isolation of phages from animals and their environment also requires about 24 h, which is less time and effort than antibiotic discovery [32][33]. These former steps are easy to achieve but numerous factors should be taken into account in the context of product development. Bacteriophages that are considered for product development must be produced with an acceptable level of purity and have to be assessed for their efficacy in vivo and the safety of the final product. To ensure the consistency and stability of phages, the procedure for their manufacture, physicochemical and biological quality tests should be defined, as well as stringent production facilities [34]. As livestock farming in SSA is quite dynamic, with farmers rearing multiple livestock species together, this represents a complex ecology between bacteria species from different livestock as well as their interactions with phages. However, this encourages the development and delivery of local phage products that would take into account these farming dynamics.

Bacteria are also less likely to develop resistance against phages when the latter are used as therapy compared to antibiotics. One of the drawbacks to this is the host range of the phages used. The host range describes the breadth of bacteria a phage is capable of infecting [35][36][37][38]. The narrow host range which is exhibited by most phages limits the number of bacterial types with which selection for specific phage-resistance mechanisms can occur [39]. Experimental data has shown that 80% of phage-resistant variants occur mostly in wide host range phages. The use of well-characterized phage cocktails is less likely to cause phage resistance compared to broad-spectrum antibiotics [40][41][42][43]. The reason for this phenomenon is that phage cocktails generally rely on different receptor-binding proteins during attachment,

allowing specific binding of a phage to a specific host through alternate routes of entry. Using single phage preparations rather than a cocktail toward a specific bacterial species only accelerates the process of mutations, thus rendering the phage product inactive [44].

3. Hurdles of Phage Research and Regulatory Aspects of Phage Development/Products in SSA with a Focus on Kenya

As phage research in Africa is gaining interest, phages that are pure, well-characterized, sequenced, and have a defined host specificity still need to be documented. Moreover, this information should be publicly available to the different government bodies regulating veterinary practices in Africa. Currently, characterization, purification, sequencing, and storage of one phage can be achieved at a cost of about EUR 500 [45], which is not sustainable for the African continent and may need collaboration between different phage research groups around the world to cut down this cost. It is important to remember that several bacterial strains are often present in an infection; hence, multiple phage types may be needed to treat different strains of one bacterial species [45], making the goal of having a phage bank consisting of characterized phages equitably impossible if support from local governments is not achieved. Hence, our group, and several others, are pleading for the creation of phage banks across Africa to cater to the need for phages that are predicted to grow over the years to come amid the alarming rate at which AMR is progressing in the sub-continent.

A point to consider during the development of phage therapies for livestock that is often overlooked is the regulatory requirements and legislation aspect that can shape the end product's design at the early stage of development. Identifying the route of administration and the relevant bacterial pathogen to target can also benefit in developing the strategy. Contrary to antibiotics legislation and regulations that have solid systems in place, phage regulation guidelines are not uniform and readily in place as a grey zone surrounds the classification of phages as biological agents, chemical agents (for enzymes derived from phages such as endolysins), veterinary medicine products, or food additive [46][47]. In the USA, phages were classified as drugs in 2011, whereas in Europe, they have been classified as medicinal products [48]. However, Georgia, one of the few countries that have maintained research and development of phage products for use in medicine, considers phages as pharmaceuticals [49]. Even in Poland, which has been a pioneer in phage therapy in Europe, phage therapy is classified as experimental treatment according to Polish law [48].

One challenge that regulators are likely to encounter is the continuous renewal of phage cocktails with novel phages to counteract the emergence of resistance in bacteria [50]. By doing this, phages need to be tested again to make sure they are lytic, do not contain toxins or AMR genes, and are safe for the animals or humans using by-products of the treated animals [51]. The regulatory framework surrounding phage licensing should be flexible enough to allow slight changes in cocktail formulations for an approved product, unless it is for a hitherto unregistered product. The current regulatory framework used for antibiotics is too long and costly to be used for phages without adapting or adjusting it [52]. Moreover, in veterinary medicine, the compassionate use of phages is not likely to be the strategy of choice, as is the case in human medicine.

Another challenge with phage products is their lack of patentability potential as is in the USA and Europe, phages cannot be patented [46][53]. However, some phage cocktails have been patented or kept as proprietary by the companies that have developed them [47]. Phage is an active treatment (because it is self-replicating) so different rules apply as the "pharmacology" of phages is different [47]. An additional concern for regulators is the co-evolving property of phages that co-evolve with their host. Furthermore, another level of complexity will be added for regulating genetically modified phages designed to evade immune recognition by the host or reduce the emergence of bacterial-resistant mutants.

Conclusion: The control of zoonotic bacteria with antibiotics marks the beginning of the arms race between the discovery of new antibiotics and bacteria. The increase of resistant bacteria in the livestock sector causes serious health problems between the animal and human interface and also significant economic losses for the farmers. Phage therapy could be an interesting alternative for the treatment of bacterial infections thereby opening up present-day approaches for bacterial treatment in the near future.

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