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A Prospective study on Environmental Factors Influencing the Development and Spread of Antibiotics Resistance Northern India

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ABSTRACT:
Antibiotic resistance and its wider implications present us with a growing healthcare crisis. Recent research points to the environment as an important component for the transmission of resistant bacteria and in the emergence of resistant pathogens. However, a deeper understanding of the evolutionary and ecological processes that lead to clinical appearance of resistance genes is still lacking, as is knowledge of environmental dispersal barriers. This calls for better models of how resistance genes evolve, are mobilized, transferred and disseminated in the environment. Here, we attempt to define the ecological and evolutionary environmental factors that contribute to resistance development and transmission. Although mobilization of resistance genes likely occurs continuously, the great majority of such genetic events do not lead to the establishment of novel resistance factors in bacterial populations, unless there is a selection pressure for maintaining them or their fitness costs are negligible. To enable preventative measures it is therefore critical to investigate under what conditions and to what extent environmental selection. for resistance takes place. In addition, understanding dispersal barriers is not only key to evaluate risks, but also to prevent resistant pathogens, as well as novel resistance genes, from reachinghumans.

Keywords:
Antimicrobial Resistance; Dissemination ; Fitness Costs; Horizontal Gene Transfer, Human health risks.
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INTRODUCTION

Antibiotic resistance genes in human pathogens such as Methicillin-Resistant Staphylococcus aureus! have become notorious because they confound the tools that are used to treat disease (FIG.1). In particular, resistance determinants in pathogens are commonly encountered after the introduction of an antibiotic to clinical use, and treating human pathogens with antibiotics directly affects the frequency of resistance to those antibiotics in these pathogens. [1-4] The presence of antibiotic resistance elements in pathogenic bacteria is made all the more problematic because of the prevalence of horizontal gene transfer, the process by which bacteria acquire genes from the environment. [5] Many of the known antibiotic resistance genes are found on transposons, integronsor plasmids, which can be mobilized and transferred to other bacteria of the same or different species.[7] There is evidence of the transfer of resistance elements to known human commensal bacteria and pathogens, and gene transfer in the human intestinal microbiome is extensive.[8] What are the sources and reservoirs of these transferable genes? Afull understanding of the pressures and circumstances that lead to the evolution and dissemination of antibiotic resistance genes in pathogens is impossible without a detailed examination of the origin and role of resistance genes in natural environments. [9] This Review discusses the environmental sources of antibiotic resistance, the functions and roles of resistance genes in microbial ecology and the ways by which those genes may be disseminated in response to human antibiotic use. Antibiotics are essential for the treatment of bacterial infections in humans and animals; it is therefore a top priority to preserve their efficacy.[9,10] For decades, clinicians and scientists have called for the prudent use of antibiotics, in an effort to slow the development and epidemic spread of resistance.[9,10]

Prudent use of antibiotics in humans demands that physicians establish that a bacterial infection is responsible for the patient's symptoms before an antibiotic prescription is written.[11] By contrast, in agriculture antibiotics are used in the absence of acute infection.[11] Some of the same antibiotics that are used to treat human pathogens, such as amoxicillin and erythromycin, are also used to treat disease, promote growth and improve feed efficiency in animals.[11] Just as in hospital settings, the agricultural use of antibiotics selects for antibiotic resistance, arguably in a more widely disseminated fashion owing to the farm-wide administration of prophylactic antibiotics in feed and water.[12] Antibiotics from both urban and agricultural sources persist in soil and aquatic environments, and the selective pressure imposed by these compounds may affect the treatment of human diseases.[12,13]As another example, the prophylactic use of antibiotics in fish farms has led to a rise in the number of resistant bacteria.[13] Strikingly, these resistant bacteria can transfer the resistance genes to human pathogens.[14] The selection pressure applied by the antibiotics that are used in clinical and agricultural settings has promoted the evolution and spread of genes that confer resistance, regardless of their origins (FIG. 2).[14] Antibiotic resistance accounts for hundreds of thousands of deaths annually and its projected increase has made the WHO recognize it as a major global health threat (WHO 2014).[15] Conventionally, the struggle against antibiotic resistance development has mainly taken place in clinical, community, and in more recent years also agricultural settings-aiming to reduce transmission and prevent selectionof resistant bacteria during antibiotic treatment.[15]

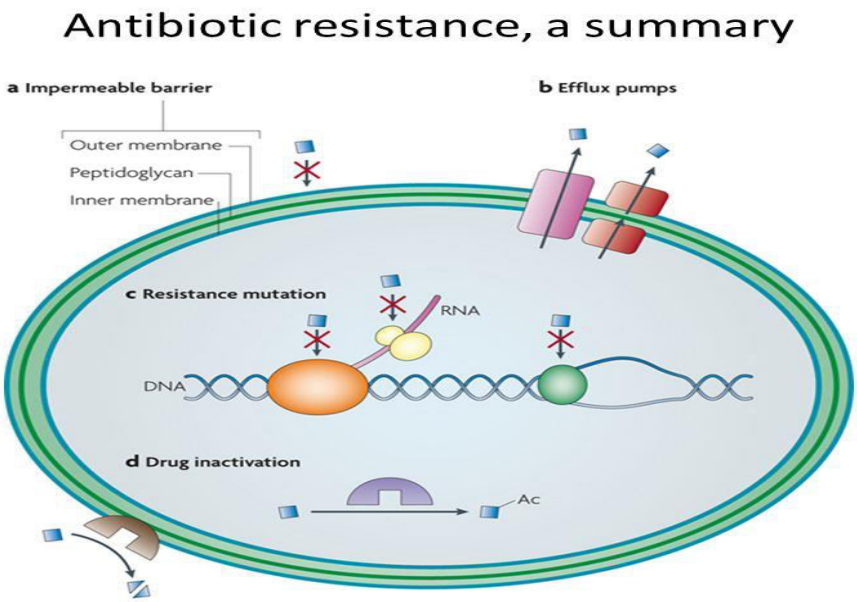

Figure 1 | Mechanisms of antibiotic resistance in a gram-negative bacterium. a | Impermeable barriers. Some bacteria are intrinsically resistant to certain antibiotics (blue squares) simply because they have an impermeable membrane or lack the target of the antibiotic. b | Multidrug resistance efflux pumps. These pumps secrete antibiotics from the cell. Some transporters, such as those of the resistance- nodulation-cell division family (pink), can pump antibiotics directly outside the cell, whereas others, such as those of the major facilitator superfamily (red), secrete them into the periplasm. c | Resistance mutations. These mutations modify the target protein, for example by disabling the antibiotic-binding site but leaving the cellular functionality of the protein intact. Specific examples include mutations in the gyrase (green), which cause resistance to fluroquinolones, in RNA polymerase subunit B (orange), which cause resistance to and in the 30S ribosomal subunit protein $12 (encoded by rpsL) (yellow), which cause resistance to streptomycin. d | Inactivation of the to rifampicin. antibiotic. Inactivation can occur by covalent modification of the antibiotic, such as that catalysed by acetyltransferases (purple) acting on aminoglycoside antibiotics, or by degradation of the antibiotic, such as that catalysed by ß-lactamases (brown) acting on ß-lactam antibiotics. Ac, acetyl group.

Over the past years, the role of the environment as an important source and dissemination route of resistance has been increasingly recognized.[16] This paper aims to conceptualize and define the factors that influence the emergence, mobilization, dissemination and maintenance of antibiotic resistance genes in the environment.[16] We have tried to accommodate both ecological and evolutionary aspects, but without any attempt to fully cover the growing literature on the

environmental dimensions of antibiotic resistance (Fig. 3).[16,17] In order to define those factors, we must first spell out some basic definitions for which ambiguous meanings exist in the literature. In this paper, we follow the operational definition of resistance, which postulates that a strain is resistant against an antibiotic if its minimal inhibitory concentration (MIC) is higher than for the corresponding parental wild-type strain.[17] Accordingly, we define a gene as a 'resistance gene' (or 'resistance factor') when its presence allows a bacterium to withstand a higher antibiotic concentration or when its absence increases susceptibility of the antibiotic, a definition that also includes many non-mobile chromosomal resistance genes. We furthermore define'novel' (or 'new') resistance genes as genes that have not previously been described to have a resistance function, regardless of if they appear in pathogens or not, and regardless of if they appear on the bacterial chromosome or on a mobile genetic element.[18] It is complicated to define ecological emergence and depending on the viewpoint several definitions of when resistance genes emerge are possible.[19] In this paper, we will consider the 'emergence' of a resistance determinant as the event where it first appears in a context where it provides operational resistance.[20] Further, we consider a gene to have undergone 'mobilization' when it appears on a mobile genetic element, such as a plasmid, transposon or integron.[18] As a consequence of these definitions, most emergence and mobilization events will in this view remain undetected at least before they result in a clinical problem, which many will probably never do.[21]

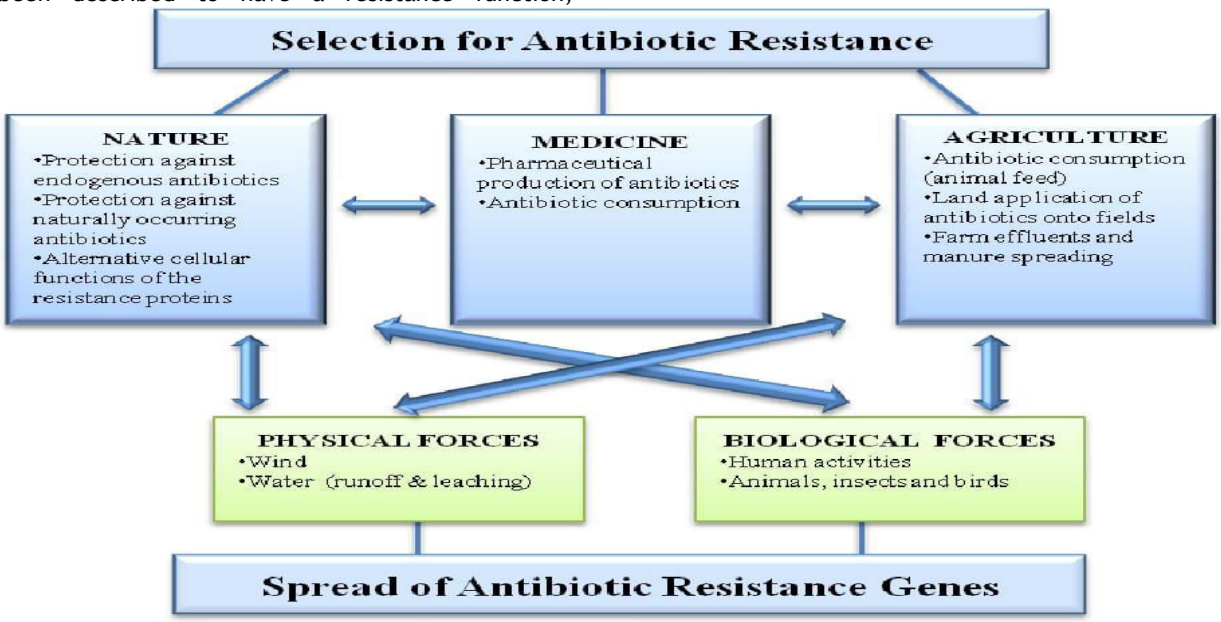

Figure 2 | Sources and movement of antibiotic resistance genes in the environment. Resistance genes exist naturally in the environment owing to a range of selective pressures in nature. Humans have applied additional selective pressure for antibiotic resistance genes because of the large quantities of antibiotics that we produce, consume and apply in medicine and agriculture. Physical and biological forces also cause widespread dissemination of resistance genes throughout many environments.

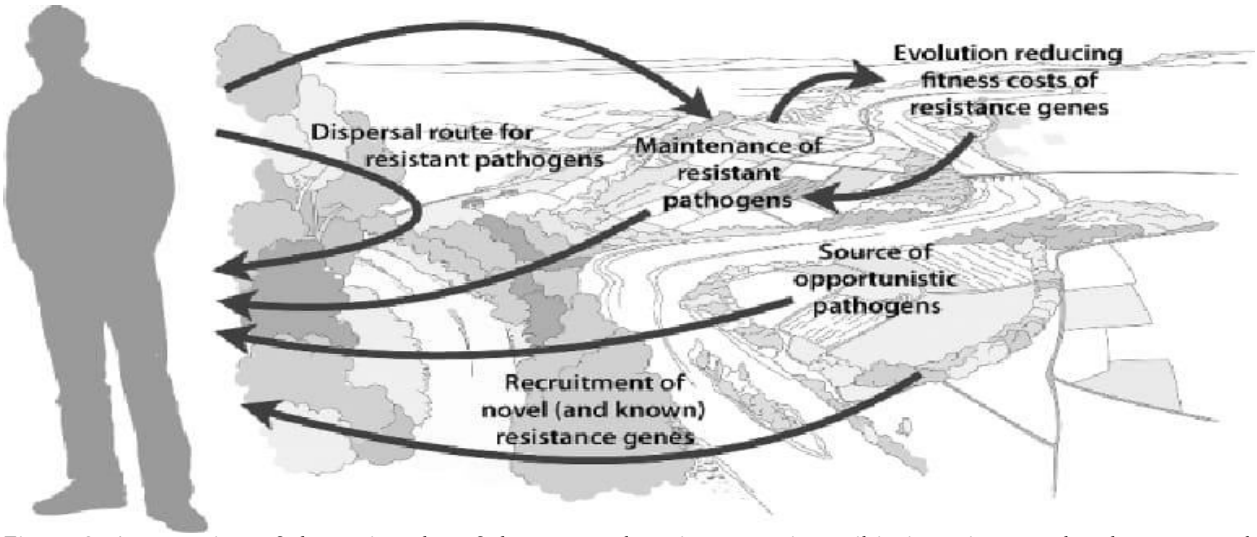

Figure 3. An overview of the main roles of the external environment in antibiotic resistance development and dissemination. Note that none of these processes requires a selection pressure for resistance to operate, although such a selection pressure would facilitate both maintenance and recruitment of resistance genes. Antibiotic exposure may select for resistant bacteria during dispersal if those bacteria are able to grow also in the external environment, which is the case for many opportunistic pathogens. Furthermore, the environment also serves as a source of opportunistic pathogens that are already resistant, or may acquire resistance genes from other human- associated bacteria in or on the human body and then cause resistant infections at a later stage. While both known and novel resistance genes may be recruited from the environmental resistance, the most severe long-term health consequences of such acquisitions are likely when genes not currently present in pathogens are added to their resistance repertoire.

THE ORIGINS OF RESISTANCE GENES

Novel antibiotic resistance factors could potentially emerge anywhere, at any time. The astounding number of bacterial cells on Earth, estimated to around 1030-a thousand billion billion billions, provide an immense genetic variability, and opportunities for mutations, rearrangements and horizontal gene transfer.[22] Thus, new resistance factors likely appear regularly, although we never detect the vast majority of these events.[23] As will be outlined below, there are nevertheless several reasons to why pathogens are not flooded by novel resistance genes.[23] For a start, resistance factors are generally associated with some fitness cost.[24] This cost may be particularly large for genes providing novel resistance functions for a bacterium, as their expression may not be sufficiently fine-tuned and their products may interfere with other cellular functions.[26] Thus, novel resistance genes will be selected against unless there is a relatively strong selection pressure to maintain them.[27] Furthermore, even if such a resistance factor would have a low or negligible fitness cost.[28] It would still be rare, and may therefore not become permanently established in the bacterial population unless there is a positive selection pressure for it.[29] This selection pressure may be weak, but unless it is present the only way by which a novel resistance factor would be retained is through genetic drift.[30]

MOBILIZATION OF RESISTANCE FACTORS

Similar to the emergence of novel resistance factors, resistance genes could be mobilized anywhere but need-unless their fitness costs are negligible-a selection pressure to be kept on a mobile genetic element until they have evolved to present smaller fitness costs to the host. [14] The subsequent question therefore becomes: where are selection pressures strong enough to promote mobilization of chromosomal resistance genes and maintenance of already mobile resistance genes? Resistance factors that have recently been mobilized onto a mobile genetic element are likely to often be associated with increased fitness costs, associated with the burden of keeping multiple copies of the same gene and the difficulty to maintain expression control of the gene on a mobile element.[17] This means that environments allowing sustained longevity of a resistance gene regardless of its cost would be of particular importance for the mobilization of resistance determinants.[18] It is then reasonable to assume that once a mobile resistance gene has gained a foothold in a bacterial community, it can subsequently evolve towards diminished fitness cost.[19]Resistance genes with considerably lower fitness costs may of course be recruited to mobile genetic elements, and sub-inhibitory concentrations of an antibiotic could then suffice to select for carriage of them.[19] It is important to consider that resistance genes have evolved in a context of competition and interaction between different species, some of which may use antibiotics as warfare agents.[21] Thus, both known and novel

resistance determinants can be selected for naturally, if they confer a competitive advantage against antibiotics producers, or allow host bacteria to survive higher concentrations of an antibiotic that they themselves produce. Such natural selection processes contribute to preserving an environmental pool of resistance genes, but only indirectly to further establishment of resistance genes in pathogens, as they are unlikely to drive mobilization of resistance factors. [22]

HORIZONTAL TRANSFER OF RESISTANCE FACTORS

Horizontal gene transfer is central for the spread of novel (and known) resistance genes, as it allows resistance to expand beyond specific clones.[20] This way, gene transfer makes resistance genes available to a much larger part of the bacterial community in a particular environment, often beyond species boundaries.[20]As for the mobilization of resistance factors, transfer of genes between bacteria can in theory occur anywhere.[21] However, for resistance genes to be horizontally transferred from environmental to pathogenic bacteria they need to, at least temporarily, share the same habitat.[21] Furthermore, horizontal gene transfer is much more likely to occur between phylogenetically closely related bacterial.[22] Finally, transfer of genetic material between bacterial cells is induced by stressors such as antibiotics and potentially also metals and biocides subsequently, antibiotic selection also contributes to establishment of transferred resistance genes in their new host.[23] Thus, resistance transfer to pathogens could be expected to be relatively frequent between human-associated bacterial[23], particularly during treatment with antibiotics.[24]In contrast, transfer of resistance genes to pathogens from environmental bacteria, which occupy other habitats and are often less phylogenetically related, would likely be less common, although environmental stressors may induce horizontal gene transfer to and from (opportunistic) human pathogens in environmental settings.[25] This means that once a resistance factor has entered a human pathogen, it is more likely to further spread between commensals and pathogens than being transferred again into another pathogen from environmental bacteria, and in any case the consequences/contribution from the latter type of event would be expected to be small.[25]Moreover, avoiding transfer of resistance between pathogens (as well as evolutionary close commensals) is likely impossible, since they share habitats, often are phylogenetically related, and carriage of mobile resistance factors generally seem to be associated with low fitness costs in pathogens. Somewhat surprisingly, the human microbiome harbors a fairly large number of resistance genes that have not (yet) been transferred to human pathogens as far aswe know.[25]

DISSEMINATION OF RESISTANT BACTERIA

The main route of exposure for humans to resistant pathogens is from other people, either in clinics or through the community setting.[26] Typical dispersal routes here are through body contact or indirect contact transmission, aerosols, and food prepared by persons carrying the pathogen These are also the typical transmission routes for infectious bacteria in general, and interventions preventing circulation of resistant pathogens among humans are essentially the same as those applied to prevent the spread of any bacterial pathogen, most importantly, proper hygiene routines constitute the principal dispersal barrier for resistant pathogens, and the significance of sanitation for preventing the spread of resistant bacteria between humans cannot be overstated. A part from transmission between humans, environmental dissemination routes for resistant bacteria have also been pointed out as potentially important for the spread of antibiotic resistance Again, environments facilitating dissemination of resistant bacteria also enable spread of non-resistant human pathogens, and generally also opportunistic pathogens Thus, sewage, wastewater treatment plants, water bodies and travel, but also air-borne aerosols, dust, and food colonized by bacteria, are important vectors enabling bacterial transmission between hosts through the environment.[29] STPs generally discharge their effluent (which has repeatedly been shown to contain resistance genes; see into water bodies.[30] Water contaminated by STP effluents is often used for irrigation of farmland, for recreational swimming and as drinking water supply (after further treatment).[31] Domestic animals often drink such surface water untreated and may subsequently spread resistant bacteria to humans.[32] However, for the dissemination of resistant bacteria, untreated sewage released into water bodies poses a considerably larger risk than STP effluents, as STPs often reduce the relative abundance of the vast majority of resistance genes, and also lower the total bacterial abundance from ten up to thousand-fold.[32]

EVOLUTIONARY PROCESSES INFLUENCING ENVIRONMENTAL ANTIBIOTIC RESISTANCE

For the long-term maintenance of antibiotic resistance genes in bacterial communities, two parallel evolutionary forces are at play: selection promoting resistance phenotypes, and selection leading to reduction of the fitness costs associated with carrying resistance genes.[32]As discussed earlier, gain and establishment of resistance genes in a bacterial population are largely dependent on a direct antibiotic selection pressure.[33] The selective forces towards maintenance of resistance genes do not only include direct antibiotic selection pressure, however.[34] Even in the absence of a direct selection pressure from an antibiotic, mobile resistance genes may be favored by co-selection by other substances present, such as metals and biocides, as the resistance determinants for some of these compounds can be co-localized to the same mobile genetic elements as antibiotic resistance genes.[35]

The implications of co-selection of antibiotic resistance by metals were recently thoroughly reviewed.[35] Similarly, exposure to other stressors than antibiotics may select for increased expression of genes encoding efflux pumps, which in turn may render bacteria less susceptible to antibiotics as well.[36]Although this may not immediately contribute to the spread of resistance factors, it could contribute to the development of more efficient resistance genes by enabling bacteria to withstand low-level antibiotic exposure for longer times, enabling a recently acquired resistance gene to evolve to be more efficient and less costly within the new host.[37]In addition, resistance genes may be maintained because they confer advantages to the cell even in theabsence of a selection pressure, in essence allowing bacteria to perform intrinsic functions more efficiently when they carry the resistance gene.[37] However, carriage and maintenance of resistance genes usually come with a cost in terms of reduced fitness, although the cost is sometimes small.[38] This cost is (apart from random reduction by genetic drift) the sole factor that acts to reduce the frequency of resistance genes in bacterial populations.[38] Random losses of resistance genes from bacterial cells happen all the time, but seldom result in complete elimination of the gene from the community, which means that once a selection pressure for resistance re-emerges (such as during antibiotic treatment), resistance development of bacterial populations previously subjected to resistance selection can be quick.[39] Selection pressure acting specifically against the carriage of resistance genes is therefore crucial for complete eradication of resistance factors from a community.[39] The fitness costs associated with carrying and expressing genes providing antibiotic resistance are largely dictated by the nature of the resistance mechanism. [39] Bacteria typically become resistant to antibiotics via-

- Upregulation of efflux pumps exporting the substance from the cell,
- Modifications to the cell wall or outer membrane, reducing permeability for the antibiotic substance,
- Expression of degradation enzymes that can render the substance harmless,
- Protection of the molecular target of the antibiotic,
- Alternative means to perform inhibited functions.[39]

Resistance mechanisms associated with efflux pumps and cell wall modifications are often caused by mutations in chromosomal DNA, although many efflux pumps are transferable between bacteria on plasmids. [38] Degradation enzymes, target protection proteins, and enzymes allowing utilization of alternative enzymatic pathways are more likely to be transmissible on mobile genetic elements as they add functions to their carrier rather than modify existing ones. In general, fitness costs associated with the latter three mechanisms are primarily associated with the cost of carrying the resistance plasmid and expressing its. [39]

THE ECOLOGY OF ANTIBIOTIC RESISTANCE DEVELOPMENT

Based on the above reasoning, we propose that four steps are central on the route to clinically important antibiotic resistance: emergence of novel resistance factors, mobilization, transfer to human pathogens, and dissemination. [3] Notably, these steps need not to happen in this particular order; transfer to human pathogens may occur before or after dissemination to the human microbiome, and certain steps in the process may repeat (Fig. 2).[11] The arsenal of resistance factors currently present in pathogens and opportunistic pathogens constitute a set of genes that are at the later stages of this route.[14]A crucial factor for a resistance gene to reach human pathogens is that it is maintained throughout all these steps. Resistance genes with high fitness costs are more likely to be lost in the absence of a selection pressure, particularly if located on a mobile genetic element. [17] Furthermore, a scenario with a constant selection pressure by antibiotics-from the environmental emergence of a resistance gene to its transfer to a human pathogen seems improbable, although one could argue that there are places in the world where this may be possible.[19] Taken together, it seems reasonable that successfully maintained resistance genes have either evolved towards low fitness cost in a mobile context (a sort of evolutionary rescue 35 on the individual gene level), or were associated with low fitness costs from the beginning . [22] Since losses of resistance genes are likely as long as they bestow their carrier with a significant fitness cost, recently mobilized genes that do not provide an obvious fitness advantage are undoubtedly sorted out early from mobile genetic elements such as plasmids.[25] This highlights the importance of environments in which resistance genes provide a strong selective advantage, for example milieus subjected to industrial pollution with antibiotics.[27] Since these environments would also present bacteria with conditions thatfavor increased mutation frequency, one consequence may be that resistance genes could be present in several slightly different variants, all selected for detoxification efficiency, of which only those with a low fitness cost are maintained when the selection pressure is removed (for example, after dispersal of the carrier to a non-polluted environment). [28]

Given how long the dispersal route from initial mobilization to human pathogens generally is for a novel resistance gene, it is not surprising that mobile resistance factors found in pathogens today are terribly hard to eliminate from bacterial populations. [33] This suggests that once a resistance gene is widely spread among human pathogens (or even among human commensals), part of the game is

lost and we are restricted to manage its spread. [37] Mitigation of the spread of resistance factors to human pathogens should therefore ideally take place before they get a foothold in the human microbiome. Thus, detection of resistance determinants in the environment those are not yet widespread among clinical bacteria should be an important component in risk assessment and management of antibiotic resistance. [39]

WHICH ENVIRONMENTS POSE THE MOST PERTINENT RISKS TO HUMAN HEALTH?

Ultimately, the most urgent reason to study antibiotic resistance in the environment is to gain further insights into health risks for humans and domestic animals that often are dependent on effective antibiotics.[5] This knowledge can then ideally be used to design interventions that could prevent or delay the recruitment of resistance factors to pathogens from environmental bacteria and reduce environmental dissemination of resistant pathogens.[8] To identify suitable mitigation strategies, we first need to define what environments and scenarios constitute the most severe risks. [8] This, however, is not completely straightforward. [11] Some have argued that the most severe risk scenarios involve known resistance genes that have previously been reported to reside on mobile genetic elements hosted by human bacterial pathogens. [12] This is a valid argument when such genes are encountered in the human microbiome, but while they are clearly of importance, finding them in environmental bacterial communities is not necessarily indicative of a high-risk situation. [19] Well-known resistance genes present on mobile genetic elements easily spread with human feces, and detection of them in the external environment may be an indication of human fecal contamination. [20]

Table 1. Human health risks associated with environmental antibiotic resistance and examples of risk environments and appropriate mitigations

Risk Scenario	Environments of particularconcern	Possible Mitigations
Emergence and fixation of novel resistance genes Mobilization and transfer of resistance genes to human pathogens Dissemination of resistant bacteria	Human and animal microbiome Intensive aquaculture polluted by Environments discharges from antibiotic manufacturing Environments polluted by discharges from antibiotic manufacturing Sewage Human and animal microbiome Human-to-human contacts, hospitals Contact environmental opportunistic pathogens, directly or through food Animal husbandry Poorly treated sewage	Reduce and optimize antibiotics usage Restrict antibiotics usage Incentivize better control of discharges Incentivize better control of discharges Disinfection of treated sewage and sludge Reduce antibiotics usage, limit transmission of pathogens Hygiene Hygiene, proper handling of fresh produce Reduce antibiotics usage, create transmission barriers between animals, humans and the external environment Improved infrastructure for sewage treatment

CONCLUSION

In this paper, we have attempted to define and formalize relevant ecological and environmental factors for antibiotic resistance development and transmission. [1] We propose that although emergence of novel resistance factors and mobilization of existing ones probably happen continuously, only few of these determinants are selected for and permanently established among bacterial populations.[9] As a consequence, those that do make it to pathogenic species are likely to be evolved towards conveying very little fitness cost to their hosts, and will thus be hard to eliminate from pathogen populations.[23] Successful mitigation strategies for environmental resistance development are therefore in principle limited to-Avoiding

 creation of settings that select for, mobilize and allow persistence of resistance genes in bacterial communities,Reducing dispersal routes for resistant bacteria to the human microbiome, and Limiting the selection pressure for resistant pathogens (i.e.) prudent use of antibiotics for humans and animals). [34] The diversity of resistance genes present in the environment suggests that there are still many more resistance genes available for pathogens to recruit. [36] Resistance genes common among the bacterial populations in the human microbiome are not likely to be eradicated, even in the absence of antibiotics selection.[38] Thus, the mobile resistance genes that are already circulating among human pathogens may easily re-emerge during antibiotics treatment. [39]

Recruitment of additional novel resistance genes into pathogens, on the other hand, has the potential to cause devastating consequences for human health, asmobile resistance genes against new antibiotics, ormore efficient resistance mechanisms against the ones that already face resistance, would further reduce treatment options. [39]

REFERENCES -

1. Mark C Enright. The evolution of a resistant pathogen the case of MRSA. Current Opinion Pharmacology (2003). 3, 474-479.
2. S P Conway , K G Brownlee, M Denton, D G Peckham Antibiotic treatment of multi drugresistant organisms in cystic fibrosis. American Journal. Respiratory Medicine (2003). 2, 321-332.
3. D J Austin , K G Kristinsson, R M Anderson. The relationship between the volume of antimicrobial consumption in human communities and the frequency of resistance. Proceedings of the National Academy of Sciences the United States of America. (1999). 96, 1152-1156.
4. Linda M Weigel , Don B Clewell, Steven R Gill, Nancye C Clark, Linda K McDougal, Susan E Flannagan, James F Kolonay, Jyoti Shetty, George E Killgore, Fred C Tenover. Genetic analysis of a high-level vancomycin-resistant isolate of Staphylococcus aureus. Science(2003) 302, 1569-1571.
5. Christopher M Thomas , Kaare M NielsenMechanisms of, and barriers to, horizontal gene transfer between bacteria. Nature Reviews Microbiology Bacteria have many mechanisms of DNA exchange, which are clearly and thoroughly discussed in this review. (2005),3, 711-721.
6. W Witte, Medical consequences of antibiotic use in agriculture. Science. (1998) 279, 996-997.
7. Frank M Aarestrup, Veterinary drug usage and antimicrobial resistance in bacteria of animal origin. Basic Clinical Pharmacology Toxicology (2005), 96, 271-281.
8. Abigail A Salyers , Anamika Gupta, Yanping Wang, Human intestinal bacteria as reservoirs for antibiotic resistance genes. Trends Microbiology,The complex microbial community of the human gut and the part it plays in gene transfer are comprehensively reviewed in this outstanding article, despite the challenges of drawing conclusions from such a diverse ecosystem., (2004). 12, 412-416
9. Stuart B Levy [1], Thomas F O'Brien. Global antimicrobial resistance alerts and implications. Clinical Infections Diseases.. A concise but powerful message about the worldwide status of antibiotic resistance in pathogens. (2005).41, S219-S220.
10. Julian DaviesMicrobes have the last word. A drastic re-evaluation of antimicrobial treatment is needed to overcome the threat of antibiotic-resistant bacteria European Molecular Biology Organization Reports(2007). 8, 616-621.
11. Bonlawar, J., Setia, A., Challa, R.R., Vallamkonda, B., Mehata, A.K., Vaishali, ,Viswanadh, M.K., Muthu, M.S. (2024). Targeted Nanotheransotics: Integration of Preclinical MRI and CT in the Molecular Imaging and Therapy of Advanced Diseases. Nanotheranostics, 8(3), 401-426. https://doi.org/10.7150/ntno.95791.
12. Pasala, P. K., Rudrapal, M., Challa, R. R., Ahmad, S. F., Vallamkonda, B., & R., R. B. (2024). Anti-Parkinson potential of hesperetin nanoparticles: in vivo and in silico investigations. Natural Product Research, 1-10. https://doi.org/10.1080/14786419.2024.2344740
13. Chakravarthy, P.S.A., Popli, P., Challa, R.R. et al. Bile salts: unlocking the potential as bio-surfactant for enhanced drug absorption. J Nanopart Res 26, 76 (2024). https://doi.org/10.1007/s11051-024-05985-6
14. Setia, A., Vallamkonda, B., Challa, R.R., Mehata, A.K., Badgujar, P., Muthu, M.S. (2024). Herbal Theranostics: Controlled, Targeted Delivery and Imaging of Herbal Molecules. Nanotheranostics, 8(3), 344-379. https://doi.org/10.7150/ntno.94987.
15. Dhamija P, Mehata AK, Tamang R, Bonlawar J, Vaishali, Malik AK, Setia A, Kumar S, Challa RR, Koch B, Muthu MS. Redox-Sensitive Poly(lactic-co-glycolic acid) Nanoparticles of Palbociclib: Development, Ultrasound/Photoacoustic Imaging, and Smart Breast Cancer Therapy. Mol Pharm. 2024 May 5. doi: 10.1021/acs.molpharmaceut.3c01086. Epub ahead of print. PMID: 38706253.
16. Eranti, Bhargav and Mohammed, Nawaz and Singh, Udit Narayan and Peraman, Ramalingam and Challa, Ranadheer Reddy and Vallamkonda, Bhaskar and Ahmad, Sheikh F. and DSNBK, Prasanth and Pasala, Praveen Kumar and Rudrapal, Mithun, A Central Composite Design-Based Targeted Quercetin Nanoliposomal Formulation: Optimization and Cytotoxic Studies on MCF-7 Breast Cancer Cell Lines. Available at SSRN: https://ssrn.com/abstract=4840349 or http://dx.doi.org/10.2139/ssrn.4840349
17. Setia A, Challa RR, Vallamkonda B, Satti P, Mehata AK, Priya V, Kumar S, Muthu MS. Nanomedicine AndNanotheranostics: Special Focus on Imaging of Anticancer Drugs Induced Cardiac Toxicity. Nanotheranostics 2024; 8(4):473-496.

doi:10.7150/ntno.96846.
https://www.ntno.org/v08p0473.htm
18. Pasala, P. K., Rcaghupati, N. K., Yaraguppi, D. A., Challa, R. R., Vallamkond, B., Ahmad, S. F., ... & DSNBK, P. (2024). Potential preventative impact of aloe-emodin nanoparticles on cerebral stroke-associated myocardial injury by targeting myeloperoxidase: In Supporting with In silico and In vivo studies. Heliyon.
19. Randhave, N., Setia, A., Challa, R. R., Vallamkonda, B., Badgujar, P., Verma, N., ... & Muthu, M. S. (2024). Autophagy Targeted Nanomedicines and NanotheranosticsIn Cancer Imaging and Therapy. Journal of Drug Delivery Science and Technology, 105945.
20. Kant, S., Kaur, H., Mishra, A.K. et al. Harmonizing Nature and Technology: Lipidic Nano-phytotherapeutics in the Management of Atopic Dermatitis. Rev. Bras. Farmacogn. (2024). https://doi.org/10.1007/s43450-024-00570-3
21. Vallamkonda, B., Satti, P., Das, D.K. et al. Enantiomeric resolution of three profen drugs using direct thin-layer chromatographic method. JPC-J Planar Chromat (2024). https://doi.org/10.1007/s00764-024-00305-z
22. Suseela, M. N. L., Mehata, A. K., Vallamkonda, B., Gokul, P., Pradhan, A., Pandey, J., ... & Muthu, M. S. (2024). Comparative Evaluation of Liquid-Liquid Extraction and Nanosorbent Extraction for HPLC-PDA Analysis of Cabazitaxel from Rat Plasma. Journal of Pharmaceutical and Biomedical Analysis, 116149. https://doi.org/10.1016/j.jpba.2024.116149
23. Gokul, Patharaj and Sobanaa, Murugesan and S, Hari Krishna Kumar and R, Prathiviraj and Pamanji, Rajesh and Lakshmi Suseela, Medapati Nikitha and Vallamkonda, Bhaskar and Setia, Aseem and Selvin, Joseph and Muthu, Madaswamy S., Decoding Antibiotic Contaminants and Their Impact in Gingee River, Puducherry: Insights from Spe-Uplc-Ms/Ms and Zebrafish Study. Available at SSRN: https://ssrn.com/abstract=4885708 or http://dx.doi.org/10.2139/ssrn.4885708
24. Bhosale, A. P., Pandey, B. S., Singh, S. P., Ojha, M., KalwalaSaritharani, Vallamkonda, B., Lokhande, V., Singh, P., Jain, A. V., & Suraj Mandal. (2024). Improved detection and quantitation of nitrosamine impurities in ophthalmic solutions using LC-MS/MS. African Journal of Biochemistry Sciences, 1212–1225. https://doi.org/10.48047/AFJBS.6.Si4.2024.1212-1225
25. Mishra, S. K., Bhardwaj, K., Mandal, S., Singh, P., Bhaskar Vallamkonda, Yadav, R. K., Jain, A. V., & Dr. Udaybhan Yadav. (2024). Challenges in validating an LC-MS/MS method for MNP quantification in Rifampicin-Containing formulations. African Journal of Biochemistry Research, 1199–1211. https://doi.org/10.48047/AFJBS.6.Si4.2024.1199-1211
26. Savita Sambhaji Patil (Pol). (2024). Development and validation of a High-Throughput Method for NDMA Quantitation in Drug products using Headspace–SIFT-MS. In African Journal of Bio-Sciences (Vol. 6, Issue 2, pp. 1542–1555). African Journal of Bio-Sciences. https://doi.org/10.48047/AFJBS.6.2.2024.1542-1555
27. Frauke Mattner , Franz-C Bange, Elisabeth Meyer, Harald Seifert, Thomas A Wichelhaus, Iris F Chaberny. Preventing the spread of multidrug-resistant gram-negative pathogens: recommendations of an expert panel of the German Society For Hygiene and Microbiology. DeutschesArzteblattInteernational Journal 2012; 109:39-45.
28. Bruce R Levin, Fernando Baquero, Pål J Johnsen. A model-guided analysis and perspective on the evolution and epidemiology of antibiotic resistance and its future. Current Opinion Microbiology 2014; 19C:83-89.
29. Johan Bengtsson-Palme , D G Joakim Larsson , Erik Kristiansson. Using metagenomics to investigate human and environmental resistomes. Jounal of Antimicrobial Chemothearapy 2017, DOI: 10.1093/jac/dkx199.
30. Antti Karkman, Timothy A. Johnson, Christina Lyra, Robert D. Stedtfeld, Manu Tamminen, James M. Tiedje, Marko Virta. High-throughput quantification of antibiotic resistance genes from an urban wastewater treatment plant. Frderation of European Medical Sciences Microbiology Ecology 2016, DOI: 10.1093/femsec/fiw014.
31. Sara Hernando-Amado,Fernando Sanz-García,PaulaBlanco,José L. Martínez,. Fitness costs associated with the acquisition of antibiotic resistance. Essays Biochemistry 2017; 61:37-48.
32. Chandan Pal , Johan Bengtsson-Palme , Erik Kristiansson ,D G Joakim Larsson . Co-occurrence of resistance genes to antibiotics, biocides and metals reveals novel insights into their co-selection potential. BMC Genomics 2015; 16:964-69.
33. B R Levin , M Lipsitch, V Perrot, S Schrag, R Antia, L Simonsen, N M Walker, F M

Stewart. The population genetics of antibiotic resistance. Clinical Infecious Diseases 1997; 24(Supply 1):S9-16.
34. Mohsen Arzanlou , Wern Chern Chai , Henrietta Venter. Intrinsic, adaptive and acquired antimicrobial resistance in Gram- negative bacteria. Essays Biochemistry 2017; 61:49-59.
35. Andrew Gonzalez, Ophélie Ronce, Regis Ferriere, and Michael E. Hochberg. *Evolutionary rescue: an emerging focus at the intersection between ecology and evolution. Philosophical Transactions of Royal Society B-Journals 2013; 368:20120404.*
36. Fernando Baquero ,Ana P. Tedim ,Teresa M. Coque. *Antibiotic resistance shaping multi-level population biology of bacteria. Frontiers Microbiology 2013, 4:15-19.*
37. Johan Bengtsson-Palme , D G Joakim Larsson. Antibiotic resistance genes in the environment: prioritizing risks. National Reviews of Microbiology 2015; 13:396-402
38. José L Martínez , Teresa M Coque , Fernando Baquero. *What is a resistance gene? Ranking risk in resistomes. National Reviews of Microbiology 2015; 13:116-23.*
39. Johan Bengtsson-Palme , D G Joakim Larsson , Erik Kristiansson, Using metagenomics to investigate human and environmental resistomes. Journal of Antimicrobial Chemotherapy 2017, DOI: 10.1093/jac/dkx199.