Chapter

Challenges of Phage Therapy as a Strategic Tool for the Control of *Salmonella Kentucky* and Repertoire of Antibiotic Resistance Genes in Africa

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Abstract

Salmonella Kentucky ST198 (*S. Kentucky* ST198) is the most ubiquitous multidrug resistant (MDR) strain posing the greatest threat to public health, livestock and food industry in Africa. The reinvention of bacteriophage (Phage) as a non-antibiotic alternative only gives a glimmer of hope in the control of MDR strains of *Salmonellae*. *S. Kentucky* ST198 posses' chromosomal and plasmid factors capable of been co-opted into phage mediated transduction and co-transduction of antibiotic resistance genes (ARGs) as well as cross-serovar transduction of ARGs. Phage DT104, DT120 and P-22 like prophages like PDT17 and ES18 together have been shown to be capable of transducing and co-transducing the classical ACSSuT resistance phenotype identified in most *S. Kentucky* ST198 strain on the continent. Also, the institution of fluoroquinolones and third generation cephalosporin for salmonellosis treatment in animals or human infected by *S. Kentucky* ST198 strain resistant to these drugs can induce *Salmonella* phage transduction of kanamycin between different *Salmonella* serovars if present. This review highlights possible risk associated with the use of known *Salmonella* phages in the control of *S. Kentucky* ST198 and the need for chromosomal and plasmid tracking of genes prior to the institution of phage therapy on the continent.

Keywords: Bacteriophages, *Salmonella Kentucky* ST198, DT104, transduction, ARGs, *Salmonella* conjugative plasmids, Africa

1. Introduction

Bacteriophages (here in after called phages are viruses that can infect a bacteria and replicate within it) are completely alien to the routine therapeutic regimens in both veterinary and human medical practices in Africa, and where phage therapies have been instituted they are mainly experimental. Phage therapy has shown to be an ecologically sustainable tool in the control of bacterial infection; scientific researches places phages to be superiorly bactericidal specific, efficacious and cost effective when compared to antibiotics and interestingly it has been proven to inhibit biofilm formation in pathogenic bacteria [1–3], customarily the production

of biofilms by bacterial cells significantly increases their resistance to antimicrobials as compared to what is normally seen by the same cells being planktonic [4]. In Africa, the indiscriminate use of antimicrobials for treatment of salmonellosis in both human medical and veterinary practices has allowed for the proliferation of multidrug resistant (MDR) determinants and the sharing of antibiotic resistant genes (ARGs) between serovars of *Salmonellae* and other bacteria population, and on a continent where Poverty, human immunodeficiency virus/acquired immune deficiency syndrome (HIV/AIDS), Malaria, Tuberculosis and other immuno-compromising diseases are prevalent, the impact of MDR salmonellosis is severe [5]. It has become pertinent that alternative strategies for control of salmonellosis and *Salmonella* associated infections be adopted especially where MDR strains of *Salmonellae* persist. *Salmonella Kentucky* (*S. Kentucky*) is amongst the most ubiquitous *Salmonella* serovar identified on the African continent in the present decade and MDR strains pose significant health risk and a threat to the livestock production, livestock trade and food industry [5]. Several MDR strains have been isolated in most regions of the continent and the abusive use of antibiotics has only enhanced its mutative MDR tendencies and acquisition of ARGs between serovars and other non-genera of *Salmonella*. The institution of phages in the treatment of salmonellosis has shown promising results because of their very low transduction frequencies in the transmission of ARGs of *Salmonella* spp. [6], they exist everywhere in the environment and are natural, economically sustainable, nontoxic and some phages have shown broad activity against numerous serovars of MDR *Salmonella* spp. [7, 8]. Felix-O1 and SE13 are examples of *Salmonella* phages with broad serovar capacities; Felix-O1, a virulent phage was proven to infect 98.2% of all *Salmonella* strain and SE13 was capable of lysing 83.6% of *Salmonella* strain it was tested with [7, 8]. While researches on the use of phages for the treatments of *S. Kentucky* in Africa are scarce, [9] reported an effective use of phage in the reduction of *S. Kentucky* colonization in different broiler farms in Egypt. Phage–host interactions through the mechanism of horizontal gene transfer have contributed significantly to genetic flux vastly responsible for the acquisition and dissemination of important bacterial phenotypes, such as enhanced colonization of the human or animal gut epithelium, AMR and toxin production [10, 11]. Thus, the identification and careful selection of phages devoid of genetic elements that could pose risk to human and animal health is critical to biocontrol applications [12]. This review proposes to highlight challenges that may arise in the institution of phages as a strategic non-antibiotic tool for the control *S. Kentucky* and repertoire of its ARGs without prior studies on their genetic make-up.

2. Antibiotic resistant gene

Antibiotic resistance genes (ARGs) are an emerging public health contaminant, posing a potential global health risk. A major factor contributing to the increased environmental burden of ARGs is the rise in intensive livestock farming [13]. The World Health Organization (WHO) defines antimicrobial resistance (AMR) as "an increase in the minimum inhibitory concentration of a compound for a previously sensitive strain" [14]. Human beings consistently use large amounts of antibiotic in the human medical contexts as well as for growth factors and prophylaxis in agriculture and livestock, culminating in the contamination of environmental microbial communities. Unfortunately, even when pathogenic bacteria are the specific targets of antibiotic use, hundreds of non-pathogenic bacteria species are affected [15]. Thus, antibiotics are present in microbial communities, not only as a result of the natural lifecycle of microorganisms but also to the usage of these

drugs in agriculture, food industry, livestock and human health [16]. The presence of antibiotic resistance genes in environmental bacteria may be responsible for different mechanisms employed to overcome the natural antibiotics present in the environment. Recently this gene pool has been named the 'resistome', and its components can be mobilized into the microbial community affecting humans because of the participation of genetic platforms that efficiently facilitate the mobilization, transmission and maintenance of these resistance genes. Evidence for this transference has been suggested and or demonstrated using cutting-edge research techniques with newly identified widespread genes in multidrug-resistant bacteria [17]. These resistance genes include those responsible for plasmid-mediated efflux pumps conferring low-level fluoroquinolone resistance (*qepA*), ribosomal methylases affecting aminoglycosides (*armA, rtmB*) and methyltransferases affecting linezolid (*cfr*) all of which have been associated with antibiotic-producing bacteria. Recently, resistance genes whose ancestors have been identified in environmental isolates that are not recognized as antibiotic producers have also been detected. These include the *qnr* and the *blaCTX* genes compromising the activity of fluoroquinolones and extended-spectrum cephalosporins, respectively [17]. Bacteria can express antibiotic resistance through chromosomal mutations or via the acquisition of genetic material through horizontal gene transfer from other bacteria or the environment. Acquisition of genetic material via horizontal gene transfer is largely driven by mobile genetic elements (MGEs), such as plasmids, transposons or bacteriophages, which play a critical role in the evolution and ecology of bacterial communities by controlling the intra-species and interspecies exchange of genetic information [18]. While the transfer of these MGEs usually occur through transformation, transduction, or conjugation, conjugation is mostly considered the most efficient mechanism employed for the exchange of genetic material among bacteria [19]. The ease of acquisition and spread of ARGs by bacteria via conjugation is frequently through conjugative plasmids and transposons, and the contribution of these elements to antibiotic resistance pool has been extensively studied in hospital, community, agricultural and environmental settings [15–17, 20, 21], but very little is known about the role of bacteriophages as vehicles for ARGs in environmental settings. Recent findings based on cutting-edge genomic technologies suggest that, in these settings, bacteriophages play a more important role in the mobilization of ARGs than previously documented [22].

3. Phage transduction: primary mechanism for the transfer of ARGs

Intensive studies of the mechanisms for horizontal gene transfer responsible for the increased spread of antibiotic resistance to foodborne bacterial pathogens have been undertaken; Conjugation, transformation, and transduction are the fundamental mechanisms by which dissemination of ARGs occurs [23]. Transduction is primarily the horizontal gene transfer mechanisms employed by most phages, and recent findings have shown phage-mediated transduction to be a significant driver in the dissemination of ARGs [24]. The concept that phage mediated transduction is a major driver of horizontal transfer of ARGs between foodborne pathogens, as well as from the environment to animals and humans, is increasingly been recognized. Phages are recognized as the most abundant organism in the biosphere, and are found in every environment regardless of their diversities, including oceans, lakes, soil, urban sewage, potable and well water, plant and animal microbial communities [25]. ARGs are often found on various MGEs, and are readily transferred horizontally by phage transduction [24]. Phages infect bacteria and either incorporate their viral genome into the host genome, replicating as part of the host (lysogenic cycle),

or replicate inside the host cell before releasing new phage particles (lytic cycle) [22, 26]. Phages can be either virulent or temperate. The mechanism of transduction has been vastly described in virulent phages (defined by their capacity to undergo lytic cycles). Following bacterial infection, there is an immediate induction of phage particles formation and lysis of the host cell but virulent phages do not integrate their DNA into the host chromosome. Temperate phages (known to undergo lysogenic cycle), integrate their DNA into the host chromosome and the prophage may remain dormant in the host until other factors like stress induces the excision of the phage from the chromosome leading to subsequent formation of phage particles and lysis of the host cell. Some phages can also adopt a pseudolysogenic state under unfavourable growth condition. In this state, their genome does not degrade but rather exist within the host cytoplasm as a plasmid and during bacterial cell division becomes incorporated into just one daughter cell [26]. Genetic materials are transferred between hosts either by generalized or specialized transduction. Virulent and temperate phages can undergo generalized transduction, here, bacterial DNA fragments are randomly packaged into the phage capsid during their lytic cycle forming a "transducing particle". These "offspring" phages do not contain phage genes, and only the capsid has a viral origin. Despite this, the transducing particle is capable of injecting the bacterial genes into a susceptible recipient cell, which can subsequently be incorporated into the host genome by recombination [5, 22, 24]. Specialized transduction is restrictive to temperate phages and results in the packaging of bacterial DNA into phages at a higher frequency; temperate phages insert their genomes into a specific region of the host chromosome. An inaccurate excision of the prophage may lead to the capture of the flanking genes adjacent to the phage integration point. If capsids carrying the rearranged phage genome with these foreign genes infect other bacteria and integrate into the host chromosome, transduction of the acquired genes will be achieved. However, the probability that the transferred genes are antibiotic resistance-related is relatively low [5].

4. Phage transduction of ARGs in *Salmonellae*

Salmonella phages have been extensively used in molecular biology for the introduction of foreign genes by generalized and specialized transduction. P-22, a well-known phage is a classical example, other P-22 like prophages ST104 or PDT17, harboured within DT104 phage type have been hypothesized to facilitate horizontal transfer of the penta-resistance genes [27, 28]. The penta-resistance genes in phage type DT104 are clustered on a 43-kb *Salmonella* genomic island-1 (SGI1), which is flanked by two type I integrons [29]. *Salmonella* genomic island 1 (SGI1) is an integrative mobilizable element that harbours a multidrug resistance (MDR) gene cluster. A research undertaken by [27] asserted that ES18 and PDT17, also a P-22 like phage, following release from DT104 could transduce ARGs. Their findings further demonstrated the transduction of *cam* and *amp* by phage PDT17 and *amp*, *cam,* and *tet*, which confer resistance to ampicillin, chloramphenicol, and tetracycline, respectively, by ES18 from a donor DT104 strain into a DT104 recipient strain lacking these resistance genes. Phage ES18 also co-transduces selected ARGs of the 71 *tet* transductants and of the 145 *cam* transductants. Interestingly, in 14 of 16 transductants, it was noticed that phage E18 could co- transduce *sul* and *str*, genes involved in resistance to sulphonamides and streptomycin, respectively, together with *amp*, *cam,* and *tet* to create the ACSSuT (ampicillin, chloramphenicol, streptomycin, sulphonamides, and tetracycline) resistance phenotype [27]. This co-transduction likely occurs because *amp* and *str* are situated on the integrons flanking SGI1, and

the phage likely packages the SGI1 and its flanking integrons [30–32]. P-22 phage has also been identified within DT120 isolates shown to be capable of generalized transduction and possess the ACSSuT resistant strain [33]. See [33] reported that carbadox, a veterinary antibacterial that posses' mutagenic and carcinogenic capabilities induced phage transduction in DT104 and DT120. Furthermore the absence of transduction in DT104 strain which had its P-22 like prophage deleted following induction with carbadox suggests that P-22 like prophages are responsible for generalized transduction. Thus; transduction and co-transduction by P22-like prophages of ARGs co-located within SGI1 in multidrug-resistant *Salmonellae* strains is a common phenomenon. Also, genome scanning proved that P22-like prophages were common in 18 *Salmonella* serovars implying that generalized transduction may be greatly underestimated [33].

5. Transduction of Ciprofloxacin and cephalosporins genes

Ciprofloxacin a fluoroquinolone and third generation cephalosporin are the drugs of choice in the treatment of invasive *Salmonella* infections [34–36]. Resistance of *Salmonella* to ciprofloxacin is due mainly to double mutations in *gyrA* and a single mutation in *parC* genes. In addition, *oqxAB* operon is suggested to be responsible for the increase in resistance observed in clinical *Salmonella* strains [37]. It was observed by [38], that Ciprofloxacin, enrofloxacin and danofloxacin induced *Salmonella* phage DT104 and DT102 transfer of a native kanamycin resistance plasmid to a strain of *Salmonella Typhimurium* by generalized transduction. Resistance to cephalosporin is mainly due to extended spectrum beta-lactamases (ESBLs), such as TEM-, SHV-, and CTX-M, or plasmid mediated AmpC β-lactamases (pAmpCs), such as CMY, encoded on transmissible conjugative plasmids [39–41], or be transferred by generalized transduction. Phage P24, induced from an isolate of *S. Typhimurium*, was propagated on a multidrug resistant strain of *S. Heidelberg* (S25). Thus, when the MDR S25 harbouring phage P24 was used as transduction donor to transfer ESBL and tetracycline resistance genes to a recipient *S. Typhimurium* isolate. PCR confirmed the presence of *bla*CMY-2, *tet*(A), and *tet*(B) in various *S. Typhimurium* transductants. Although the tetracycline genes were not co-transduced with *bla*CMY-2, their transduction frequency was equivalent, indicating generalized transduction and evidently reporting the transfer of ARGs by phage-mediated transduction between different *Salmonella* serovars. This finding likely expresses that cross-serovar transduction occurs frequently because phages can bind to various surface protein receptors on different species and serovars [42]. The LPS, FliC, OmpC, OmpF, OmpA, are examples of phage receptors present in *Salmonella* [43]. In the previous study [42] it was observed that 13 inducible phages recovered from 31 *Salmonella* serovars were capable of propagating on two or more *Salmonella* serovars including those often responsible for foodborne outbreaks such as *S. Heidelberg, S. Enteritidis, S. Typhimurium* and *S. Kentucky*. Finally, the findings of [42] demonstrate the spread of antibiotic resistance in *Salmonellae* by phage mediated transduction.

6. Transduction of R-factor genes

R-factors which are a group of conjugative plasmids that harbour one or more antibiotic resistance determinants and represents another form of MGE that can be transferred horizontally by phage mediated transduction [24]. Conjugative plasmids are also self-transmissible, affording them the capacities to increase the

spread of ARGs. The origin of transfer (*ori*T), MOB genes, and the mate-pair formation (MPF) genes are the essential components for conjugation [44, 45]. In order for conjugation to occur, a protein complex called a 'relaxasome' responsible for processing plasmid DNA to prepare it for transfer must form at the *ori*T [46–48] and the mechanism for R-factor-phage acquisition and propagation of ARGs may be random. R factors in close proximity to P22-like prophages could be integrated into the head of the assembling phage during induction from its host, thus contributing to the spread of ARGs within bacteria capable of causing foodborne illnesses, in the intestinal flora of livestock and in the environment [24].

7. Virulent factors of *S. Kentucky* ST198 that may potentiate phage-mediated transduction of ARGs

Salmonella Kentucky ST198 is a global contaminant and an emerging risk for foodborne illness, although first identified in Egypt it has now been isolated in several countries across the different regions in Africa [5, 49], with reservoirs in various animals and food [49–54]. Successes have been recorded with the institution of phages in controlling the spread of MDR *Salmonella Kentucky* [9, 55]; these findings however rarely discuss the tendencies of phage-host mediated propagation of ARGs or other MDR determinants. *S. Kentucky* ST198 belongs to a single lineage, which is predicted to emerged circa 1989 following the acquisition of the AMR-associated *Salmonella* genomic island (SGI) 1 (variant SGI1-K), that confers resistance to ampicillin, streptomycin, gentamicin, sulfamethoxazole and tetracycline [56]. This MDR *Salmonella Kentucky* clone has undergone substitution mutations in the quinolone-resistance-determining regions (QRDRs) of DNA gyrase (gyrA) and DNA topoisomerase IV (parC) genes, such that most strains carry three QRDR mutations which together confer resistance to ciprofloxacin. Its molecular characterization further shows a chromosomal genomic island carrying resistance genes that confer resistance to β-lactam antibiotics, carbapenems, quinolones, aminoglycosides, co-trimoxazole (trimethoprim-sulfamethoxazole), and to Azithromycin. Extended-spectrum cephalosporins (ESCs) resistance has also been associated with *S. Kentucky* ST198 [57–60]. Genetic basis for this resistance showed an extended-spectrum b-lactamase (ESBL) [61]. The aforementioned resistant properties evidently can allow for the transfer of native kanamycin resistance plasmid to strains of *S. Typhimurium* or other *Salmonella serovar* by generalized transduction as treatment with these antibiotics as reported by [38] can induce *Salmonella* phage DT104 and DT102 transmission of a native kanamycin resistance plasmid and other ARGs between serovars of *Salmonella* by generalized transduction. *S. Kentucky* also exhibits an extensive MDR pattern with diverse resistance profile cutting across human, environmental and poultry micro biomes [57]. A penta-resistant profile (SSuTCipNa) was observed in *S. Kentucky* from human, environmental and poultry samples with a deca-resistant profile, ACKSSuSxTAmcCipNa in poultry [57, 58]. *Salmonella* Phages ES18, PDT17, DT104, DT120 and other P22-like prophages like ST104 or PDT17 haboured within DT104 have been proven to be participatory in the transduction and co-transduction of genes for ACSSuT resistance phenotype [33], making *S. Kentucky* ST198 a luxurious menu for the transduction of these genes complemented by other factors that may helps in phage-mediated transduction of ARGs between serovars of *Salmonella* and other enterobacteriaceae. Several conjugative plasmids have also been detected in *S. Kentucky* ST198; IncA/C conjugative plasmids have been isolated in *S. Kentucky* ST198 that contain up to 10 ARGs for more than five classes of antibiotics. The most common ARGs carried by IncA/C are *str*AB (aminoglycosides), *sul*2 (sulfonamides), *tet*AR (tetracycline),

*bla*CMY-2 (β-lactams), *floR* (chloramphenicols) and *bla*CTX-M-25 (cephalosporin). Other genes for resistance to aminoglycosides, tetracyclines, trimethoprim, chloramphenicols, and have also been identified [49, 62–64]. *S. Kentucky* ST198 also contains an IncF plasmid [65]. IncF plasmids can carry multiple types of replicon associated genes, such as FIA, FII, or FIB [66]. IncF plasmids have been observed to contain ARGs, exhibiting resistance to fluoroquinolone [67], they have also been associated with *str*AB, *tet*A, *tet*C, *tet*D, *aph*A (aminoglycosides), and *sul*2 (sulphonamides) resistance [68, 69]. Another plasmid of importance carried by *S. Kentucky* ST198 is the IncHI plasmid and has been associated with *qnr* genes (fluoroquinolones) and ESBL genes [70]. The integration of one of more of these conjugative plasmids that may be in close proximity to P22-like prophages would facilitate their packaging into the core of the assembling phage during induction from its host, thus contributing to the spread of antibiotic resistance between generic and non-generic bacteria in the intestinal flora of livestock and human and in the environment causing foodborne illnesses and outbreaks. Although the mode of acquisition of Plasmids ARGSs in *Salmonella* may seem random, their proliferation in a population is usually not random. Consequently, surveillance is a necessary tool, not just for *Salmonella* and other important human and animal pathogens, but for the plasmids they carry. Therefore, the tracking of plasmids and the genes they carry would allow for a better understanding of co-selection of ARGs and the associations of plasmids with *Salmonella* serotypes [71]. Finally since *Salmonellae* phages can bind to several protein receptors in *Salmonellae* and other members of the enterobacteriaceae family thereby permitting cross-serovar and inter-specie transduction of ARGs [43], it has become necessary that measures or protocols that can hinder such developments be adopted in order to forestall the spread of *Salmonellae* associated foodborne outbreaks.

8. Conclusion

The renewed and profound interest in phage therapy as a non-antibiotic measure for combating MDR strains of *Salmonellae* is a testament to their efficacy, but clearly *Salmonella Kentucky* ST198 posse's virulent factors that can potentiate phage-mediated cross-serovar transduction and co-transduction of ARGs and MDR determinants, therefore investigative laboratory protocol should therefore be sought to identify these determinants prior to the institution of phages in the treatment of non-repressive salmonellosis.

Conflict of interest

Author declares none.

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