REVIEW ARTICLE

Environmental hotspots for antibiotic resistance genes

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Abstract

Bacterial resistance toward broad-spectrum antibiotics has become a major concern in recent years. The threat posed by the infectious bacteria and the pace with which resistance determinants are transmitted needs to be deciphered. Soil and water contain unique and diverse microbial communities as well as pools of naturally occurring antibiotics resistant genes. Overuse of antibiotics along with poor sanitary practices expose these indigenous microbial communities to antibiotic resistance genes from other bacteria and accelerate the process of acquisition and dissemination. Clinical settings, where most antibiotics are prescribed, are hypothesized to serve as a major hotspot. The predisposition of the surrounding environments to a pool of antibiotic-resistant bacteria facilitates rapid antibiotic resistance among the indigenous microbiota in the soil, water, and clinical environments via horizontal gene transfer. This provides favorable conditions for the development of more multidrug-resistant pathogens. Limitations in detecting gene transfer mechanisms have likely left us underestimating the role played by the surrounding environmental hotspots in the emergence of multidrug-resistant bacteria. This review aims to identify the major drivers responsible for the spread of antibiotic resistance and hotspots responsible for the acquisition of antibiotic resistance genes.

KEYWORDS
antibiotic resistance genes, antimicrobial resistance, clinical settings, environmental hotspots

1 | INTRODUCTION

Soil is an important rich source of nourishment for the survival of many microbial forms. Soil consists of four basic components namely, mineral particles (45%), water (25%), air (25%), and organic matter (5%) (Brady et al., 2008). The organic matter, in turn, contains humus (80%), roots (10%), and organisms (10%). Soil microorganisms include viruses, bacteria, actinomycetes, fungi, algae, and protozoa, and their prevalence is affected by soil temperature, moisture content, and available carbon sources. To gain an advantage in the soil environment many microbes have evolved survival mechanisms, such as producing antibiotics, to suppress competitors. A concomitant benefit is the suppression of other disease-causing microorganisms. Indeed, soil microorganisms (Actinomycetes) are the source of most antibiotics used to treat humans today. The presence of these antibiotics in the soil has led to the development of antibiotic resistance mechanisms, both in bacteria that produce antibiotics and those that do not have this capacity (D'Costa et al., 2006).

The decades between the 1930s and 1960s were considered the golden era of antibiotics due to the number of antibiotics discovered

(Nathan & Cars, 2014). Indeed, the discovery and use of antimicrobial agents in clinical practice are some of the great achievements in medicine, significantly increasing the life span of patients. However, the pace of discovery of new antibiotics has significantly slowed emerging resistant pathogens (Nathan, 2004). Moreover, in the 21st century, antibiotic resistance has evolved as one of the biggest public health threats (Munita & Arias, 2016). There are different drivers and "hotspots" contributing to the spread of antibiotic resistance in both household and clinical settings. In clinical settings, monitoring wards and hospital wastewater for antibiotic resistance are key sites of interest (Cacace et al., 2019; Karkman et al., 2018; Khan et al., 2019). However, no information currently exists regarding the spread of resistance from bacteria in the hospital to the environmental soil. The mechanism for bacteria developing resistance is acquired through gene mutation or horizontal gene transfer. This enables organisms to acquire resistance to a single antibiotic and being receptive to many mobile genetic elements. Moreover, organisms may acquire resistance to multiple antibiotics becoming multidrug-resistant, resulting in difficulty treating patients in clinical environments (Munita & Arias, 2016). This review describes some of the major drivers responsible for the spread of antibiotic resistance and hotspots responsible for the acquisition of antibiotic resistance genes. It also briefs the possibilities of potential exchange of resistance genes between different soil bacteria, commensals, and clinical pathogens. Human pathogens capable of acquiring such resistance determinants are generating a challenge for the treatment of multidrug-resistant infections.

2 | THE DISCOVERY OF ANTIBIOTICS AND EMERGENCE OF ANTIBIOTIC RESISTANCE

Table 1 summarizes the different classes of antibiotics based on their origin and structure. It highlights the timeline of the discovery of different antibiotics based on the year they were first reported in the literature and when they were introduced clinically. The initial finding was the discovery in 1928 by Alexander Fleming, where the development of _Staphylococcus_ bacteria was inhibited by a specific species of mold known as _Penicillium notatum_, which led to the discovery of the first "Modern Day" antibiotic, penicillin (Jeśman et al., 2011). It still took more than a decade to introduce penicillin for the treatment of bacterial infections. Before the idea of bacterial inhibition by secondary metabolites of mold, in 1921, Fleming observed that lysozymes in systemic fluids could dissolve bacteria.

During the late 1930s, a group of synthetic drugs called sulfonamides became the first systematically used chemical substances for the prevention and treatment of bacterial infections and proved to be a revolution in antibiotic therapy. However, the success of these antibiotics was temporary. This is because the organisms that encountered the first commercially produced antibiotics gradually began to attain resistance to single antibiotics. Resistance to penicillin among _Staphylococcus_, specified by an enzyme (penicillinase)

that degraded the antibiotic, is one of the most notable examples (Barber, 1947; Davies, 2012). The industry invested heavily in the production of antibiotic derivatives against the developed resistant strains (e.g., introducing methicillin, modified aminoglycosides, and other beta-lactamase-resistant penicillins and cephalosporins). Over time, however, bacteria developed ways to circumvent these antibiotics and this ended the so-called golden era of antibiotics (Davies, 2012). Researchers in Japan were the first to report the transfer of multidrug resistance by elements referred to as "R factors" in 1959 (Macuch et al., 1967). Other international studies subsequently confirmed this mechanism (Kruse & Sørum, 1994; Linton et al., 1974). Efforts to prevent or eliminate horizontal gene transfer were unsuccessful (Davies, 2014). As resistance increased, most pharmaceutical companies closed down their antibiotic research and development programs, thus creating a void in the discovery of antibiotics (Harbarth et al., 2015). The last class of antibiotics approved for clinical use was the Diarylquinolines (e.g., Bedaquiline) in 2012. Salvarsan, introduced in 1910, is no longer used clinically. Fusafungin, a mixture of enniatins, was withdrawn from the market in February 2016 based on the recommendation of the European Medicines Agency (Hutchings et al., 2019). One of the current scientific challenges is to discover new active antibiotics against clinically relevant antibiotic-resistant bacteria.

3 | DRIVERS DISSEMINATING ANTIBIOTIC RESISTANCE GENES

With extensive use of different drugs over time, microorganisms have emerged bearing additional kinds of resistance mechanisms leading to multidrug resistance. There are several eloquent reviews on this topic (Alekshun & Levy, 2007; Ayukekbong et al., 2017; Choudhury et al., 2012; Colodner et al., 2004; Gashaw et al., 2018; Nikaido, 2009; Tanwar et al., 2014). Alekshun and Levy (2007) highlight that these resistance mechanisms include novel penicillin-binding proteins, enzymatic mechanisms of drug modification, mutated drug targets, enhanced efflux pump expression, and altered membrane permeability (Alekshun & Levy, 2007). Figure 1 illustrates that bacterial antibiotic resistance genes can be transferred through several horizontal gene transfer mechanisms.

Horizontal gene transfer (HGT) is one of the most widely known and prevalent processes among bacterial populations for the transfer of genes and enriching themselves with new characteristics or traits (Khan & Rao, 2019). HGT is a process of swapping genes between organisms that are not in a parent–offspring relationship (Soucy et al., 2015). This exchange of genetic material can cause both beneficial as well as adverse consequences. Although HGT plays a crucial role in biodiversity, innovations, and evolution (Jain et al., 2003; Ochman et al., 2000; Soucy et al., 2015), a recent review by Emamalipour et al. (2020) argued that HGT plays a role in the development of pathological conditions in case of disease (Emamalipour et al., 2020). Adding on to the significance of HGT, it plays a significant role in evolutionary genetics. It rescues prokaryotes from Muller's ratchet,

TABLE 1 Different classes of antibiotics based on their origin and the timeline of discovery

Antibiotics from Actinomycetes

Class	Discovery reported	Introduced clinically	Example	Source
Aminoglycosides	1944	1946	Kanamycin A	*Streptomycin kanamyceticus*
Tetracyclines	1948	1948	Tetracycline	*Streptomyces aureofaciens*
Amphenicols	1947	1949	Chloramphenicol	*Streptomyces venezuelae*
Macrolides	1952	1952	Erythromycin	*Saccharopolyspora erythraea*
Tuberactinomycins	1951	1953	Viomycin	*Streptomyces puniceus*
Glycopeptides	1954	1958	Vancomycin	*Amycolatopsis orientalis*
Lincosamides	1962	1963	Clindamycin	*Streptomyces lincolnensis* (Semi-synthetic derivative of lincomycin)
Ansamycins	1959	1963	Rifamycin SV	*Amycolatopsis rifamycinica* (Semi-synthetic derivative of lincomycin)
Cycloserines	1955	1964	Seromycin	*Streptomyces orchidaceus*
Streptogramins	1953	1965	Pristinamycin	*Streptomyces pristinaespiralis*
Phosphonates	1969	1971	Fosfomycin	*Streptomyces fradiae*
Carbapenems	1976	1985	Meropenem	*Streptomyces cattleya* (Synthetic molecule based on Thienamycin)
Lipopeptides	1987	2003	Daptomycin	*Streptomyces roseosporus*
Lipiarmycins	1975	2011	Fidaxomicin	*Dactylosporangium aurantiacum* subsp. *hamdenesis*

Antibiotics from other bacteria

Class	Discovery reported	Introduced clinically	Example	Source
Polypeptides	1939	1941	Gramicidin A	*Bacillus brevis*
Bacitracin	1945	1948	Bacitracin A	*Bacillus subtilis*
Polymyxins	1950	1959	Colistin	*Paenibacillus polymyxa*
Mupirocin	1971	1985	Mupirocin	*Pseudomonas fluorescens*
Monobactams	1981	1986	Aztreonam	*Chromobacterium violaceum* Synthetic molecule based on SQ 26,180

Antibiotics from Fungi

Class	Discovery reported	Introduced clinically	Example	Source
Penicillin	1929	1943	Amoxicillin	*Penicillium chrysogenum* Semi-synthetic derivative of Penicillin
Fusidic acid	1958	1962	Fusidic acid	*Fusidium coccineum*
Enniatins	1953	1963	Fusafungine	*Fusarium lateritium*
Cephalosporins	1948	1964	Cefacetrile	*Acremonium chrysogenum* Semi-synthetic derivative of Cephalosporin C
Pleuromutilins	1951	2007	Retapamulin	*Pleurotus mutilus* (Semi-synthetic derivative of pleuromutilin)

Synthetic Antibiotics

Class	Discovery reported	Introduced clinically	Example
Arsphenamines	1907	1910	Salvarsan
Sulfonamides	1932	1936	Mafenide

TABLE 1 (Continued)

Synthetic Antibiotics			
Class	Discovery reported	Introduced clinically	Example
Salicylates	1902	1943	4- Aminosalicylic acid
Sulfones	1908	1945	Dapsone
Pyridinamides	1952	1952	Isoniaizid
Nitrofurans	1945	1953	Nitrofurantoin
Azoles	1959	1960	Metronidazole
Fluoro(quinolones)	1962	1962	Ciprofloxacin
Diaminopyrimidin	1950	1962	Trimethoprim
Ethambutol	1962	1962	Ethambutol
Thioamides	1956	1965	Ethionamide
Phenazines	1954	1969	Clofazimine
Oxazolidinones	1987	2000	Linezolid
Diarylquinolines	2004	2012	Bedaquiline

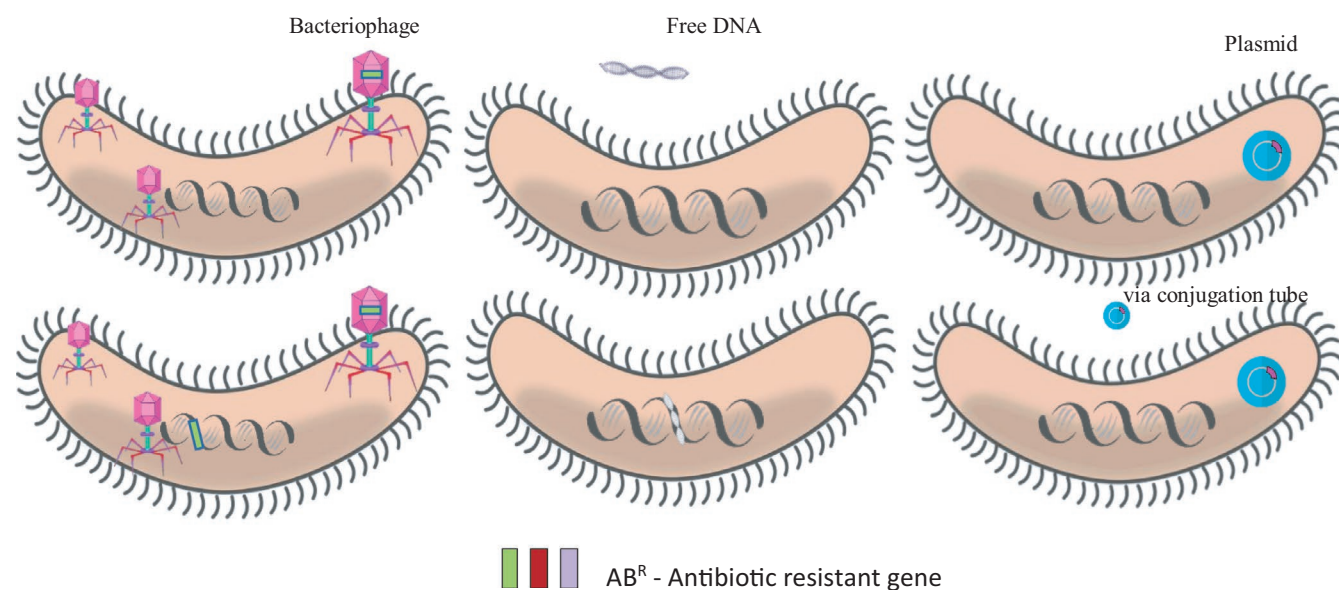Adapted from Hutchings et al. (2019).

FIGURE 1 Acquisition of antibiotic resistance—the mechanism of horizontal gene transfer between different bacterial populations. There are three methods of transfer of genetic material: (1) transduction (via bacteriophage), (2) transformation (via free deoxyribonucleic acid (DNA)), and (3) conjugation (via plasmid). The antibiotic-resistant gene becomes incorporated into the chromosome by recombination and/or transposition. Adapted from Alekshun and Levy (2007)

an irreversible phenomenon, where the absence of recombination in asexual reproduction results in the accumulation of harmful mutations (Takeuchi et al., 2014; van Dijk et al., 2020). It is the primary mechanism for the spread of antibiotic resistance in bacteria and is achieved through the processes of conjugation (via plasmid and conjugative transposons), transduction (via bacteriophages), or transformation (via incorporation into the chromosome of chromosomal DNA, plasmid, and other naked DNA) (Levy & Marshall, 2004). It also plays a crucial role in the development of drug-resistant microorganisms and the transfer of virulence genes. Gene transfer agents, first discovered in the 1970s (Solioz & Marrs, 1977), are another less common mode of gene transfer. It is a combination of bacteriophage transduction and natural transformation (Lang et al., 2012). They are small virus-like particles responsible for the transfer of their entire genome between host cells (Solioz & Marrs, 1977). Among the three main mechanisms involved in HGT, transformation rarely occurs between bacterial species for the transfer of drug resistance genes. However, conjugation involving mobile genetic elements like transposons and plasmids is the most efficient and important method involved in the spread of antibiotic resistance (Price et al., 2019).

Gene transfer commonly occurs within the same genus, but it has also been observed between very different genera via the aforementioned methods (Evans et al., 2020; Jiang & Paul, 1998; Redondo-Salvo et al., 2020). Lacroix and Citovsky (2016) have also reviewed the transfer of DNA from bacteria to Eukaryotes (Lacroix & Citovsky, 2016). For a gene transferred from another species to survive a long time in the recipient lineage, it is important to have a survival advantage either to the recipient or itself (Gogarten & Townsend, 2005).

Horizontal gene transfer is expedited by transposons and/or plasmids. These are the drivers responsible for the spread of antibiotic resistance (Khan & Rao, 2019). Microorganisms transfer resistance genes rapidly, spreading antibiotic resistance between the strains. The environment is considered to be the main source of mobile genetic elements, which are key elements responsible for the transfer of resistance genes (Knöppel et al., 2017). Recently, a study by Katale et al. (2020) revealed the presence of multiple sequence types isolated from the drug-resistant bacteria and their replicon plasmid types from human, animal, and environmental sources. These findings highlighted a remarkable genetic diversity. Moreover, the presence of diverse antimicrobial resistance genes suggests an increased likelihood of multiple sources of resistant bacteria or a possible exchange of strains or gene flow among different strains due to transfer via mobile genetic elements (Katale et al., 2020). Next-generation sequencing may be used as a better tool for the detection of evolving antibiotic resistance threats (Crofts et al., 2017). Performing whole genome sequencing on enteric bacteria, Kumar et al. (2017) confirmed the presence of multiple mobile genetic elements and horizontal gene transfer in six extensive enteric pathogens which were resistant to drugs (Kumar et al., 2017).

A deeper understanding of the significance of HGT has turned the focus of scientists to study the frequency of gene transfers. The frequency of gene transfers is of paramount importance, especially in the human microbiome. Although it is challenging to determine the frequency of HGT, an analysis that tried to explore gene transfers in the human microbiome revealed a surprising extend of gene transfer in human microbiota compared to other environments. The results showed a 25% greater frequency of HGT between pairs of organisms associated with humans than between pairs of organisms in different hosts or terrestrial or aquatic environments. Additionally, there was a 50-fold likelihood of gene transfer between pairs of human-associated organisms which were isolated from the same body site. This indicated that environmental fluctuations, which promote adaptive changes, were more prevalent in a holobiont ecology, especially human holobiont. The study highlighted that ecology governs HGT (Smillie et al., 2011).

One of the most important traits transferred via HGT is resistance to antibiotics. Antibiotic resistance can be achieved through intrinsic or acquired mechanisms. Intrinsic mechanisms are those specified by naturally occurring genes found on the host's chromosome and efflux systems. Acquired mechanisms involve gene mutations and/or transfer of resistance determinants via horizontal gene transfer. Such mutations in genes may cause resistance by (a) altering the target protein to which the antibacterial agent binds, modifying or eliminating the binding site; (b) upregulating the production of enzymes that inactivate the antimicrobial agent; (c) down-regulating or altering an outer membrane protein channel that is required by the drug to bind and for cell entry, or (d) upregulating pumps that expel the drug from the cell. Despite a plethora of studies on the science behind resistance, limited information is available on the rise of resistant pathogens across the globe (Kumar et al., 2017).

4 | HOTSPOTS FOR THE SPREAD OF ANTIBIOTIC RESISTANCE

Antibiotic-resistant bacteria have been known to exist in the environment for many years. For example, there is evidence of their presence in caves up to 4 million years ago (Bhullar et al., 2012). Antibiotic-resistant bacteria have been found in permafrost 30,000 years old (Finley et al., 2013), and they have also been detected in the gastrointestinal tract of individuals from Amazon tribes who have never been exposed to antibiotics (Gibbons, 2015). Certain bacteria are commensals and colonize people, however, they may cause disease when they traffic from their normal sites (e.g., skin and gastrointestinal tract) to areas of the body that they should not be (the bloodstream, organs, etc.). The drug-resistant organisms and the antibiotics in the animal feed reach the environment through animal waste (Berendsen et al., 2015; Wichmann et al., 2014). The resistant organisms live close to each other in the soil, and this facilitates horizontal gene transfer through the transfer of genetic determinants (Christensen et al., 1998). In the case of humans, there is a definitive link between the consumption of antibiotics in animals and the development of antibiotic resistance in humans (O'Neill, 2016).

Antibiotics are released into the environment via several pathways, such as clinical settings, human or animal waste, the use of products containing antibacterials, and through food and fodder which are supplemented with antibiotics and given to animals (Figure 2) (Gelband et al., 2015). They can even spread by contact with infected workers handling and processing meat or farm workers (Elbossaty, 2017; Michael et al., 2014). It is important to explore the different environmental hotspots facilitating the robust dissemination of resistance in pathogenic and non-pathogenic bacteria. In this context, it is important to identify some of the major drivers which provide a favorable environment to hasten the process of antimicrobial resistance. Hotspots for antibiotic-resistant bacteria include wastewater systems, pharmaceutical manufacturing sites, food and animal production sites in agriculture and aquaculture, and clinical settings such as hospitals (Berendonk et al., 2015).

4.1 | Wastewater treatment plants

The selection of multi-resistant bacteria and their spread into the surrounding environment from wastewater treatment plants is
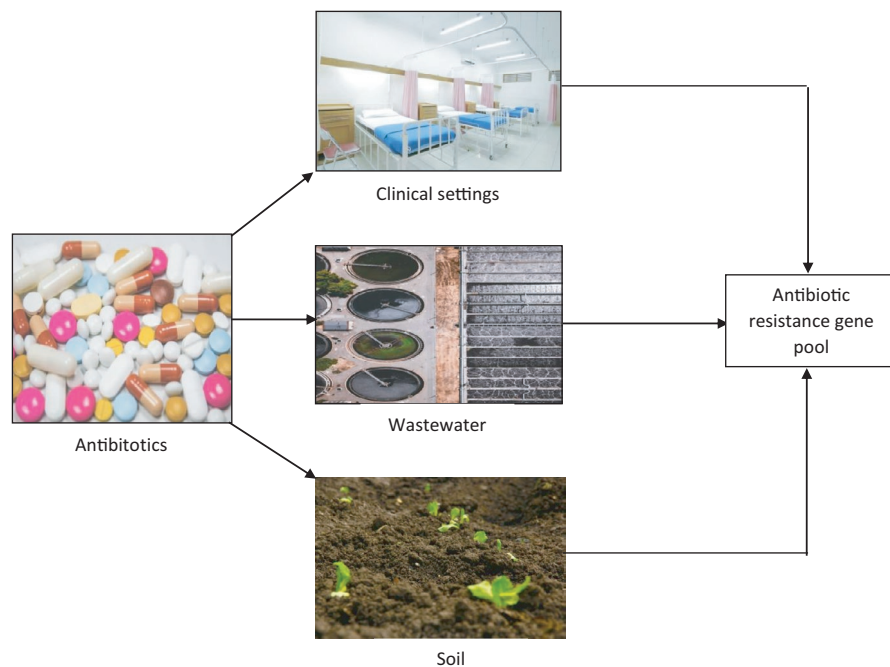
FIGURE 2 Antibiotic contamination or waste enters a variety of different environmental settings. Antibiotics are accumulated in these environments and select for bacteria that encode resistance genes for their survival generating a hotspot environment for resistance genes. These hotspots contribute to the spread of antibiotic resistance genes in the environment

favored by the concomitant presence of antibiotics, heavy metals, antibiotic-resistant bacteria, and antibiotic-resistant genes (Di Cesare et al., 2016). Municipal wastewater systems serve as a hotspot for bacteria resistant to antibiotics (Berendonk et al., 2015; Di Cesare et al., 2016) and as a direct source responsible for the spread of resistance into the environment (Czekalski et al., 2014; Di Cesare et al., 2016; Gao et al., 2012; LaPara et al., 2011; Rizzo et al., 2013). Improper disposal of antibiotics and medical wastes from hospitals, leakage in septic systems, etc., facilitate the entry of antibiotic residues into the soil and groundwater. Though some antibiotics may degrade after reaching the environment, others can remain active for prolonged periods in wastewater treatment plants (Rizzo et al., 2013). High concentrations of antibiotics have been detected in water samples and sediments from the Liuxi River in Guangzhou, China, and fishponds are suggested to be the reservoirs of antibiotic residues and antibiotic-resistant genes (Xiong et al., 2015). Xu et al. (2016) showed the occurrence and abundance of antibiotic-resistant genes in drinking water treatment plants and distribution systems in China. Tap water supplied from these drinking water reservoirs to the residential areas contained resistance genes against some important classes of antibiotics considered useful in clinical practice (β lactams, aminoglycosides, and macrolide lincosamide streptogramin B) (Xu et al., 2016).

Studies have also reported the presence and dissemination of antibiotic-resistant genes in environmental soil following the continuous release of reclaimed water irrigation containing antibiotic residues and antibiotic resistance genes (Wang et al., 2014). Poorly treated sewage also facilitates the transfer of resistant genes to human pathogens and the dissemination of antibiotic-resistant bacteria (Bengtsson-Palme et al., 2018). Even minute concentrations of antibiotics are sufficient to select for highly resistant bacteria, and this has been demonstrated in laboratory settings (Gullberg et al.,

2011; Wistrand-Yuen et al., 2018). As water-containing antibiotics move through the sediments and soil, gradients of antibiotic concentration form.

4.2 | Manufacturing industry

Antibiotic manufacturing industries release a significant concentration of antibiotics into the environment (Bengtsson-Palme et al., 2018). Improper disposal of pharmaceutical wastes pollutes the natural environments like grasslands, water, and air (Sahoo et al., 2010). In Hyderabad, India, approximately 100 pharmaceutical manufacturing plants are involved in the supply of drugs to different parts of the world. However, a single plant processes the wastewater from all these manufacturing sites. Indicative of how easily drug contamination can occur following usage and incorrect disposal, Fick et al. (2009) previously reported in 2009 how processed effluent from the water treatment plant ended up in nearby surface water (lakes) as well as ground and drinking water (wells). These workers documented ciprofloxacin and cetirizine levels in the water which exceeded the human therapeutic concentration in blood plasma; indeed, the highest levels ever detected in surface and well waters at that time (Fick et al., 2009). The high levels of antibiotics in the water suggest likely mixing of water with the microbial population in the soil, creating a very favorable environment for the transfer of resistant genes between different species contributing to the spread of resistance (Gelband et al., 2015). A high number of multidrug-resistant bacteria have been detected in treatment plants that harbor multiple bacteria from the environment, human pathogenic bacteria as well as normal commensal bacteria which do not cause any disease (Marathe et al., 2013).

4.3 | Human and animal wastes – sanitary practice

Administering antibiotics to humans and animals results in antibiotics being excreted in the urine or feces, and thus, end up in the environment (Daghrir & Drogui, 2013; Sarmah et al., 2006). Additionally, animal feed containing antibiotics may directly seep into the soil or may be excreted through animals into the soil (Sarmah et al., 2006). It is known that wildlife represents a further reservoir of antibiotic-resistant genes in the environment (Swift et al., 2019; Wellington et al., 2013). Animal waste and fertilizers (manure) can also be a source of resistant genes and bacteria entering the soil and groundwater (Sarmah et al., 2006). In the Netherlands, Berendsen et al. (2015) presented a multiclass method for the detection of around 20 classes of antibiotics in feces. Their analysis revealed that greater than 30% of collected pig and cattle feces contained the residue of at least one antibiotic. Three different antibiotics were recovered from pig samples while eight different classes were recovered from cattle feces (Berendsen et al., 2015). Such a study has not been conducted in a country like India, where it is common to see overcrowded shelter houses with more cattle and improper disposal of animal wastes. It is speculated that these livestock farms also serve as hotspots for the transfer of antibiotic resistance genes (Taneja & Sharma, 2019).

Smillie et al. (2011) identified bacteria from human food and farm animals and concluded that resistance transfers were more evident among human-associated bacteria compared to non-human-associated bacteria. The study identified unique antibiotic-resistant genes (belonging to the gene family—*aac, aph, catA, erm, sul, and tet*) transferred between human and farm isolates. The transferred genes comprised of nine gene families and targeted at least one antibiotic used in agriculture. Smillie et al. (2011) speculated that in the case of these mobile traits like antibiotic resistance, it is genes and not the genomes that serve as a unit of evolution and proliferation. Hence, livestock-associated bacteria obtained from farm animals or human food, comprising of antibiotic resistance genes conferring resistance to both clinical antibiotics and agricultural drugs like amikacin, gentamycin B, kanamycin, lividomycin, neomycin, paromomycin, streptomycin, lincosamide, macrolide, streptogramin B, sulfonamide, and tetracycline can contribute to clinical resistance without infecting humans directly (Smillie et al., 2011).

4.4 | Commensal bacteria

Commensal bacteria supply the host with essential nutrients and help protect the host from opportunistic pathogens; they are also important contributors to the pool of antibiotic resistance genes present in the surrounding environment. There is evidence for the transfer of antibiotic resistance genes between commensal bacteria and bacterial pathogens in the human intestine (Salyers et al., 2004). For example, fecal *E. coli* (commensal) has been universally acknowledged and explored as an indicator for the surveillance and spread of acquired antibiotic resistance genes among pathogens in community settings (Bartoloni et al., 2006; Lester et al., 1990; Nys et al., 2004; Shakya et al., 2013; Singh et al., 2018).

In a recent study among children aged 1–14 years who were not previously exposed to antibiotics, Singh et al. (2018) found a close correlation between demographic factors and an increased incidence of commensal *E. coli* which were resistant to antibiotics. This highlights the overuse and misuse of antibiotics leading to a transmissible threat of multidrug resistance between commensals and pathogenic isolates (Singh et al., 2018). In another study by Infante et al. (2005), fecal samples tested for the presence of antibiotic resistance genes revealed a high prevalence of *sul*-genes in commensal *E. coli* isolated from healthy children of Bolivia and Peru. This highlights the potential risks posed by commensal bacteria for the spread of antimicrobial resistance among pathogens (Infante et al., 2005). Antibiotic-resistant *E. coli* (very high resistance to ampicillin and cotrimoxazole) isolated from the fecal microbiota of neonates less than a month of age, who had no previous history of antibiotic therapy, emphasize the rapidly growing threat of antibiotic resistance (Tule & Hassani, 2017). Analysis of the rates of transmission of antibiotic resistance between commensal bacteria and pathogenic members of Enterobacteriaceae (*E. coli* O157, and *Salmonella* spp) has also revealed that members of the Enterobacteriaceae family are capable of exchange, transfer, and dissemination of resistant genes from commensal microbiota to zoonotic pathogens and vice versa (Blake et al., 2003).

4.5 | Clinical settings and surroundings

Clinical settings/hospitals have the highest level of antimicrobial consumption and hence are considered hotspots of AMR. Hospital effluents are expected to harbor a high number of resistant bacteria and genes. It was found that the effluent of Indian hospitals had high enough antibiotic concentrations to cause genotoxic modifications in bacterial strains (Diwan et al., 2010). Mutiyar and Mittal (2014) reported the dangerous levels of antibiotic residues (fluoroquinolones, sulfonamides, and tinidazoles) recovered from one of the hospital effluents in India (Mutiyar & Mittal, 2014). Although 80%–85% of antimicrobial residues in the hospital effluents can be removed efficiently by proper wastewater treatment (Duong et al., 2008), less than 45% of wastewater treatment systems work efficiently among all the healthcare facilities in India (Taneja & Sharma, 2019; World Health Organization, 2015).

Studies have shown that clinically relevant antibiotic resistance genes such as cefotaxime-hydrolyzing beta-lactamase (*blaCTX-M*), quinolone resistance determinant (*qnrA*), and New Delhi metallo-beta-lactamase (*blaNDM*) originating in the environmental bacteria *Kluyvera spp.*, *Shewanella algae*, and *Erythrobacter litoralis*, respectively (Berendonk et al., 2015; Nordmann & Poirel, 2005; Oliver et al., 2001; Zheng et al., 2011), contribute to the challenge of effectively treating bacterial infections. However, anthropocentrism (a philosophical viewpoint that humans are the most important forms of life) has led

to the view that these genes have evolved as antibiotic resistance genes, naturally (Walsh, 2013). The clinical importance of the soil resistome is that we know there can be an interchangeable transfer of resistance between the soil bacteria and pathogens, irrespective of whether resistance genes are moving from the clinic to the soil or vice versa (Forsberg et al., 2012). Cycoń et al. (2019) previously described alterations in the metabolizing capability of microorganisms in the soil present with antibiotics; namely, altered ability to metabolize different sources of carbon, and also their enzyme activity. The antibiotics also affected microbial biodiversity—biomass and the abundance of different categories; namely, gram-positive bacteria, gram-negative bacteria, and fungi in the microbial population. Exploring the array of antibiotic resistance genes in the soil had led to the discovery of new enzymes and genes which contribute to the emergence of antibiotic resistance in bacteria. The impact of antibiotics on microbial activity and biodiversity remains a great challenge (Cycoń et al., 2019).

Several examples in the literature have provided evidence for the transfer of antibiotic-resistant genes in clinical environments via horizontal gene transfer. For example, Lerminiaux and Cameron (2019) concluded conjugation is the primary mode of horizontal gene transfer in clinical settings. Their review also offered that conditions for the spread of resistance genes via natural transformation and transduction existed in clinical environments (Lerminiaux & Cameron, 2019). Kac et al. (2004) conducted a study in a 17-bed cardiac surgery intensive care unit in a large university teaching hospital to analyze the environmental contamination by extended-spectrum α-lactamase producing Enterobacteriaceae and compare the clinical and environmental strains. Molecular analysis revealed four species (three *Klebsiella oxytoca* and one *Enterobacter cloacae*) to be identical or having close similarities between clinical and environmental strains (Kac et al., 2004).

Another study has provided evidence for the exchange of antibiotic resistance genes between the bacteria in the environment and clinical pathogens (Forsberg et al., 2012). Most of the clinical pathogens belonged to be Proteobacteria (Rizzatti et al., 2017) and were cultured from the soil. Forsberg et al. (2012) described the presence of resistance gene cassettes in multidrug-resistant soil bacteria. Gene cassettes against five classes of antibiotics: β-lactams, amphenicols, aminoglycosides, tetracyclines, and sulfonamides were identified, which were present in clusters and shared identical nucleotide sequences with clinical pathogens. Mobile genetic elements flanked these gene clusters, suggesting they were acquired via horizontal gene transfer (Forsberg et al., 2012). Though studies of HGT in environments like wastewater treatment plants have progressed, no studies have identified and quantified the rate and drivers of HGT in clinical environments. Hence, there is a need to track these factors in clinical environments, where pathogens exacerbate the problem (Lerminiaux & Cameron, 2019).

5 | CONCLUSION

The drivers and hotspots of antibiotic resistance together increase the burden of the spread of resistance determinants. There is currently a paucity of information regarding the impact of antibiotics on environmental microorganisms and the spread of resistance from clinical settings to the environment and vice versa. Soil harbors many microorganisms which are the primary source of antibiotics. It is, therefore, important to bridge the gap of scientific knowledge on these environmental microorganisms—specifically bacteria—habituated in the soil around clinical sites where many antibiotics are prescribed for medicinal use. The assessment of indices such as soil temperature, pH and moisture, and determination of resistant genes present in organisms isolated from the soil surrounding clinical settings over time, will help us understand the evolution of microorganisms and the impact of antibiotics on these organisms in clinical settings. Exploring these research gaps may further help us tackle the problem of antibiotic resistance which is considered one of the biggest threats to public health today.

CONFLICT OF INTEREST
None declared.

AUTHOR CONTRIBUTIONS
Shalini Kunhikannan: Conceptualization (equal); Writing-original draft (lead). **Colleen J. Thomas:** Conceptualization (equal); Supervision (equal); Writing-review & editing (equal). **Ashley E. Franks:** Conceptualization (equal); Project administration (equal); Supervision (equal); Writing-review & editing (equal). **Sumana Mahadevaiah:** Conceptualization (equal); Supervision (equal); Writing-review & editing (equal). **Sumana Kumar:** Conceptualization (equal); Supervision (equal); Writing-review & editing (equal). **Steve Petrovski:** Conceptualization (equal); Project administration (equal); Supervision (equal); Writing-review & editing (equal).

ETHICS STATEMENT
None required.

DATA AVAILABILITY STATEMENT
Not applicable.

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