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Exploring resistance mechanisms of bacteria to antibiotics: Insight into determinants and clinical correlations

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Abstract
The rise of antibiotic-resistant pathogenic bacteria poses a global public health threat. Beyond clinical settings, antibiotic resistance genes are widespread in various bacterial populations in the environment. To comprehend the development of antibiotic resistance in pathogens, it is crucial to explore significant reservoirs of resistance genes, which may include determinants providing self-resistance in soil bacteria producing antibiotics and genes encoding intrinsic resistance mechanisms in non-producing environmental bacteria. Although the presence of resistance determinants in soil and environmental bacteria doesn't initially endanger human health, the potential transfer of these genes to pathogenic bacteria through mechanisms like plasmids and integrons could lead to substantial problems. Therefore, there is an urgent need to investigate the distribution of resistance determinants, elucidate resistance mechanisms, and identify environmental factors promoting their spread. This review delves into self-resistance mechanisms in Streptomyces, producer soil bacteria, and examines the interplay between resistance determinants in producer soil bacteria, non-producer environmental bacteria, and clinical isolates. Overall, this article presents a conceptual framework for understanding the intricacies of antibiotic resistance emergence in clinical settings. Access to such knowledge is essential for researchers to construct models depicting the dissemination of resistance genes and to develop interventions preventing the recruitment of additional genes into pathogens.

Keywords: Antibiotic resistance, Producer bacteria, Resistance gene dissemination, Self-resistance mechanisms

1. Introduction

Penicillin, the first natural antibiotic, was discovered accidentally by Alexander Fleming in 1928, but its development for use occurred in the late 1930s, inhibiting cell wall synthesis and proving effective against Gram-positive bacteria (Hopwood, 2007) [1]. Subsequent to penicillin, scientists, including Rene Dubos and Selman Waksman, actively searched for antibacterial agents in soil microorganisms, with actinomycetes proving most promising. In 1943, streptomycin, produced by Streptomyces griseus, was discovered, inhibiting protein synthesis and proving effective against Gram-negative bacteria and the tubercle bacillus (Hopwood, 2007) [1].

This discovery marked the start of the golden age of antibiotic development (1940–1990), involving academic institutions and pharmaceutical companies worldwide. Currently, antibiotics affecting various bacterial cell processes are known. Antibiotics, a diverse group of compounds with distinct structures and mechanisms of action, can be categorised into seven major groups. These include β-lactams, which hinder cell wall synthesis; aminoglycosides, targeting protein synthesis; macrolides, also affecting protein synthesis; tetracyclines, another group influencing protein synthesis; daptomycin, which disrupts cell membrane function; platensimycin, impacting fatty acid biosynthesis; and glycopeptides, inhibiting cell wall synthesis.

It's noteworthy that organisms producing antibiotics possess inherent self-resistance mechanisms against their own antimicrobial agents. Furthermore, the coexistence of antibiotic-producing and non-producing bacteria is thought to have driven the co-evolution of resistance mechanisms in non-producing environmental bacteria.

Certainly, in the face of the ongoing global challenge of antibiotic resistance, it becomes imperative to delve into the origins of these resistance factors within pathogens. This comprehensive review article aims to present a current understanding of antibiotic self-resistance mechanisms prevalent in soil bacteria belonging to the Streptomyces genus, particularly those with a role in antibiotic production. Additionally, the article explores the interconnections between resistance determinants identified in both producer and non-producer soil bacteria, as well as their links to clinical pathogenic bacteria.

Remarkably, this review attempts a thorough examination of self-resistance in producer bacteria, filling a gap in existing literature, as opposed to the extensively documented antibiotic resistance mechanisms observed in clinical isolates (Munita and Arias, 2016) [2]. Consequently, the article does not delve deeply into the resistance mechanisms of clinical isolates, with a decision to keep these sections separate due to the current disparity in the understanding levels of resistance mechanisms between producers and clinical isolates.

To enhance the reader's understanding, critical information about clinical isolates is nonetheless presented in a distinct section following the discussion on self-resistance in Streptomyces. This segregation reflects the considerable difference in our understanding of resistance mechanisms between these two categories. The concluding segments of this review address the origins of resistance determinants in clinical strains and contemplate potential mechanisms facilitating their mobilisation.

It's important to note that, despite meticulous efforts to encompass all available literature, the vast and continually expanding nature of information on each discussed topic may inadvertently result in omissions. The article strives to be inclusive, and, wherever feasible, references to supplementary literature and review articles are provided for readers interested in further exploration.

1.2. Autonomous Defensive Mechanisms in Producer Organisms

Bacteria that produce antibiotics have evolved intricate self-defence mechanisms against their own antibacterial compounds. These defence mechanisms are often diverse and can be concurrently present to ensure comprehensive protection against the biologically active substances they generate. Notably, the genetic components responsible for self-resistance are typically situated in close proximity to the genes involved in antibiotic biosynthesis, and their activation is coordinately regulated (Mak *et al.*, 2014) [3]. The subsequent section outlines the primary biochemical categories of these self-defence mechanisms observed in antibiotic-producing organisms, offering specific examples for each category.

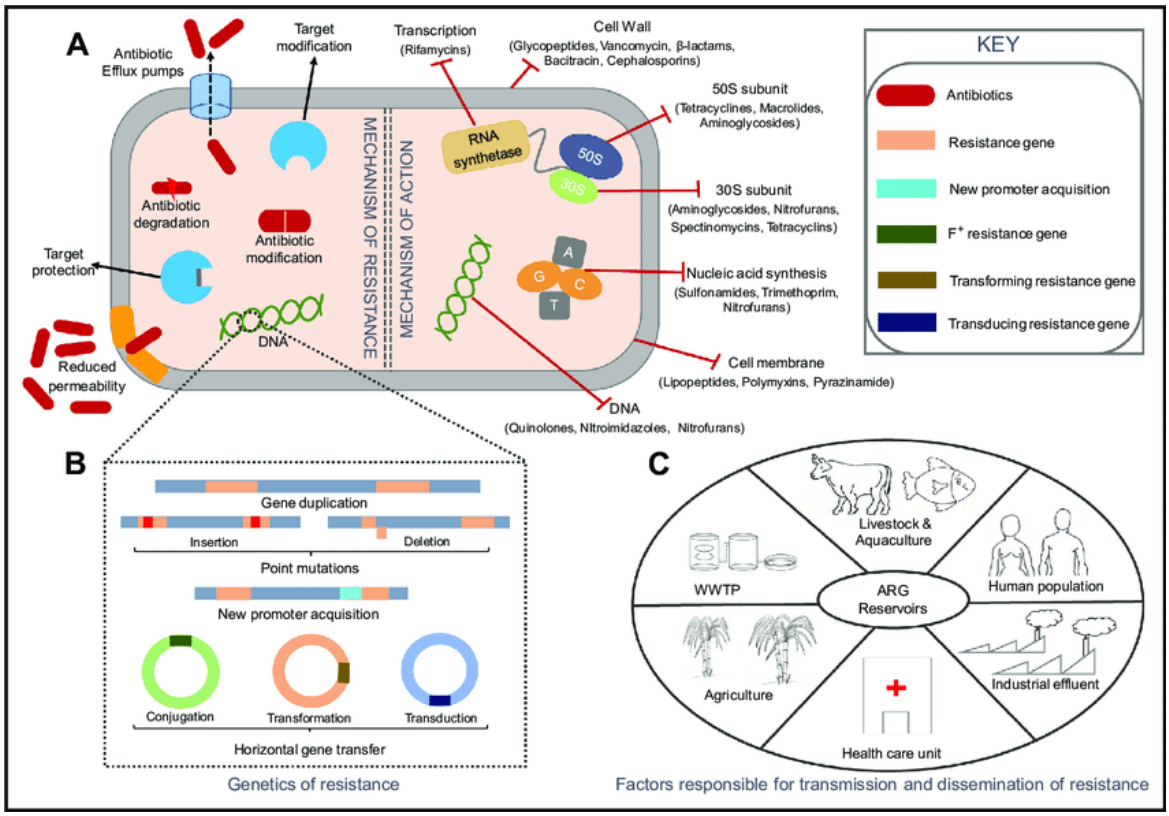

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Fig 1. Pathway for antibiotic resistance dissemination. (A) Illustrative summary of the various ways antibiotics function and the subsequent pathways through which antibiotic resistance develops. (B) Detailed understanding of the genetic foundation of antibiotic resistance and the methods through which it spreads. (C) Diagrammatic representation of the genetic elements that control the transfer of acquired antibiotic resistance genes.

1.2.1. Efflux of Antibiotics

Antibiotic efflux is a prevalent self-resistance mechanism often observed alongside other protective strategies, such as antibiotic modification or target alteration. A well-examined instance of antibiotic efflux is found in Streptomyces *peucetius*, a producer of the anticancer antibiotics daunorubicin (Dnr) and doxorubicin (Dox). These antibiotics impede DNA replication by intercalating with the DNA strands. In S. *peucetius*, efflux of Dnr and Dox is facilitated by an ATP Binding Cassette (ABC) family transporter known as DrrAB, encoded by the drrAB genes within the antibiotic biosynthesis gene cluster.

The DrrAB pump consists of two subunits, each comprising the ABC protein DrrA and the integral membrane protein DrrB. DrrA functions as the catalytic nucleotide binding domain (NBD), while DrrB acts as the carrier protein and forms the transmembrane domain (TMD). Detailed molecular and biochemical studies have been conducted on the DrrAB system, revealing its ability to efflux Dox in an ATP or GTP-dependent manner. Originally considered a dedicated transporter for Dnr and Dox, recent investigations have shown that the DrrAB pump exhibits broad substrate specificity. It can transport various compounds, including well-known multidrug resistance (MDR) pump substrates like ethidium bromide, Hoechst 33342, verapamil, and vinblastine. Interestingly, the DrrAB system shares similarities with the mammalian ABC multidrug transporter P-glycoprotein (Pgp), a major contributor to chemotherapy failure in human cancer cells. Studies indicate that critical aromatic residues within DrrB, contributed by multiple helices, form a common drug-binding pocket, resembling the aromatic residue-based mechanism for substrate recognition observed in mammalian Pgp. This suggests a shared evolutionary origin and mechanism for polyspecificity conserved across significant evolutionary distances.

Remarkably, within the oxytetracycline-producing bacterium Streptomyces *rimosus*, the OtrC efflux system serves as an intriguing illustration of a self-resistance mechanism displaying broad-spectrum resistance. In this organism, self-protection is achieved through two efflux proteins: OtrB (formerly TetB), situated within the biosynthesis cluster, and OtrC, located outside the cluster (Mak et al., 2014) [3]. OtrB, a member of the major facilitator superfamily (MFS) of transport proteins, remains relatively enigmatic in terms of its mechanism of action and substrate specificity, with limited knowledge available on these aspects (Ohnuki *et al.*, 1985; Reynes *et al.*, 1988; Mak *et al.*, 2014) [4, 5, 3]. The OtrC protein, on the other hand, belongs to the ABC family and, akin to DrrAB, imparts resistance against a spectrum of antibiotics and multidrug resistance (MDR) substrates. This includes resistance to ampicillin, oxytetracycline, doxorubicin, ethidium bromide, ofloxacin, and vancomycin (Yu *et al.*, 2012; Mak *et al.*, 2014) [6, 3]. Notably, the DrrAB and OtrC systems exhibit significant homology and display a high degree of sequence conservation in previously identified motifs, such as the DEAD and LDEVLF motifs of DrrA (Zhang e*t al.*, 2010, 2015) [8, 9] and the EAA-like motif in DrrB (Kaur *et al.*, 2005; Yu *et al.*, 2012) [7, 6]. This observed similarity suggests close evolutionary connections between efflux systems found in distinct producer organisms.

1.2.2. Target Modification Mechanism

Target modification serves as a built-in defence mechanism against various classes of antibiotics, including β-lactams, glycopeptides, macrolides, lincosamides, streptogramins (MLS), and aminoglycosides. The β-lactam antibiotics share a structural resemblance with peptidoglycan precursors, facilitating their association and subsequent acylation of the active site serine, leading to inhibition (Yeats *et al.*, 2002) [10]. Despite being Gram-positive, Streptomyces species exhibit high resistance to penicillins, attributed to either the overproduction of penicillin-binding proteins (PBPs) or the synthesis of low-affinity PBPs (Ogawara, 2015) [11]. Bacteria typically possess three classes of PBPs (A, B, and C) (Ogawara, 2015) [11], and an analysis of β-lactam-producing bacteria biosynthesis clusters often reveals the presence of PBP genes, suggesting their involvement in self-resistance (Liras and Martin, 2006; Ogawara, 2015) [12, 11]. Notably, Streptomyces species exhibit an above-average number of PBPs, surpassing 10 on average, encompassing both Classes A and B. Some of these PBPs exhibit low affinity for β-lactams, possibly due to the absence of a serine/threonine protein kinase domain (STPK) (now known as PASTA), which binds β-lactams (Ogawara and Horikawa, 1980; Nakazawa et al., 1981; Coque *et al.*, 1993; Paradkar et al., 1996; Yeats *et al.*, 2002; Ishida *et al.*, 2006; Ogawara, 2016a) [13, 14, 18, 15, 16, 10, 17]. Glycopeptides like vancomycin and teicoplanin impede cell wall transpeptidation and transglycosylation by associating with peptidoglycan precursors (D-Ala-D-Ala). Antibiotic resistance arises from altering the peptidoglycan precursor to D-Ala-D-Lac or D-Ala-D-Ser, resulting in a significant reduction in affinity for glycopeptides (Bugg et al., 1991; Billot-Klein *et al.*, 1994) [19, 20]. Vancomycin resistance genes, initially identified in clinical strains, commonly include the vanA cluster (vanHAX) on the transposon Tn1546. Some systems also employ VanY, a D, D-carboxypeptidase, to generate tetrapeptides incapable of glycopeptide binding (Binda *et al.*, 2014) [21]. Related core vanHAX clusters have been observed in producer organisms, indicating an evolutionary connection between resistance mechanisms in producers and pathogens (Marshall *et al.*, 1997, 1998) [22, 23].

1.2.3. Antibiotics Modification

Antibiotic modification is a widely employed strategy to neutralise the efficacy of antibiotics, particularly in the case of aminoglycoside antibiotics such as kanamycin, gentamicin, and streptomycin, as well as chloramphenicol and β-lactams. Numerous aminoglycoside modification enzymes (AMEs) are identified, including N-acetyl transferases (AAC), O-phosphotransferases (APH), and O-adenyltransferases (ANT). These enzymes acetylate, phosphorylate, or adenylylate the aminoglycoside antibiotics, rendering them ineffective. The existence of these modification enzymes was first observed in producer bacteria, specifically in Streptomyces species, during the early 1970s. The biochemical reactions performed by these enzymes in producer bacteria closely mirror those observed in antibiotic-resistant clinical strains (Walker and Walker, 1970; Benveniste and Davies, 1973) [25], there is not always a straightforward correlation between the synthesis of aminoglycosides and the presence of modification enzymes in Streptomyces producers. In certain cases, species may lack antibiotic production despite containing modification enzymes, and vice versa. However, an exception is observed in streptomycin resistance, where a clear link exists between antibiotic synthesis and the involvement of modification enzymes in self-resistance. Specifically, in the producer S.

griseus, streptomycin resistance is associated with the modification enzyme streptomycin 6-phosphotransferase. This enzyme, situated at the end of the biosynthetic pathway, converts streptomycin into an inactive precursor, streptomycin 6-phosphate. Importantly, the gene encoding this enzyme is co-regulated with biosynthesis genes, establishing a direct connection between its expression and the overall process of antibiotic production (Shinkawa *et al.*, 1985; Mak *et al.*, 2014) [28, 3].

1.3. Insight into Resistance of Antibiotics in Clinical Isolates

The discovery and development of antibiotics marked a remarkable milestone in the history of chemotherapy, providing effective treatments for infectious diseases. However, the extensive and indiscriminate use of antibiotics over the past seven decades has led to a concerning trend—the emergence of resistant strains to virtually every introduced antibiotic. The initial instances of resistance date back to the late 1930s, with the rapid development of resistance observed in the case of early antimicrobial agents like sulfonamides (Davies and Davies, 2010) [27]. Even before the widespread clinical use of penicillin, the discovery of penicillinase in 1940 within Staphylococcus aureus and Streptococcus pneumoniae hinted at pre-existing resistance mechanisms in the natural environment (Davies and Davies, 2010; Ogawara, 2016b) [27, 28]. Subsequent introductions of new antibiotics, such as methicillin, aimed at combating penicillin-resistant strains, led to the emergence of Methicillin-resistant Staphylococcus aureus (MRSA) (Davies and Davies, 2010) [27]. These instances highlight a recurring pattern wherein the use of each antibiotic eventually gives rise to resistant strains, underscoring the remarkable adaptability and plasticity of bacterial genomes.

The high frequency of spontaneous mutations and the widespread exchange of DNA among bacteria significantly contribute to this adaptability. The Centers for Disease Control and Prevention reports that antibiotic resistance is responsible for 23,000 deaths annually in the United States alone. Recent developments, particularly the emergence of Multi-Drug Resistant (MDR) and Extremely Drug Resistant (XDR) strains in pathogens like Mycobacterium tuberculosis, S. aureus, and Acinetobacter *baumannii*, pose a serious threat. These strains, often referred to as 'superbugs,' limit treatment options and raise concerns due to their increased resistance and virulence. Examples include Methicillin-resistant Staphylococcus aureus (MRSA) and vancomycin-resistant enterococci (VRE), as well as environmentally intrinsic bacteria like Pseudomonas aeruginosa and A. *baumannii* that can transform into opportunistic pathogens (Wright, 2007; Miller *et al.*, 2014) [29].

1.4. Antibiotic Resistance Mechanism in Clinical Isolates

In the realm of antibiotic resistance observed in clinical isolates, two primary categories can be distinguished: intrinsic and acquired resistance mechanisms. Intrinsic mechanisms are inherent to an organism's chromosome and typically involve non-specific efflux pumps, which likely evolved as a generalised response to environmental toxins. Additionally, intrinsic resistance may manifest through antibiotic-inactivating enzymes or permeability barriers. Examples of intrinsic mechanisms include the AcrAB/TolC efflux pump in Escherichia coli, known for its broad substrate specificity, and the outer membrane-imposed permeability barrier causing vancomycin resistance in E. coli and other Gram-negative bacteria. While intrinsic mechanisms confer a baseline level of antibiotic resistance in the original host, there is a risk that normal commensal flora or environmental bacteria with intrinsic mechanisms can become opportunistic pathogens in immunocompromised patients.

On the other hand, acquired resistance mechanisms are typically acquired through horizontal gene transfer (HGT). This category includes plasmid-encoded specific efflux pumps, such as TetK and TetL in Staphylococcus aureus, as well as enzymes capable of modifying either the antibiotic itself or its target. Acquired resistance mechanisms pose a more significant threat to human health because they involve a shift from chromosomal to plasmid-mediated determinants. This transition results in enhanced expression and dissemination of resistance, making acquired mechanisms a serious concern. An illustrative example is the mobilisation of the chromosomal β-lactamase gene ampC to a plasmid, leading to its global dissemination. This change in context amplifies the impact of resistance determinants, emphasising the urgency of addressing acquired antibiotic resistance for public health.

1.5. Origin of Resistance in Clinical Isolates

Scientists and clinicians continue to grapple with the perplexing origins of antibiotic resistance genes in clinical settings. The notion that resistance genes in pathogens might be obtained from antibiotic-producing organisms through horizontal transfer was initially proposed in the 1970s by Benveniste and Davies. This proposal was based on the observation that aminoglycoside-modifying enzymes found in actinomycetes shared biochemical activities with those in pathogenic strains. Another compelling example of the close relationship between antibiotic resistance genes in clinical isolates and those in antibiotic-producing bacteria is illustrated by the vanHAX genes. These genes exhibit significant protein sequence similarity and maintain a conserved arrangement within the gene cluster, as observed by Barna and Williams in 1984 and Marshall *et al.* in 1998 [28].

Despite the strong indications supporting the possibility of gene transfer from producers to pathogens, establishing a direct link between producers and pathogens has proven challenging. Rarely have resistance genes in pathogens been traced back to their producer counterparts. This difficulty arises primarily from the high sequence divergence and distinct G+C content observed in resistance genes of producers compared to those in pathogens, even when they employ similar mechanisms, as noted by Forsman et al. in 1990 and Marshall *et al.* in 1998 [28]. These findings collectively imply an evolutionary connection between determinants of producers and pathogens, but not necessarily a recent, direct gene transfer from producers, as highlighted by Forsman et al. in 1990, Marshall *et al.* in 1998 [28], and Aminov and Mackie in 2007 [33]. However, the transfer from producers might have taken place in the distant past, facilitated by a succession of closely associated carriers. For instance, it could have initially moved to closely related non-producing actinomycetes within the soil and subsequently progressed to proteobacteria and more remote pathogenic strains, as indicated by Marshall *et al.* in 1998 [28].

The divergence in G+C content between the two groups of organisms may be attributed to the extended time horizon in

this context. However, an emerging perspective, supported by a growing body of recent literature, posits that resistance genes present in non-producer environmental bacteria might have played a pivotal role in shaping the evolution of antibiotic resistance in pathogens (Aminov and Mackie, 2007) [33]. Contrary to initial beliefs, resistance genes appear to be more widespread in non-pathogenic microbial populations within the environment (D'Costa *et al.*, 2006; Nesme *et al.*, 2014; Surette and Wright, 2017) [34, 35, 36]. Notably, a study examining 500 Streptomyces strains isolated from soil revealed surprising multidrug resistance to 7 or 8 of the 21 tested antibiotics, underscoring widespread resistance mechanisms among contemporary organisms (D'Costa *et al.*, 2006) [34]. Recent genome sequence analyses further indicate that intrinsic resistance mechanisms are prevalent across all microbes (Fajardo *et al.*, 2008; Cox and Wright, 2013) [37, 38]. Additionally, homologs of resistance determinants found in clinical isolates are commonly present in non-pathogenic Gram-positive and Gram-negative bacteria (Seoane and Garcia Lobo, 2000; Mukhtar *et al.*, 2001; Sugantino and Roderick, 2002) [39, 40, 41]. Compelling evidence also suggests that antibiotic resistance gene sequences are ancient and predate the use of antibiotics (D'Costa *et al.*, 2011; Bhullar *et al.*, 2012; Warinner *et al.*, 2014; Perron *et al.*, 2015; Kashuba *et al.*, 2017) [36, 43, 44, 45, 46]. Analysis of microbial DNA from ancient human remains and permafrost reveals the existence of gene sequences homologous to those conferring resistance to various antibiotics in clinical strains (Warinner et al., 2014; Olaitan and Rolain, 2016; D'Costa *et al.*, 2011) [44, 47, 36]. These findings, along with similar studies in isolated caves, collectively suggest the presence of a continuum of resistance genes in environmental, producer, and pathogenic organisms, giving rise to the concept of a 'resistome'—the collection of antibiotic resistance genes found in all microorganisms (Wright, 2007) [29]. Consequently, a comprehensive understanding of resistance origins necessitates consideration of the pan-microbial genome, encompassing antibiotic producers, pathogens, cryptic genes, and precursor genes (Wright, 2007; Nesme and Simonet, 2015) [29].

In summary, it can be reasonably concluded that both producer and non-producing environmental organisms harbour extensive reservoirs of resistance genes that have the potential to be transferred to clinically relevant strains. While the evidence for the transfer of resistance determinants through the routes is somewhat limited, there are noteworthy instances supporting this phenomenon. Several reports describe direct genetic exchange from producer to non-producer organisms and from environmental organisms to clinical pathogens. For instance, a study identified the transfer of otrA and otrB gene sequences, part of the oxytetracycline biosynthesis cluster in Streptomyces, to mycobacteria variants, indicating a potential role of these organisms as carriers in the soil. Furthermore, this study revealed the presence of tetracycline resistance genes in Streptomyces and mycobacteria variants that were almost identical to those in Staphylococcus aureus, suggesting bidirectional movement of resistance genes between producer and non-producer organisms.

In another investigation, bioinformatics analysis provided evidence for the recent inter-phylum transfer of chloramphenicol and lincomycin efflux genes (cmx and lmrA) from Actinobacteria to Proteobacteria. This mechanism may subsequently lead to the transfer of these genes to clinical isolates. The most compelling evidence for recent transfers from non-pathogenic environmental bacteria to clinical strains comes from independent reports. One report demonstrated that the CTX-M ESBL gene, prevalent in pathogenic bacteria worldwide, closely resembled the CTX-M gene in the genome of non-pathogenic environmental Kluyvera species, implying a recent transfer of the gene to clinical strains. Another report highlighted the origin of the quinolone resistance determinant qnr in Klebsiella from the genome of non-pathogenic environmental Vibrio and Shewanella species. Additionally, evidence was presented for the transfer of the aph6 gene, encoding the Aph(30)-VI amikacin modification enzyme, from the chromosome of the environmental Acinetobacter *guillouiae* to a plasmid in A. *baumannii*, subsequently spreading to members of the Enterobacteriaceae family and Pseudomonas species. These examples collectively provide definitive proof of genetic transfer from environmental organisms and underscore how an intrinsic resistance gene within the genome of a non-pathogenic organism can lead to a widespread pandemic when mobilised to a conjugative plasmid or a phage and transferred to a clinically relevant strain.

1.6. Determinants of Antibiotic Resistance Genes

It is widely acknowledged that the environment plays a crucial role in the development of antibiotic resistance among pathogenic organisms. This progression typically involves four stages: the emergence of novel resistance genes, their mobilisation, transfer to pathogens, and dissemination. Although emergence and mobilisation events are frequent, the actual establishment of novel genes in populations depends on environmental factors such as selective pressure, fitness cost, and dispersal (Bengtsson-Palme et al., 2018) [48]. Among these factors, selection emerges as the most pivotal in maintaining resistance genes and mobile genetic elements (MGEs) throughout each stage of the acquisition process. Notably, antibiotic producers serve as a natural setting where resistance genes can be naturally selected in a competitive environment, preserving a reservoir of resistance genes within that niche (Laskaris et al., 2010). However, the primary source of potent selective pressure is the widespread and indiscriminate use of antibiotics by humans. This practice leads to the dominance of bacteria strains that are resistant or multiply resistant, not only among human pathogens but also in environments affected by human activities, such as pollution from antibiotic manufacturing facilities (Larsson, 2014). Additionally, specific settings identified as hot-spots, where both human-associated and environmental bacteria coexist, offer significant opportunities for the exchange of resistance genes and the selection of resistance (Bengtsson-Palme *et al.*, 2018) [48]. In essence, the pervasive use of antibiotics by humans, combined with various environmental contexts, creates a formidable selective pressure that promotes the persistence and longevity of resistance genes, influencing their prevalence and impact on microbial populations. The described environments are not only conducive to the transfer of resistance genes to pathogens but also facilitate the transfer of resistance from pathogens to environmental bacteria or opportunistic pathogens. This transfer may lead to the persistence and potential reemergence of resistance genes in the future (Ashbolt *et al.*, 2013; Martínez *et al.*, 2014; Bengtsson-Palme *et al.*, 2018) [49, 48, 50]. Recent studies indicate that even antibiotic

concentrations well below the minimum inhibitory concentration for sensitive bacteria can exert selective pressure (Gullberg *et al.*, 2011, 2014) [51, 52]. Additionally, contaminants such as heavy metals can co-select for antibiotic resistance (Pal *et al.*, 2015; Andersson and Hughes, 2017) [53, 57].

There is compelling evidence demonstrating that human activities over the past 70 years have substantially increased the abundance of resistance genes in bacterial populations. A study comparing pre-antibiotic era microbes with contemporary environmental bacteria in archived soils from 1940 to 2008 in the Netherlands revealed a significant rise in genes conferring resistance to tetracycline, erythromycin, and β-lactams over time (Knapp *et al.*, 2010) [55]. Intriguingly, there was also an increased mobilisation of β-lactamase genes from the chromosome to plasmids (Barlow *et al.*, 2008) [56].

A novel hypothesis suggests that antibiotic use strongly selects for the 'capture' of antibiotic resistance genes by mobile genetic elements (including plasmids, transposons, and integrons), acting as a potent force in microbial evolution (Gillings, 2014; Surette and Wright, 2017) [57, 36]. Other reports propose that antibiotic selection promotes competence in S. pneumoniae (Prudhomme *et al.*, 2006), induction of prophages in S. aureus (Goerke *et al.*, 2006), and enrichment of antibiotic resistance genes in phages present in the gut microbiome (Modi *et al.*, 2013) [60], all processes that could accelerate horizontal gene transfer (HGT). Notably, a recent study indicated that sub-inhibitory concentrations of different antibiotics can influence the ratio of transducing particles to virulent phages upon induction, suggesting an impact on gene packaging into phage particles (Stanczak-Mrozek *et al.*, 2017) [61].

Antibiotic exposure has been linked to increased rates of mutations, recombination, and integrase activity (Maiques *et al.*, 2006; Lopez *et al.*, 2007; Blazquez *et al.*, 2012) [62, 63, 64], compounding the multifaceted effects of excessive antibiotic usage on the emergence and enrichment of antibiotic resistance in bacterial populations. In conclusion, strategies aimed at mitigating these effects should focus on reducing selective pressure by minimising unnecessary antibiotic use and avoiding settings that favour selection and persistence, thereby preventing the further acquisition of novel resistance genes by pathogens.

1.7. Conclusion, Knowledge Gaps, and Directions for Future Research

Bacteria belonging to the Streptomyces genus, known for their antibiotic-producing capabilities, and non-pathogenic environmental bacteria play crucial roles as reservoirs for antibiotic resistance determinants. These genetic elements can be transferred to clinical strains through diverse horizontal gene transfer (HGT) mechanisms, such as the transformation of naturally competent bacteria, involvement of phages, and the utilisation of conjugative plasmids, transposons, and integrons. Regardless of limitations to the exchange of genetic materials between different kinds of bacteria, widespread gene transfer from chromosomes of soil and environmental bacteria to the mobilizable elements in clinical isolates seem to have occurred. While there is ample evidence of recent transfers of resistance genes from environmental bacteria to clinical strains, the direct transfer from producers to clinical strains has limited supporting evidence. Nevertheless, the potential for transfer from producer bacteria to other actinomycetes in the soil creates a possible pathway for subsequent transfer to proteobacterial clinical strains.

To gain a deeper understanding of the factors facilitating the dissemination of resistance genes and to elucidate the relationships between antibiotic resistance genes in producer, environmental, and pathogenic bacteria, new and improved strategies for sampling and screening microbial populations and metagenomic libraries are essential. Enhanced algorithms and the application of bioinformatics approaches are crucial for establishing relationships between resistance determinants in different environmental niches. Additionally, incorporating more genome sequencing data will help address gaps in our knowledge regarding intermediate stages and carriers for mobilisation.

Two databases, namely the Antibiotic Resistance Database (ARDB) and the Comprehensive Antibiotic Resistance Database (CARD), compiled over the last decade, offer computational tools for rapidly predicting antibiotic resistance genes and their targets in newly sequenced genomes. These databases, as demonstrated in a recent bioinformatics study (Jiang *et al.*, 2017), are expected to unify information on resistance genes and their products across thousands of bacterial species isolated from clinical or environmental sources, along with their associated mobile genetic elements. This will enable researchers in the field to efficiently mine and utilise this comprehensive information on resistance genes.

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