Antibiotic Resistance Spreads Internationally Across Borders

Tamar F. Barlam and Kalpana Gupta

Introduction

Antibiotic resistance (ABR) poses an urgent public health risk. High rates of ABR have been noted in all regions of the globe by the World Health Organization.[1] ABR develops when bacteria are exposed to antibiotics either during treatments in humans or animals or through environmental sources contaminated with antibiotic residues (Figure, Panel A). Spread beyond those administered antibiotics occurs through direct contact with the infected or colonized person or animal, through contact or ingestion of retail meat or agricultural products contaminated with ABR organisms, or through the environment. ABR bacteria spread from individuals to populations and across countries (Figure, Panel B).

Antibiotic Use in Human Medicine

It is well described that use of antibiotics in human medicine results in ABR organisms that can disseminate internationally. In a 2013 report, the Centers for Disease Control and Prevention (CDC) labeled *Clostridium difficile*, carbapenem-resistant *Enterobacteriaceae*, and drug-resistant *Neisseria gonorrheae* as the most urgent ABR threats in the U.S.[2] Those three compelling examples, however, support the need for worldwide mitigating actions.

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A recent report of *C. difficile* genetic epidemiology describes a perfect storm between antibiotic resistance, antibiotic use, and global spread.[3] The study used whole genome sequencing to evaluate the worldwide spread of fluoroquinolone-resistant *C. difficile* 027/NAP1/BI in health care facilities. This specific hypervirulent *C. difficile* strain is associated with significant morbidity and mortality. The investigators found that there were two genetically distinct lineages, both with mutations conferring high-level fluoroquinolone resistance. One lineage, FQR1, originally emerged in the northeastern U.S. and was subsequently transmitted to the Republic of Korea and Switzerland. The second lineage, FQR2, was found to have rapidly disseminated across continents, starting in North America and traveling to continental Europe, the UK and Australia. Acquisition of fluoroquinolone resistance is considered to be the key element driving the global spread of these lineages, fueled by liberal use of fluoroquinolones and international travel.[4]

Carbapenem-resistant *Enterobacteriaceae* (CRE) mediated by New Delhi metallo-beta-lactamase-1 (NDM-1) was first reported in 2008[5] and is another example of clinically important ABR driven by antibiotic use and disseminated by international travel across continents. *Enterobacteriaceae* are bacteria well known for their propensity to develop resistance and to cause serious, including fatal, disease. Infections caused by *Enterobacteriaceae* containing extended-spectrum beta-lactamases (ESBL), e.g., CTX-M, were described prior to NDM-1. ESBL limit the utility of many antibiotics; carbapenems are often the drugs of last resort. The emergence of CRE mediated by NDM-1 has further limited therapeutic options to a critical level.[6]

Figure

Development and Spread of Antibiotic Resistance

Figure The development of antibiotic-resistant organisms (A) through human and non-human use of antibiotics is followed by spread (B) from local to global environments.

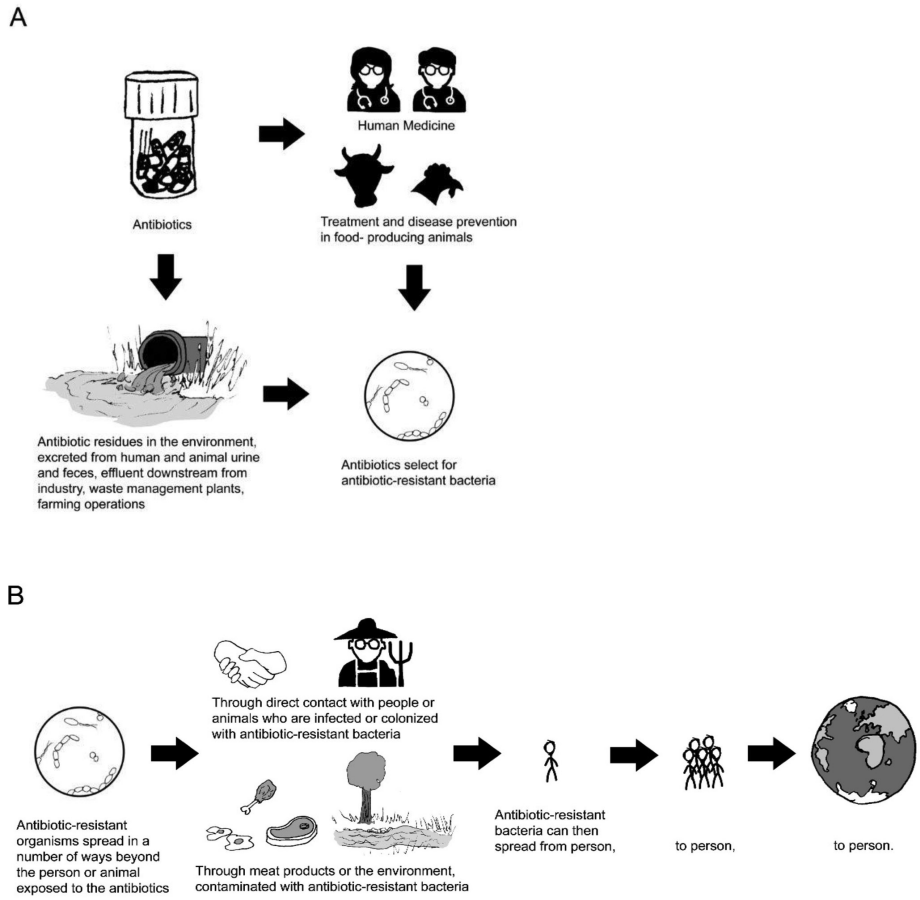

Illustration by Nathaniel Barlam

NDM-1 was initially identified from a man who traveled from his residence in Sweden to New Delhi, India and was hospitalized for treatment of an infection. On his first day back in Sweden, a highly resistant *Klebsiella pneumoniae* isolate was grown from his urine. Subsequently an *Escherichia coli* isolate was grown from stool; both species were found to carry NDM-1. Molecular studies confirmed that the NDM-1 gene was located on transferable plasmids (extrachromosomal genetic material) in the *K. pneumoniae* and *E.coli* isolates, supporting the concept that resistance spread between the species. Further studies have identified NDM-1-positive *Enterobacteriaceae* in almost every continent.[7] Travel, including medical tourism, is a key feature of the international spread of these highly resistant bacteria within health care facilities.[8] Importantly, hospitals are not the only reservoir for NDM-1 bacteria; studies have demonstrated that samples of pooled water from streets as well as from drinking water can also be positive for the bacteria.

N. gonorrheae causes gonorrhea, a common and clinically important sexually transmitted disease. Until now, the bacteria have been extremely susceptible to fluoroquinolones, and these infections were relatively

easy to treat. However, fluoroquinolone resistance in *N. gonorrheae* is spreading worldwide and impacting choices for therapy. Soaring rates of antibiotic resistance have been reported in every continent, with evidence of high-level fluoroquinolone resistance (MICs of ciprofloxacin >1.0 mg/L).[9] The story progresses even further with emergence of multi-drug resistant *N. gonorrheae*, further restricting treatment options. Treatment failure due to antibiotic resistance has led to a change in CDC guidelines that now recommend an injectable agent, ceftriaxone, in combination with either azithromycin or doxycycline.

Antibiotic Use in Food-Producing Animals

Development and spread of ABR bacteria is also promoted by use in food-producing animals (FPA). In food-animal production, antibiotics are not only administered to treat sick animals, but also given routinely to prevent illness (prophylaxis) or promote growth. That nontherapeutic antibiotic use is given for much of the animal's life, often at sub-inhibitory doses which particularly select for ABR (see below). ABR bacteria that develop in FPAs reach human hosts through several pathways. ABR *Salmonella*, *Campylobacter*, enterococci, and *E. coli* commonly contaminate retail meats[10] and are linked to human infections. Those meats are distributed both locally and globally. For example, a Swiss study identified and characterized 24 ESBL-producing *Enterobacteriaceae* from both domestic and imported poultry meat.[11] A study from the UK cultured chicken breasts for *E. coli* and identified isolates producing the CTX-M ESBL enzyme. One of 62 UK samples contained this enzyme compared with 10 of 27 imported samples. The South American poultry meat contained CTX-M-2, the dominant genotype in human infections in South America but a rare cause to date of those infections in the UK.[12]

There is strong evidence that contaminated meats contribute to ABR *Salmonella* and *Campylobacter* gastroenteritis, but whether they cause extra-intestinal infections, such as urinary tract infections (UTIs) caused by *E. coli*, is more controversial. A recent systematic review examined the evidence that extra-intestinal human infections with ESBL *E. coli* originated from FPAs. The authors reviewed evidence for both transmission of the ABR bacterial organisms (whole bacterium transmission or WBT) and transfer of ABR genetic material between bacteria (mobile genetic elements or MGE). Six studies supported WBT between poultry meat and humans. Transfer of MGE between bacteria from different species of animals (poultry, pigs, and cattle) and human *E. coli* was found in 13 geographically diverse studies. Seventeen studies did not support WBT, although 8 of those studies did find MGE-mediated transmission. Three of 4 observational epidemiological studies found ABR transmission between FPA and human *E. coli*. Overall, the literature supports a connection, but further study is needed to quantify the frequency and magnitude of the issue.[13]

ABR bacteria in FPAs can also spread directly to farmers and then to the community. A study in the 1980s examined the spread of nourseothricin, an agent used to medicate pigs that has no equivalent in human medicine. The farmers had nourseothricin-resistant *E. coli* in their gut, as did the members of that community. In addition, nourseothricin-resistant *E. coli* UTIs were identified.[14] In a more recent example, a Danish study compared farms with and without high 3rd and 4th generation cephalosporin use for the presence of ESBL-producing isolates in humans and pigs. Nineteen of 195 human participants were colonized, and 18 of those 19 had direct animal contact. In 10 farms, the same resistance gene was detected in both pig and human feces; in four farms, the isolates were proven identical in pigs and humans by many scientific methods, i.e., enzyme analysis, phylotype, PFGE type and multilocus sequence typing.[15]

New research emphasizes how sub-inhibitory levels of antibiotics are an important contributor to ABR by selecting for pre-existing resistant strains, by generating genetic and phenotypic variability, and by acting as signaling molecules to influence bacterial activities such as biofilm formation and gene expression. These resistant strains grow as well as organisms that are fully susceptible to antibiotics; thus, resistance imparts no fitness cost.

Antibiotic-Resistant Bacteria and the Environment

Resistant bacteria contaminate the environment due to industrial sources and large farming operations. For example, researchers sampled river sediment

upstream and downstream from a treatment plant that processed human, animal, and industrial waste. The samples downstream from the plant contained a marked increase in genes encoding the plasmid-mediated ESBL enzyme, CTX-M, in multiple species including *E. coli* and *Aeromonas*. They also demonstrated that the gene could be transferred easily between different bacterial species abundant in waste effluent.[16] In addition to ABR bacteria, actual antibiotic drug residues are commonplace as 20-80% of antibiotics are excreted in active forms into the environment from urine and feces.[17] Drug residues are found in waste water and sludge from farming operations but also in rivers, lakes, and drinking water. ABR bacteria and antibiotic drug residues have also been found in flies[18] and dust[19] originating from industrial farms.

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Infection and Asymptomatic Colonization in Returning Travelers

When spread of ABR bacteria results in overt infection, there are opportunities for containment through proper treatment, infection control, and surveillance. However, ABR infections can persist and spread due to asymptomatic colonization. An outbreak of carbapenem-resistant *K. pneumoniae* at the U.S. National Institutes of Health Clinical Center resulted in 18 infections and 11 deaths, more than three weeks after the index patient was discharged. The authors determined the ability of the organism to silently colonize patients in the hospital contributed to the ongoing outbreak.[21]

Strong evidence shows that people can become asymptomatically colonized with resistant pathogens after international travel. In a recent study, German travelers were studied for fecal colonization with ABR *Enterobacteriaceae* before and after travel to one of 53 different countries. ESBL-producing *E. coli* and *K. pneumoniae* were present in 6.8% of study volunteers pre-travel but post-travel, 30.4% were colonized with ESBL *E. coli* and 8.6% were colonized with ESBL *K. pneumoniae*. Travel to India and SE Asia had the highest acquisition rates for those ABR bacteria. Although half the individuals had a clinical bout of gastroenteritis associated with the travel, the rest were totally asymptomatic. Colonization persisted for six months in 8.6%.[22] In another study conducted in the Netherlands with a similar pre- and post-travel design, metagenomics DNA was extracted from fecal samples of volunteers. ESBL *Enterobacteriaceae* increased from 9% pre-travel to 33.6% post-travel, while quinolone-resistance genes qnrB and qnrS increased from 6.6% and 8.2% pre-travel to 36.9% and 55.7% post-travel, respectively.[23] Fecal colonization is of special interest because of the potential for MGE transfer of ABR among the diverse bacterial species within the gut microbiome. Colonization with other ABR bacteria, such as methicillin-resistant *Staphylococcus aureus*, in travelers has also been reported.[24]

Summary

Resistant bacteria selected by medical, agricultural, and industrial use spread globally through travelers, the export of animals and retail products, and the environment. Resistant bacterial strains may not persist if there is no selective pressure from local antibiotic use. However, this is not true for most ABR bacteria, where low fitness cost of the resistance and co-selection of other markers linked on MGE sustain those bacteria.[25] Thus, it is essential that nations work together to identify how to reduce emergence and amplification of resistant bacteria through sensible antibiotic treatment guidelines and restrictions. Concerted efforts for surveillance — to understand when new threats are brought across borders — and infection control — to minimize the spread and risk for outbreaks of ABR organisms — require global cooperation and solutions.

References

1. World Health Organization, *Antibiotic Resistance: Global Report on Surveillance* (2014), *available at* <http://apps.who.int/iris/bitstream/10665/112642/1/9789241564748_eng.pdf> (last visited May 8, 2015).
2. Centers for Disease Control and Prevention (CDC), "Antibiotic Resistance Threats in the United States. 2013," *available at* <http://www.cdc.gov/drugresistance/threat-report-2013/> (last visited May 8, 2015).
3. D. N. Gerding and F. C. Lessa, "The Epidemiology of *Clostridium difficile* Infection Inside and Outside Health Care Institutions," *Infectious Disease Clinics of North America* 29, no. 1 (2015): 37-50.
4. M. He et al., "Emergence and Global Spread of Epidemic Healthcare-Associated *Clostridium Difficile*," *Nature Genetics* 45, no. 1 (2013): 109-113.
5. D. Yong et al., "Characterization of a New Metallo-β-Lactamase Gene, *bla*NDM-1, and a Novel Erythromycin Esterase Gene

Carried on a Unique Genetic Structure in *Klebsiella Pneumoniae* Sequence Type 14 from India," *Antibiotic Agents and Chemotherapy* 53, no. 12 (2009): 5046-5054.
6. A. P. Johnson and N. Woodford, "Global Spread of Antibiotic Resistance: The Example of New Delhi Metallo-b-Lactamase (NDM)-Mediated Carbapenem Resistance," *Journal of Medical Microbiology* 62, part 4 (2013): 499-513.
7. J. S. Molton et al., "The Global Spread of Healthcare-Associated Multidrug-Resistant Bacteria: A Perspective from Asia," *Clinical Infectious Diseases* 56, no. 9 (2013): 1310-1318.
8. B. A. Rogers et al., "Country-to-Country Transfer of Patients and the Risk of Multi-Resistant Bacterial Infection," *Clinical Infectious Diseases* 53, no. 1 (2011): 49-56.
9. D. Yong et al., "Epidemiological Characteristics and Molecular Basis of Fluoroquinolone-Resistant *Neisseria Gonorrhoeae* Strains Isolated in Korea and Nearby Countries," *Journal of Antibiotic Chemotherapy* 54, no. 2 (2004): 451-455; M. Pérez-Losada et al., "Distinguishing Importation from Diversification of Quinolone-Resistant *Neisseria Gonorrhoeae* by Molecular Evolutionary Analysis," *BMC Evolutionary Biology* 7, article 84 (2007).
10. F. M. Aarestrup et al., "Resistance in Bacteria of the Food Chain: Epidemiology and Control Strategies," *Expert Review of Anti-Infective Therapy* 6, no. 5 (2008): 733-750.
11. H. Abgottspon et al., "Characteristics of Extended-Spectrum Cephalosporin-Resistant *Escherichia coli* Isolated from Swiss and Imported Poultry Meat," *Journal of Food Protection* 77, no. 1 (2014): 112-115.
12. R. E. Warren et al., "Imported Chicken Meat as a Potential Source of Quinolone-Resistant *Escherichia coli* Producing Extended-Spectrum Beta-Lactamases in the UK," *Journal of Antibiotic Chemotherapy* 61, no. 3 (2008): 504-508.
13. B. Lazarus et al., "Do Human Extra Intestinal *Escherichia coli* Infections Resistant to Expanded-Spectrum Cephalosporins Originate from Food-Producing Animals? A Systematic Review," *Clinical Infectious Diseases* 60, no. 3 (2015): 439-452.
14. R. Hummel et al., "Spread of Plasmid-Mediated Nourseothricin Resistance Due to Antibiotic Use in Animal Husbandry," *Journal of Basic Microbiology* 26, no. 8 (1986): 461-466.
15. A. M. Hammerum et al., "Characterization of Extended-Spectrum Beta-Lactamase (ESBL)-Producing Escherichia coli Obtained from Danish Pigs, Pig Farmers and Their Families from Farms with High or No Consumption of Third- or Fourth-Generation Cephalosporins," *Journal of Antibiotic Chemotherapy* 69, no. 10 (2014): 2650-2657.
16. G. C. A. Amos et al., "Waste Water Effluent Contributes to the Dissemination of CTX-M-15 in the Natural Environment," *Journal of Antibiotic Chemotherapy* 69, no. 7 (2014): 1785-1791.
17. D. I. Andersson and D. Hughes, "Microbiological Effects of Sublethal Levels of Antibiotics," *Nature Reviews Microbiology* 12, no. 7 (2014): 465-478.
18. J. P. Graham et al., "Antibiotic Resistant Enterococci and Staphylococci Isolated from Flies Collected Near Confined Poultry Feeding Operations," *Science of the Total Environment* 407, no. 8 (2009): 2701-2710.
19. G. Hamscher et al., "Antibiotics in Dust Originating from a Pig-Fattening Farm: A New Source of Health Hazard for Farmers?" *Environmental Health Perspectives* 111, no. 13 (2003): 1590-1594.
20. See Andersson and Hughes, *supra* note 17.
21. E. S. Snitkin et al., "Tracking a Hospital Outbreak of Carbapenem-Resistant *Klebsiella pneumoniae* with Whole-Genome Sequencing," *Science Translational Medicine* 4, no.148 (2012): 148ra116.
22. C. Lübbert et al., "Colonization with Extended-Spectrum Beta-Lactamase-Producing and Carbapenemase-Producing *Enterobacteriaceae* in International Travelers Returning to Germany," *International Journal of Medical Microbiology* 305, no. 1 (2015): 148-156.
23. C. J. Von Wintersdorff et al., "High Rates of Antibiotic Drug Resistance Gene Acquisition after International Travel, The Netherlands," *Emerging Infectious Diseases* 20, no. 4 (2014): 649-657.
24. Y. P. Zhou et al., "The Role of International Travel in the Spread of Methicillin-Resistant *Staphylococcus aureus*," *Journal of Travel Medicine* 21, no. 4 (2014): 272-281.
25 D. I. Andersson and D. Hughes, "Antibiotic Resistance and Its Cost: Is It Possible to Reverse Resistance?" *Nature Reviews Microbiology* 8, no. 4 (2010): 260-271.