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Role of Bacteriophages and Plasmids in Antibiotic Resistance Gene Transfer

Review Article

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Abstract

Introduction: The constant increase in the antibiotic resistance poses substantial threat to the human health, increase ion health care costs and serious complications in treating bacterial infections. Purposes of the review is to investigate the mechanisms through which bacteriophages and plasmid help in acquiring antibiotic resistance in bacteria the transfer of resistance. And to assess the effects of these processes for the possible control of resistance in bacteria.

Methodology: The Antibiotic resistance genes (ARGs) in bacterial populations are transmitted through two main sources that are Bacteriophages and plasmids. Through transduction, Bacteriophages facilitate horizontal gene transfer (HGT) at much higher frequencies that result in evolutionary resistant strains, while to spread antibiotic resistance across and within bacterial species the plasmids play a key role. Tandem Duplication along with maintenance of plasmid copy number results in hetero resistance which results in multidrug resistance.

Conclusion: Understanding the ways to cope with transfer of plasmid and mechanisms of bacteriophage inactivation can reduce risk of multi drug resistant strains transfer. Further studies and experiments are required to properly understand spread of Antibiotic-Resistant Bacteria (ARB) and ARGs which would ultimately help in development of strategies to counter resistance against antibiotics.

Introduction

Antibiotics played a major role as a lifesaving drug and helped in control of infection spread.(1) There is a common practice to administer antibiotics in animals and humans even before the actual detection of the causative agent(2). Antibiotics are used in all regions of the world, so antibiotic resistance is global issue. In the past decade, there was a 65% increase in consumption of antibiotics in Pakistan. Considering 1000 people per day, antibiotics usage has reached to 19.6 DDD from previous DDD that was 16.2. Resistance against antibiotics has reached to an alarming level due to the widespread misuse of antibiotics(3). First decade of 21st Century has seen a 35% increase in antibiotic consumption worldwide(4).In the transfer of antibiotic resistance genes in bacteria, the interaction between plasmids and bacteriophages is complex. Bacteriophages usually integrate into the genome of bacteria in latent form which is known as lysogeny. In this situation, bacteriophages mostly remain dormant, until they enter in the lytic cycle. In lytic cycle they cause the bacterial cell to rupture and then they are released in the form of multiple new viral particles(5). Any resistance genes, even as a plasmid or in the genome of host bacteria, can be transmitted to new bacterial cells during this process(6). This is the form of horizontal gene transfer, and it results in increase of the genetic diversity of populations of microbes. It also promotes the spread of resistance traits in conjugative and non-conjugative bacterial species(7).

In clinical settings, the resistant bacteria usually carry multiple resistance genes and has acquired hospital infections sometimes called superbugs(8). If this bacteria transfer plasmids or spread through bacteriophages, it ultimately leads to the development of extensively drug resistant (XDR) and multidrug resistant (MDR) strains(9). In addition, the increasing use of antibiotics in agriculture and animal husbandry further accelerates the transfer of resistance genes, both in the environment and among human pathogens. Examination of faeces samples of animals determined that the animal's Gut has become the major accumulating source of antibiotic-resistant bacteria (10). ARGs are carried with the help of plasmids and bacteriophages can also distribute it to further species resulting in fast spread of resistant genes. For example, transposons and integrons are mobile genetic sequences that can integrate new genes and are commonly correlated with plasmids(11), resulting in enhancing their ability to promote and transfer supplementary resistance sequences(12).

The transfer of plasmids and the spread of resistance genes mediated by transduction are not limited to only bacterial populations in a single host but can also occur across different microbial species, which include both the commensal and pathogenic bacteria(13). In the background of zoonotic transmission(14), this inter specie flow of gene is a serious concern, where ARBs transfer between humans and animals through the food chain, direct contact or by environmental means(15). Whole set of mobile genetic sequences within a bacterial community is called mobilome. In the global spread of ARGs and ARB, mobilome has emerged as a vital player recently(16). Both plasmids and bacteriophages contribute to the spread of resistance in the humans, environment and in the hospitals(17). This also complicates the drug resistance matter, as bacteria is adopting miraculously to acquire new resistance traits(9). Antibiotic Resistance Gene (ARG) is the root causes of antibiotic resistance(18). Bacteriophage play a key role to transfer ARG. Plasmid exchange at genetic level is another notable way of gene transfer and results in development of strong antibiotic resistant bacterial strains.(19).

Review of Literature

Transfer of Antibiotic Resistance Genes

The animal husbandry accounts for roughly 50% of antibiotics used worldwide(20). Therefore, the faeces and intestines of chickens and animals and are the major resources of antibiotic resistant strains of bacteria and ARGs(21). Furthermore, animal husbandry plays a chief role to transfer antibiotic resistant bacteria (ARB) and ARG(21). adopting alternative health practices , like better hygiene ,designing specific guidelines to limit antibiotic use in animals, can reduce reservoirs of ARGs in husbandry and agriculture settings(22). Mobile genetic elements (MGEs) have also a critical role in distribution of drug-resistant strains as MGEs can move between and within DNA molecules(23). These can also move as integrated conjugative sequences between different bacterial cells, Insertion sequences (IS) and transposons (Tn) together with Plasmids MGEs play vital role in easing horizontal transfer of genetic material(23), and in enhancing the spread of resistant genes (24) (25). These elements together pay a key role in transfer of ARG(26)

Bacteriophages

Bacteriophage otherwise called as phage. It is the virus that attacks bacteria causing infection in bacteria and reproduces inside them(27). Phage transfer genetic material between bacteria through transduction of genetic material. Antagonistic coevolution(AC) between bacteriophages and bacteria regulate bacterial strains and help in maintaining diversity among microbes(28). On average, Bacteriophages infect 10^{24} bacterial cells every second, and almost all carry physiologically similar plasmids(29). And transfer of plasmid through phage results in transfer of ARGs among different microbes (30). Through bacteriophages, the horizontal gene transfer (HGT) between different bacteria is the prime powerful tool for microbial evolution(31).

Genetic transduction, carried out through two known mechanisms which include specialized transduction (ST) and generalized transduction (GT) (27). In the process of GT, phage can package and transfer any bacterial DNA to another bacterium while in case of ST, a phage can only transfer of specific part of genes between bacterium (32). A newly discovered mode of transduction is also found known as lateral transduction, is probably the most effective way to transfer huge parts (hundreds of thousands of bases long) of bacterial genome which transfer genes at extremely higher frequencies(33) from one bacterium to another(34) . This discovery explains the high rate of transfer of multiple drug-resistant genes and different microbial strains. Together with promoting the ARG transfer, phage can also enhance bacterial resistance(35). Development of Pseudomonas resistance by filamentous phage is an example, that promote resistance by forming liquid crystal structures around antibiotics (36).

Studies conclude that phage is the environmental vector for transferring ARGs horizontally as multiple phage that carry ARGs are present abundantly in the environment (37). There is a limit of phage identification as PHAST (phage-search-tool, 2011) and its successor PHASTER (phage-search-tool- enhanced 2016 are most widely used we- tools for identification of bacteriophages in bacterial genomes (38). Bacteriophages cannot be easily inactivated as many bacterial cells are prone to inactivation(39). But are vulnerable to autoclaving(40), UV and standard disinfection procedures specified by Hospitals(41)

Plasmids

Plasmids are considered as vector of horizontal gene transfer. Plasmids also play crucial part in evolution of bacterial because accessory genes are activated by horizontal gene transfer(42). The role of plasmids is far beyond than only the gene delivery platforms because Islands of polyploidy is present in cells, and many cells have multiple copies of plasmids, so plasmids play critical part in bacterial evolution resulting in resistant microbes(43). Moreover, Plasmid Copy Number (PCN) strongly promotes rate of evolutionary trait (44), numerous transmutations in replicated genes can increase the copy number (45, 46, 47). Agrobacterium tumefaciens (48, 49) and Yersinia spp.(50) are the example of plasmid virulence of two distant species. These pathogens have constantly maintained plasmids which are must for their infectiousness. Target cells emit different signals which result in regulation of copy number of plasmid (51). Bacterial hetero-resistance, a phenomenon in which bacterial subpopulation clones show much lower susceptibility to administered antibiotics than their main population(52). Hetero-resistant bacteria can attain high-level of antimicrobial resistance by amplification of gene dosage in genes that maintain low level of resistance. Tandem duplications in genes were also involved in most cases of hetero-resistance (53). When antibiotic resistance gene was plasmid encoded, there was consequent increase in tandem duplication with increase in plasmid copy number (54). Conventional antimicrobic sensitivity tests are not able to detect the resistant sub-populations and might lead in failed treatments.(55). Some plasmids also carry antibiotic resistance cassettes which are obtained from different source plasmids, which results in drug resistance to multiple antibiotics(56, 57). This poses a serious health concern(58, 59). For instance, plasmids carrying the mcr1 (colistin resistance gene) are reported to recombine with plasmids carrying other determinants of resistance like bla-CTX-M, bla-NDM (β-lactamase genes)(57). Bacterial conjugation widely results in spread of multi resistance plasmids.(60)

HGT is also considered as foremost source of resistance in microbes(61). The identification of plasmids on basis of their properties and their correlation with several bacterial species provides critical knowledge that is vital to recognise the role of plasmids in the transmitting antimicrobial resistance (AMR)(62).A common genetic pool is shared with the help of HGT which allows taxonomically different organisms to mitigate together and also describes why closely related bacteria greatly differ in genetic sequences(63). Genes are transmitted at very high frequencies through multiple mechanisms, majorly conjugation (which includes plasmid conduction and mobilization), also vesiduction, transduction and transformation (64) (65). Magnitude of gene transfer in the human gut through plasmid is the focus of recent researchers(66). bacterial samples and whole-genome sequencing from social donors have demonstrated that the transfer of plasmids is imminent(67), plasmids also transfer naturally even in the absence on antibiotic resistance(68, 69).

Comparison of Results

The active interaction between ARGs, bacteriophages, and plasmids highlights the complexity of transmission of AMR and the demanding requirement for integrated strategies to address the growing global health challenge of antibiotic resistance(24, 27, 40, 42). Studies confirm the critical role of Mobile Genetic Element in the spread of antimicrobial resistance and a requirement of in depth understanding of the mechanisms by which MGEs facilitate gene transfer. Transfer of ARGs is greatly influenced by mobile genetic elements (MGEs), plasmids, transposons, and bacteriophages. All of which contribute effectively towards horizontal gene transfer (HGT) between bacteria.

Table 1 Comparison of Results

Aspect	Animal Husbandry	Mobile Genetic Elements (MGEs)	Bacteriophages	Plasmids
Role in Antibiotic Resistance	Major contributor to the spread of ARBs and ARGs, accounts to ~50% of global antibiotic use(20).	Role in horizontal gene transfer (HGT) of ARGs between and within bacterial cells.	Facilitate transfer of ARGs via GT and ST(27).	Vector for horizontal gene transfer (HGT) of ARGs.
Source of ARGs	Faeces and intestines of animals and poultry serve as major sources of ARBs and ARGs.	Includes insertion sequences (IS), transposons (Tn), and conjugative sequences(23).	Can transfer genetic material (including ARGs) via transduction.	Can carry multiple resistance genes, increasing bacterial adaptability and contribute to multi-drug resistance.
Transfer Mechanism	ARGs spread via faecal contamination, contact with animals, and contaminated water sources(21).	Facilitate HGT within and between bacterial species.	Generalized transduction (GT) and Specialized transduction (ST)(27).	Facilitate transfer of ARGs via conjugation and can be mobilized by other MGEs(24).
Impact on Bacterial Evolution	Contributes to rapid evolution of ARBs, promoting multi-drug resistance (MDR)(56, 57).	Increase bacterial rapid gene transfer, including drug resistance genes.	Promote bacterial evolution via HGT, accelerating the spread of ARGs via lateral transduction.	Contribute to bacterial evolution by increasing adaptation via copy number variation and tandem duplications(43).
Examples of ARG Spread	ARGs spread from animals to humans through direct contact or via the food chain.	Transposons carry ARGs between bacteria, enhancing resistance traits across species(25).	Lateral transduction transfers large genomic segments, including multiple resistance genes.	Plasmids carrying ARGs (e.g., mcr1, blaCTX-M) can recombine, spreading resistance to multiple antibiotics(57).
Influence Spread of Resistance	Misuse of antibiotics in animal husbandry facilitate the spread of ARGs.	Enhance the spread of resistance genes across bacterial populations, leading to widespread resistance.	Increase resistance spread via high-frequency transduction, especially of multi-drug-resistant genes(64).	Promote the spread of multi-drug resistance through conjugation and variation in gene copy numbers.
Control Measures	Improving hygiene and implementing specific guidelines to limit antibiotic use can reduce ARG reservoirs in agriculture.	Understanding and controlling the mechanisms of MGEs is key to combating the spread of ARGs(23).	Phage therapy, UV, and autoclaving can help reduce the impact of bacteriophages.	Limiting plasmid transfer and understanding plasmid mediated resistance are key control strategies.
Conjugation & Horizontal Gene Transfer	Animal-to-human transmission of ARGs facilitated by direct contact or contaminated food sources.	Crucial for the horizontal transfer of ARGs, promoting resistance across bacterial species.	Facilitate intercellular horizontal gene transfer, contributing to the evolution of microbial populations(44).	Transfer ARGs via conjugation and can increase evolutionary potential via gene dosage variation(53).
Environmental Impact	Contributes significantly to environmental contamination with ARBs and ARGs.	Found abundantly in the environment, facilitating the transfer of ARGs across different bacterial species.	Abundant in the environment and act as environmental vectors for ARG transfer.	Naturally transfer ARGs within the environment, even without antibiotics present, contributing to resistance spread.
Tools for Identification	Through sequencing technologies and bioinformatic tools.	Through sequencing technologies and bioinformatic tools.	Tools like PHAST and PHASTER help identify bacteriophages in bacterial genomes(38).	Genome sequencing and characterizing their resistance gene profiles(67).
Vulnerability to Inactivation	Resistant bacteria from animal sources may survive in harsh environmental conditions (e.g., in soil and water)(10).	MGEs like transposons are typically stable and resistant to inactivation but can be disrupted by specific agents(25).	Resistant to inactivation by bacterial resistance mechanisms but can be inactivated by autoclaving, UV, and disinfection(39).	Through specific genetic interventions or antimicrobial agents targeting resistance mechanisms.

Animal husbandry is a source of ARB and contributes significantly to spread the ARBs and ARGs. With better hygiene practices, and restrictions on misuse of antibiotics is proposed as possible solutions. Bacteriophages by lateral transduction can transfer huge portions of resistant genes and multi drug resistant genes at high frequencies resulting in acceleration of resistance spread. Plasmids are vector of horizontal gene transfer and not only carry antibiotic resistance cassettes but also increase adaptation in bacteria by variation in copy number and tandem duplications. This leads to hetero resistance. Bacteriophages, plasmids together with other MGEs highlights the complexity of AMR transmission and the need for strategies to mitigate the global threat of antibiotic resistance. Studies further emphasise on how MGEs facilitate in horizontal gene transfer and agree on the contribution of plasmid and bacteriophages in horizontal gene transfer(23, 26). Sources acknowledge plasmids and bacteriophages along with MGEs as major vectors for the spread of ARGs, and also point up the ability of plasmids in spreading multiple drug-resistant genes.

Conclusion

Antibiotic resistance is widely spread through bacteriophages and plasmids which is a complex, and complicated problem. Bacteriophages facilitate the process of transduction, while the plasmids enable conjugation, both contribute highly to HGT and the propagation of ARGs in the populations of bacteria. To address this issue, a multidimensional approach is required that must involve controlled antibiotic administration and management, environmental management to reduce the spread, and vast research and study about HGT and mobile genetic elements. Bacteriophages and plasmids enhance genetic variability at much higher rates than chromosomes of bacteria. Plasmids and bacteriophages tend to increase their ability to evolve. Additionally, Plasmids are able to store genetic variations for thousands and thousands of bacterial generations. Although it is somehow approachable to control and inactivate bacteriophages by UV(40), and other hygienic means we can restrict the spread of ARG and ARB. Phage therapy is also being explored as a potential measure that can combat resistant infections. Further studies and experiments are required to find the ways to cope with multi drug resistance.

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