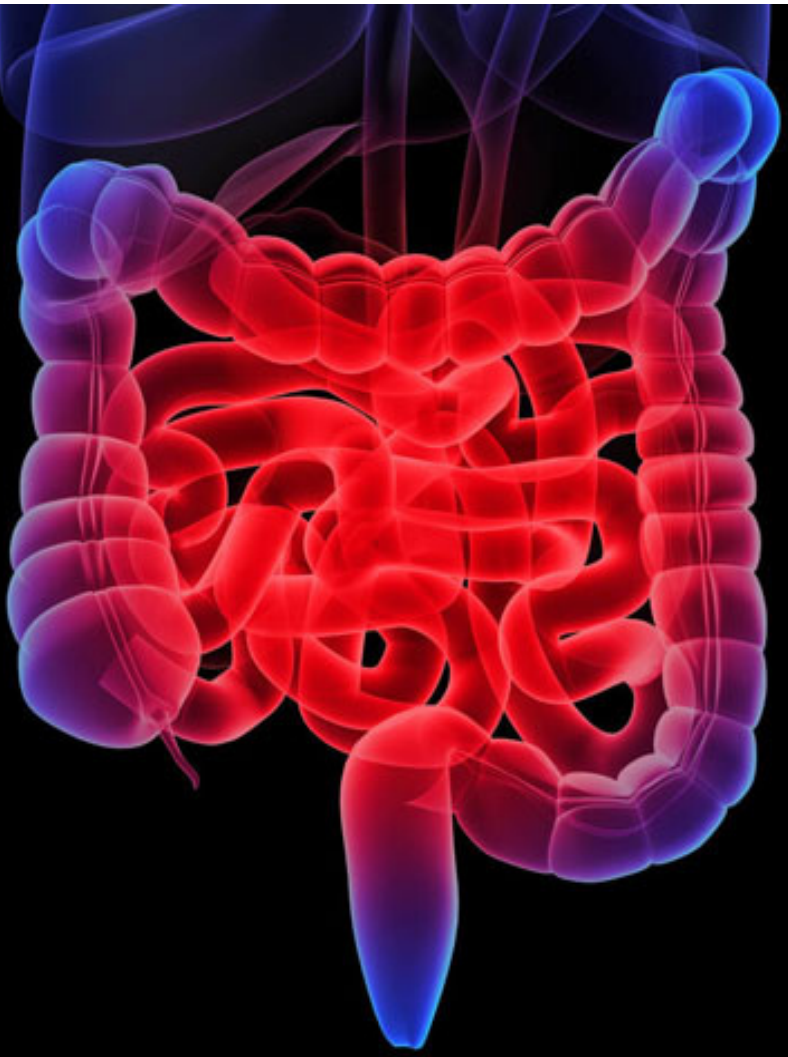

INFLAMMATORY BOWEL DISEASE

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Emergence of antibiotic resistance in bacteria

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Introduction

Antibiotic Resistant Bacteria (ARB) occurrence and spread is causing problems related to public health all over the world and therefore is a reason of concern for the public [1]. Bacteria inherit resistance or develop resistance to some antibiotics through spontaneous mutation or incorporation of resistant genes [2]. Which are normally associated with mobile elements such as integrons, transposons and plasmids.

Most common and easiest way for bacteria to develop resistance for antibiotics in both environment and host is incorporation of resistant gene through the process of horizontal gene transfer [3]. In this process, the genetic material is incorporated by an organism from another organism without being its progeny [4]. The main biochemical mechanisms of antibiotic resistance involves inactivation of antibiotics, changes in membrane permeability, modification of antibiotic target molecule or survival by alternative “bypass” metabolic pathway [5].

Environmental consequences due to excessive use and release of antibiotics from various sources [6] such as human and animal wastes, surface waters receiving lands runoff, polluted estuaries [7], contaminated water supplies, human sewage [8] and medicine industries [9] favours the development of resistance for antibiotics in coliform and fecal coliform populations.

Moreover, the surplus antibiotic use has led progressive adaptation of bacteria to combat powerful drugs. Bacterial strains that are resistant for antibiotics spread in the environment through air as well as water and may affect human health. Collection of all genes that directly or indirectly imparts antibiotic resistance is known as environmental resistome [10]. Various studies provide evidence for existence of resistome in environmental strains for example, class A β-lactamases have been estimated to evolve approximately 3.4 billion years ago. Environmental reservoirs of resistance genes “Resistome” are changing due to anthropogenic activities and increasing the possibility of resistance genes development into pathogens which are clinically important. Several antibiotic resistance genes have been detected from natural water bodies as well as affluent samples as shown in table-1.

Table 1: Antibiotic Resistance Genes from Environmental Sources.

Antibiotic resistance	Antibiotic resistance genes	Source of isolation
Tetracycline resistance genes	tetC,tetH, tetM, tetO, tetQ, tetW ,tetZ	lagoons and groundwater
Tetracycline resistance genes	tetA	drinking water samples
Ciprofloxacin resistance gene	gyrA	drinking water samples
Methicillin resistance gene	mecA	drinking water samples
Macrolide resistance	mef A	Groundwater
Ampicillin resistance	ampC	artificial recharge systems of groundwater
Vancomycin resistance	vanA	municipal wastewater

Koike et al [11], Soge et al [12], Volkmann et al [13], Bockelmann et al [14]

Antibiotics

Antibiotics are the compounds produced by microorganisms and capable of inhibiting bacterial growth. Synthetic or semi-synthetic drugs used in treatment of bacterial infections are also termed antibiotics.

An antibiotic inhibits certain essential processes of bacterial cells or metabolism. Recognition of target site and the antibiotic concentration at target is important for achieving efficient inhibition of bacterial activity [15]. Antibiotics are classified into five major classes on basis of their mode of action shown in (Figure 1).

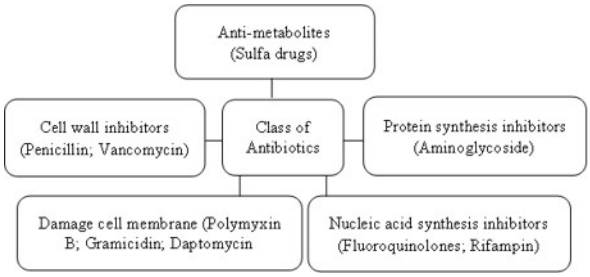

Figure 1: Classes of antibiotics on basis of their mode of action.

Antibiotic resistant bacterial pathogens

The use of antimicrobial agents has lead to more efficient treatment processes against various diseases but has simultaneously also lead to the emergence of new threat to the world. Studies have shown that the use of antibiotics have lead to emergence of antibiotic resistant bacterial pathogens. Antibiotic resistance is a form of drug resistance where microorganisms usually species of bacteria are able to survive even after exposing them to one or more antibiotics. Multiple antibiotic resistant pathogens are termed as Multidrug Resistant (MDR) or superbugs [16]. Which carry several resistant genes.

Global emergence of antibiotic resistant bacteria and the resistant genes has become a major health issue [17]. Resistance to antibiotics has lead to higher morbidity, mortality and has imposed huge cost on the society [18]. Over the years, development of resistance to one or more clinical antibiotics has been reported in nearly every bacterial pathogen [19]. Extended Spectrum Beta-Lactamase (ESBL), Multidrug-Resistant *A. Baumannii* (MRAB), Vancomycin-Resistant *S. aureus* (VRSA), Vancomycin-Intermediate *S. aureus* (VISA), and Vancomycin-Resistant *Enterococcus* (VRE) are the most commonly used acronyms for describing the causative agents or the infection.

Antibiotic resistance genes (ARG)

Several studies reported that the resistance genes obtained from human pathogens have distinct functions in their original host [20,21]. It has been assumed that microorganisms producing antibiotics does not have its activity on them but may function as a resistance genes when transferred to human pathogens. It had been also observed that some genes do not show resistance in the hosts when expressed in low level but can confer resistance when found in plasmids with high copy number, also strong promoter increases their expression [22]. Gene not developing in its original host for conferring resistance can be appropriate for showing resistance once it is transmitted to human pathogen [23]. Depending on the specificity of substrates, an efflux pump or an enzyme confers resistance to antibiotics.

Human Pathogens Have Gained Resistance Genes from Environmental Bacteria

Plasmid studies of pre and post antibiotic periods have shown that resistance genes which were initially absent in human pathogens are acquired by bacteria [24]. The Shewanella algae, carries quinolone resistance genes (qnrA) on its chromosome and blaCTX-M from Kluyvera [25] this provides the confirmation that human pathogens have gained resistance genes from environment bacteria. Resistant bacteria in animals enter into the environment by excretion and secretion of materials such as milk, urine, saliva, semen, faeces etc. Most common human pathogens such as *Enterobacter faecium*, other *Enterobacter species*, *Pseudomonas aeruginosa, Escherichia coli, Klebsiella pneumonia, Staphylococcus aureus* and *Acinetobacter baumannii* have shown resistance to antibiotics [26]. More than 80% species of *Shigella*, *Salmonella* and *Campylobacter* have shown resistance to trimethoprim-sulfamethoxazole [27].

Acinetobacter, a gram negative bacteria have been reported to be most resistant bacteria to control and treat [28]. *A. baumannii* have been considered multi-drug resistant bacteria possessing all resistance mechanisms. Most resistance genes which have been obtained from *Salmonella sp., Pseudomonas sp*. and *E. coli* and mobile gene elements have been detected in chromosome of *A. baumannii* [29].

Drug resistance in pathogens during therapies

Disease causing agents have now developed resistance to the antibiotics used in therapies [30]. There are several reports available in history providing evidence for initial development of drug resistant strains soon after the drug introduction for treatment. These involve streptomycin resistant *Mycobacterium tu-*

berculosis [31], Multiple Drug Resistant (MDR) enteric bacteria such as *E. coli, Shigella* and *Salmonella* [32], *ampicillin resistant Haemophilus influenza* as well as *Neisseria gonorrhoeae*, chloramphenicol and tetracycline resistant *Haemophilus*. Such resistant strains not only possess severe clinical problems but also cost lives especially in developing countries. MDR enteric disease agents such as *Vibrio cholerae*, *Salmonella enteritidis* and *Shigella flexneri* pose a threat on public health in developing countries [33]. Even in developed countries like United Kingdom and United States, 40-60% strains of noscomial *S. aureus* are methicillin resistant and are also MDR. Several resistances for antibiotics in bacteria are documented in (Table 2).

Table 2: Antibiotic Resistance in Enteric Pathogens Determined by Disc Diffusion.

Bacteria	Resistance for antibiotics
Aeromona sp.	Erythromycin, Penicillin, Vancomycin
Bacillus sp.	Penicillin, Erythromycin
Campylobacter sp.	Ampicillin, Erythromycin
Corynebacterium	Penicillin, Vancomycin
Clostridium difficile	Clindamycin, Ciprofloxacin, Levofloxacin.
Enterococcus sp.	Penicillin, Cotrimoxazole
E. coli	Ampicillin, Ciprofloxacin, Nalidixic acid, Tetracycline, Chloramphenicol, Streptomycin, Co-trimoxazole, Gentamycin, Trimethoprin/ Sulphamethoxazole, Norfloxacin,
Klebsiella pneumonia	Carbapenem
Lactobacillus sp.	Chloramphenicol, Cotrimoxazole, Penicillin, Vancomycin
Salmonella typhi and S. paratyphi A	Ciprofloxacin
Shigella sp.	Sulfonamide, Tetracycline, Chloramphenicol, Ampicillin, Streptomycin, Trimethoprin/ Sulphamethoxazole
Streptococcus pneumonia	Cefotaxime, Erythromycin, Chloramphenicol, Trimethoprim/ Sulphamethoxazole
Vibrio cholerae	Chloramphenicol, Nalidixic acid, Trimethoprin/ Sulphamethoxazole.

Sharma and Thapaliya [34], Michaud et al. [35], Alzahrani and Gherbawy [36], Sang et al. [37], Arnold et al. [38], Kapil et al. [39], Sang et al. [40], Lalitha et al. [41]

Development of antibiotic resistance

Resistance for antibiotics can develop by mutations [42] or by procurement of resistant genes through Horizontal Gene Transfer (HGT) [43]. The acquisition of a resistant gene via horizontal gene transfer is the most common and the easiest way for bacteria to develop resistance for antibiotics both in the host and environment [44].

Mutation in target coding genes and proteins activating pre-antibiotics may confer resistance. Resistance genes are developed when directly transferred to all bacterial progeny through DNA Replication is termed as vertical gene transfer or vertical evolution. These mechanisms are considered passive mechanism of resistance and do not affect the active antibiotic resistance mechanism.

Active resistance, the major mechanism of antimicrobial resistance, is the result of an evolutionary pressure to develop a mechanism against an antimicrobial agent so that the bacterial populations formerly sensitive to antimicrobials can become resistant. This type of resistance results from changes in the bacterial genome. Microorganisms exhibit resistance to antimicrobials through drug inactivation or modification, alteration of target site, alteration of metabolic pathway and reduced drug accumulation [5]. Major process responsible for spread of acquired resistance through mutation is by horizontal gene transfer or by clonal expansion [45].

Lateral or Horizontal Gene Transfer (HGT) is a process where genetic material can be transferred between bacteria of the same or different species [46]. Genetic exchange in bacteria through HGT can occur through three processes which include transduction, transformation or conjugation.

Multidrug resistance (MDR) in drinking water

Multidrug resistance cases and deaths of typhoid fever due to municipal water consumption were reported in Tajikistan [47]. Also infections due to multidrug resistant *Salmonella typhi* were reported in Nepal due to consumption of non-chlorinated water. Infections can only be controlled or eliminated when safe drinking water is provided to the public [48]. Heavy metals also generate a common source of antibiotic Resistance bacteria in small numbers [49].

The MDR in drinking water is a main concern in both developed and developing countries as this is a major route for bacterial transmission to the people. Excessive use of antibiotics has resulted in development of resistant bacteria or gene, which spread between humans throughout the world such as New Delhi metallo-beta-lactamase-1 (NDM-1) resistance [50]. The NDM-1 gene present in chlorinated municipal drinking water in India is a major health concern [51]. This gene has been identified in pathogenic bacteria such as *Shigella boydii, V. Cholera* and *A. caviae* and show resistance for all types of β-lactams, including carbapenems. It coexists with vectors, which transfers genes, transmits rapid and simultaneous resistance to important clinical antibiotics and thus is of major concern to public health.

Incomplete metabolism of antibiotics in humans or disposal of unused antibiotics results in release of antibiotics in large amounts into municipal wastewater, which finally enters into natural environment compartments [52]. Antibiotic resistance genes and resistant bacteria are released into streams through wastewater effluents [53], where these ARGs rebound during sequential procedure for treatment.

Main causes of development of antibiotic resistance in bacteria

Overuse and widespread use of antibiotics (evolutionary pressure) has resulted in bacteria to become more resistant. Improper clinical practices and procedures followed during drug treatment, selling of antibiotics without prescription and unsound practices in pharmaceutical manufacturing industry contribute in creating antibiotic resistant strains [54].

Antibiotics are useless against viruses but are most commonly prescribed for common cold. It has been observed that poor hand hygiene by hospital staff is associated with spread of resistance in organisms [55] as decrease in rate of AR resistant organisms had been observed with increase in hand washing [56].

In United States, in the year 1997, of the total antibiotics used half was used in animals and half in humans however in year 2013, up to 80% of antibiotics are used mainly for animals [57]. Fluoroquinolones, macrolides and cephalosporin is of generation third and fourth requires urgent risk management for their usage in food animals [58].

Industrialization of antibiotic production has increased the abundance of resistance genes. Antibiotics up to millions of tones have been released into the environment due to industrialization through wastewater effluent, aquaculture, land application of animal wastes and crop disease treatment. Certain drug making centres have been identified as "hot spots" that release antibiotics, antibiotic resistant bacteria as well as antibiotic resistance genes in high levels into ground, surface and drinking water. In sewage sludge and treated effluents, antibiotics concentration ranges from ng/µL to µg/L. Minimum inhibitory concentration of antibiotics in range of mg/L has been detected in treated wastewater from drug manufacturing [59] and µg/L range in untreated hospital effluents. Hospital effluents and wastewater treatment are "hot spots" for enhancement and transference of resistant bacteria [60].

Antibiotics use for raising food animals at high density have drawn special attention towards many public health agencies due to increase in resistance and decrease in water quality. Some antibiotics like penicillin and tetracycline are used in both humans and animals. Antibiotics are used for therapeutic, prophylactic, metaphylactic and for growth promotion. As upto 70 percent of antibiotics are provided to animals in farms not for treating disease but to promote growth [61]. Use of antibiotics in aquaculture has resulted in increase in food production and treatment of fish infection by adding antibiotics directly in water resulting in decrease in water quality.

It was stated early that antibiotics have suppressive role in natural ecosystems [62]. Several studies have suggested that antibiotics at low concentrations in natural ecosystems may have other functions such as cell to cell signaling and not necessarily related with killing of competitors [63]. There has been evident reports which highlights the role of genes acquired by human pathogens are behaving as resistance genes but have different functions initially in their original hosts. The antibiotic production and their release in the environment at high levels might have resulted in change in their functional role.

Controlling environmental antibiotic resistant genes (ARGs) and antibiotic resistant bacteria (ARB)

Antibiotics have been used as growth promoters for animal production. However, limiting their use for this purpose would directly help in controlling environment ARGs and ARB. Erythromycin resistance in Enterococcus faecium was decreased upto 47% from 90% in pigs, 13% from 76% in danish broiler chickens during 1997-2000 period demonstrating the role of regulations in reversing the resistance for antibiotics in food animals [64]. Good nutrition and low density of animals, immunization of animals in farms and fishes with low cost vaccines would further limit the need for antibiotics. In Norway Salmon vaccines were adopted between 1987 and 2007 which resulted in reduction of upto 99% use of antimicrobial agents and rise in production of fishes [65]. Control on antibiotics usage in medical and agriculture will reduce the AR genes spread in environment. Ban on certain antibiotics in Scandinavia has led to lower prevalence of antibiotic resistance in populations of animal bacteria [66]. According to World Health Organization (2013) anti-microbial resistance is a complicated problem which is controlled by several interconnected factors therefore coordinated action are required to control them. Local surveillance data on occurrence and antibiotic type used, ARB and ARG in aquatic systems and soil related disease information will help in risk assessment and management [67]. Risk management approach should be followed to minimize resistant bacteria in animal facilities and aquaculture. Manure digestion and composting methods should be used for degradation of residual antibiotics in animal manure [68]. Role of natural environment in transmission of antibiotic resistance must be considered especially in transmission to human pathogens.

The World Health Organization (WHO) considers AR to be the greatest threat to the development, food security and global health and thus management of this problem is on a high priority worldwide. A global action plan was formulated for the effective prevention and treatment of infectious diseases using safe and effective remedies with five strategic objectives by the WHO .These objectives include the spread of proper understanding about antimicrobial resistance and to improve research and surveillance at all levels with sustainable investment and global support [69].

As a global challenge for world health the economic burden on all the countries on the issue have been realized by World Economic Forum [70] and World Bank [71] with the report stating a potential economic impact on GDP and a threat to our economic future.

Conclusion

The understanding of the extensive spread of antibiotic resistance in pathogenic bacteria and the resulting ineffectively of treatments procedures, subsequent death toll and economic considerations is a matter of urgent concern and action. All the stakeholders who are directly or indirectly involved in manufacture and use of antibiotics for human or animal must take outmost caution and care while managing antibiotics with optimal use and appropriate disposal.

References

1. Schwartz T, Kohnen W, Jansen B, Obst U. Detection of antibiotic- resistant bacteria and their resistance genes in wastewater, surface water, and drinking water biofilms. FEMS Microbiol Ecol. 2003; 43: 325-335.
2. Tenover FC. Mechanisms of antimicrobial resistance in bacteria. Am J Med. 2006; 119: 3-10.
3. Salyers AA, Gupta A, Wang Y. Human intestinal bacteria as reservoirs for antibiotic resistance genes. Trends Microbiol. 2004; 12; 412-416.
4. Kemboi WK, Raphael W, Ramesh F. Horizontal gene transfer of drug resistance genes between Salmonella and Escherichia coli. Int J Bioassays. 2014; 3: 3066-3072.
5. Kapoor G, Saigal S, Elongavan A. Action and resistance mechanisms of antibiotics: A guide for clinicians. Journal of Anaesthesiology Clinical Pharmacology. 2017; 33. 300-305.
6. Kummerer K. Antibiotics in the aquatic environment-a review-part I. Chemosphere. 2009; 75: 417-434.
7. Samra ZQ, Naseem M, Khan SJ, Dar N, Athar MA. PCR targeting of antibiotic resistant bacteria in public drinking water of Lahore, Metropolitan, Pakistan. Biomed. Environ Sci. 2009; 22: 458-463.

8. Dolejska M, Frolkova P, Florek M, Jamborova I, Purgert M, et al. CTX-M-15- producing Escherichia coli clone B2-025b-ST131 and Klebsiella spp. isolates in municipal wastewater treatment plant effluents. J Antimicrob Chemother. 2011; 66: 2784-2790.

9. Larsson DGJ, de Pedro C, Paxeus N. Effluent from drug manufactures contains extremely high levels of pharmaceuticals. J Hazard Mater. 2007; 148: 751-755.

10. Perry JA, Wright GD. The antibiotic resistance "mobilome": searching for the link between environment and clinic. Front Microbiol. 2013; 4: 138.

11. Koike S, Krapac IG, Oliver HD, Yannarell AC, Chee-Sanford JC, et al. Monitoring and source tracking of tetracycline resistance genes in lagoons and groundwater adjacent to swine production facilities over a 3-year period. Appl Environ Microbiol. 2007; 73: 4813-4823.

12. Soge OO, Michael A, Giardino AM, Lana C, Lvanova IC, et al. Low prevalence of antibiotic resistant gram-negative bacteria isolated from rural-south western Ugandan groundwater. Water SA. 2009; 35: 343-348.

13. Volkmann H, Schwartz T, Bischoff P, Kirchen S, Obst U. Detection of clinically relevant antibiotic-resistance genes in municipal wastewater using real-time PCR (TaqMan). J Microbiol Methods. 2004; 56: 277-286.

14. Bockelmann U, Dorries H, Ayuso-Gabella MN, de Marcay MS, Tandoi V. Quantitative PCR monitoring of antibiotic resistance genes and bacterial pathogens in three European artificial groundwater recharge systems. Appl Environ Microbiol. 2009; 75: 154-163.

15. Martinez JL, Baquero F. Emergence and spread of antibiotic resistance: setting a parameter space. Ups J Med Sci. 2014; 119: 68-77.

16. CDC. Commercially bottled water. Centers for disease control and prevention, Atlanta. USA. 2014.

17. Munir M, Wong K, Xagoraraki I. Release of antibiotic resistant bacteria and genes in the effluent and bio solids of five wastewater utilities in Michigan. Water Res. 2011; 45: 681-693.

18. Ashbolt NJ, Amezquita A, Backhaus T, Borriello P, Brandt KK, et al. Human Health Risk Assessment (HHRA) for environmental development and transfer of antibiotic resistance. Environ. Health Perspect. 2013; 121: 993-1001.

19. Todar K. Bacterial Resistance to Antibiotics. Todar's Online textbook of Bacteriology. University of Wisconsin-Madison. 2006.

20. Martinez JL. The role of natural environments in the evolution of resistance traits in pathogenic bacteria. Proc Biol Sci. 2009; 276: 2521-2530.

21. Martinez JL, Fajardo A, Garmendia L, Hernandez A, Linares JF, et al. A global view of antibiotic resistance. FEMS Microbiol Rev. 2009; 33: 44-65.

22. Dantas G, Sommer MO. Context matters-the complex interplay between resistome genotypes and resistance phenotypes. Curr Opin Microbiol. 2012; 15: 577-582.

23. Baquero F, Alvarez-Ortega C, Martinez JL. Ecology and evolution of antibiotic resistance. Environ Microbiol Rep. 2009; 1: 469-76.

24. Datta N, Hughes VM. Plasmids of the same Inc groups in Enterobacteria before and after the medical use of antibiotics. Nature. 1983; 306: 616-617.

25. Canton R, Gonzalez-Alba JM, Galan JC. CTX-M enzymes: origin and diffusion. Front Microbiol. 2012; 3: 110.

26. Boucher HW, Talbot GH, Bradley JS, Edwards JE, Gilbert D, et al. Bad bugs, no drugs: no ESKAPE! An update from the infectious diseases society of America. Clin Infect Dis. 2009; 48: 1-12.

27. Gordillo ME, Singh KV, Murray BE. In vitro activity of azithromycin against bacterial enteric pathogens. Antimicrob Agents Chemother. 1993; 37: 1203-1205.

28. Maragakis LL, Perl TM. Acinetobacter baumannii: epidemiology, antimicrobial resistance, and treatment options. Clin Infect Dis. 2008; 46: 1254-1263.

29. Fournier P, Vallenet D, Barbe V, Audic S, Ogata H, et al. Comparative genomics of multidrug resistance in Acinetobacter baumannii. PLoS Genet. 2006; 2: 7.

30. Adegoke AA, Mvuyo T, Anthony O, Steve J. Studies on multiple antibiotic resistant bacterial isolated from surgical site infection. Sci Res Essays. 2010; 5: 3876-3881.

31. Rodrigues OD, Cezario CR, Filho GPP. Ventilator-Associated Pneumonia (VAP) caused by Multidrug-Resistant (MDR) Pseudomonas aeruginosa vs. other microorganisms at an adult clinical-surgical intensive care unit in a Brazilian University Hospital: Risk factors and outcomes. Int J Med Med Sci. 2009; 1: 432-437.

32. Subha A, Devi RV, Ananthan S. AmpC b-lactamase producing multidrug resistant strains of Klebsiella spp. and Escherichia coli isolated from children under five in Chennai. Indian J Med Res. 2003; 177: 13-18.

33. Welch JT, Fricke FW, Patrick F, McDermott FP, White GD, et al. Multiple antimicrobial resistance in plague: An emerging public health risk. PLoS ONE. 2007; 2: 309.

34. Sharma M, Thapaliya HP. Antibiotic profiling of heavy metal resistant bacterial isolates from the effluent of a garment industry in Lalitpur. Nepal Our Nature. 2009; 7: 203-206.

35. Michaud Bourgault A, Nguyen T, Frenette C, Kelly M, Vibien A. A predominantly clonal multi-institutional outbreak of Clostridium difficile. 2005; 12: 353.

36. Alzahrani AM, Gherbawy YA. Antibiotic resistance in Escherichia coli strains isolated from water springs in Al-Ahsa region. Afr J Microbiol Res. 2011; 5: 123-130.

37. Sang WK, Oundo V, Schnabel D. Prevalence and antibiotic resistance of bacterial pathogens isolated from childhood diarrhea in four provinces of Kenya. J Infect Dev Ctries. 2012; 6: 572-578.

38. Arnold RS, Thom KA, Sharma S, Phillips M, Johnson JK, et al. Emergence of Klebsiella pneumoniae Carbapenemase (KPC)-Producing Bacteria. South Med J 2011; 104: 40-45.

39. Kapil A, Renuka K, Das BK. Nalidixic acid susceptibility test to screen ciprofloxacin resistance in Salmonella typhi. Indian J Med Res. 2002; 115: 49-54.

40. Sang WK, Oundo V, Schnabel D. Prevalence and antibiotic resistance of bacterial pathogens isolated from childhood diarrhea in four provinces of Kenya. J Infect Dev Ctries. 2012; 6: 572-578.

41. Lalitha MK, Pai R, Manoharan A, Appelbaum PC. Multidrug-resistant Streptococcus pneumonia from India. The Lancet. 2002; 359: 445.

42. Gillings MR, Stokes HW. Are humans increasing bacterial evolvability?. Trends Ecol Evol. 2012; 27: 346-352.

43. Boto L, Martinez JL. Ecological and temporal constraints in the evolution of bacterial genomes. Genes. 2011; 2: 804-828.

44. Salyers AA, Gupta A, Wang Y. Human intestinal bacteria as reservoirs for antibiotic resistance genes. Trends Microbiol. 2004; 12: 412-416.

45. Baquero F, Coque TM, Canton R. Allodemics. Lancet Infect Dis. 2002; 2: 591-592.

46. Kemboi WK, Raphael W, Ramesh F. Horizontal gene transfer of drug resistance genes between Salmonella and Escherichia coli. Int J Bioassays. 2014; 3: 3066-3072.

47. Mermin JH, Villar R, Carpenter J, Roberts L, Samaridden A, et al. A massive epidemic of multidrug-resistant typhoid fever in Tajikistan associated with consumption of municipal water. J Infect Dis. 1999; 179: 1416-1422.

48. Forsberg KJ, Reyes A, Wang B, Selleck EM, Sommer MO, et al. The shared antibiotic resistome of soil bacteria and human pathogens. Science. 2012; 337: 1107-1111.

49. Sieler C, Berendonk TU. Heavy metal driven co-selection of antibiotic resistance in soil and water bodies impacted by agriculture and aquaculture. Front Microbiol. 2012; 3: 399.

50. Wilson ME, Chen LH. NDM-1 and the role of travel in its dissemination. Curr Infect Dis Rep. 2012; 14: 213-226.

51. Walsh TR, Weeks J, Livermore DM, Toleman MA. Dissemination of NDM-1 positive bacteria in the New Delhi environment and its implications for human health: an environmental point prevalence study. Lancet Infect. Dis. 2011; 11: 355-362.

52. Nagulapally SR, Ahmad A, Henry A, Marchin GL, Zurek L, et al. Occurrence of ciprofloxacin, trimethoprim, sulfamethoxazole and vancomycin resistant bacteria in a municipal wastewater treatment plant. Water Environ Res. 2009; 81: 82-90.

53. Zhang Y, Marrs CF, Simon C, Xi C. Wastewater treatment contributes to selective increase of antibiotic resistance among Acinetobacter spp. Sci Total Environ. 2009; 407: 3702-3706.

54. Larsson DGJ, Fick J. Transparency throughout the production chain-a way to reduce pollution from the manufacturing of pharmaceuticals?. Regul Toxicol Pharmacol. 2009; 53: 161-163.

55. Girou E, Legrand P, Soing-Altrach S, Lemire, A, Poulain C, et al. Association between hand hygiene compliance and methicillin-resistant Staphylococcus aureus prevalence in a French rehabilitation hospital. Infect Control Hosp Epidemiol. 2006; 27: 1128-1130.

56. Swoboda SM, Earsing K, Strauss K, Lane S, Lipsett PA. Electronic monitoring and voice prompts improve hand hygiene and decrease nosocomial infections in an intermediate care unit". Crit Care Med. 2004; 32: 358-363.

57. Martin K. Antibiotics Becoming Useless All Over the World, Why?. The Real News. 2014.

58. Finley RL, Collignon P, Larsson DGJ, McEwen SA, Li X, et al. The scourage of antibiotic resistance: the important role of the environment. Clin Infect Dis. 2013.

59. Larsson DGJ, de Pedro C, Paxeus N. Effluent from drug manufactures contains extremely high levels of pharmaceuticals. J Hazard Mater. 2007; 148: 751-755.

60. Andersson DI, Hughes D. Persistence of antibiotic resistance in bacterial populations. FEMS Microbial Rev. 2011; 35: 901-911.

61. Pruden A, Larsson J, Amezquita A, Collignon P, Brandt KK, et al. Management options for reducing the release of antibiotics and antibiotic resistance genes to the environment. Environ Health Perspect. 2013; 121: 878-885.

62. Waksman SA, Woodruff HB. The soil as a source of microorganisms antagonistic to disease-producing bacteria. J Bacteriol. 1940; 40: 581-600.

63. Fajardo A, Martinez JL. Antibiotics as signals that trigger specific bacterial responses. Curr Opin Microbiol. 2008; 11: 161-167.

64. Aarestrup FM, Seyfarth AM, Emborg HD, Pedersen K, Hendriksen RS, et al. Effect of abolishment of the use of antimicrobial agents for growth promotion on occurrence of antimicrobial resistance in fecal enterococci from food animals in Denmark. Antimicrob Agents Chemother. 2001; 45: 2054-2059.

65. Heuer OE, Kruse H, Grave K, Collignon P, Karunasagar I, et al. Human health consequences of use of antimicrobial agents in aquaculture. Clin Infect Dis. 2009; 49: 1248-1253.

66. Bengtsson B, Wierup M. Antimicrobial resistance in Scandinavia after a Ban of Antimicrobial growth promoters. Anim Biotechnol. 2006; 17: 147-156.

67. Ashbolt NJ, Amezquita A, Backhaus T, Borriello P, Brandt KK, et al. Human Health Risk Assessment (HHRA) for environmental development and transfer of antibiotic resistance. Environ Health Perspect. 2013; 121: 993-1001.

68. Masse DI, Saady NMC, Gilbert Y. Potential of biological processes to eliminate antibiotics in livestock manure: an overview. Animals. 2014; 4: 146-163.

69. WHO 2018: http://www.who.int/news-room/fact-sheets/detail/antibiotic-resistance

70. World economic forum. 2018. http://reports.weforum.org/global-risks-2018/anti-microbial-resistance/

71. World Bank. "Drug-Resistant Infections: A Threat to Our Economic Future". Washington, DC: World Bank. 2017.