Antibiotic resistance - will sewage testing help to control it?

Antimicrobial resistance (AMR) might result in a post-antibiotic area, in which common infections and minor injuries can kill. Can public health assist in surveillance AMR?

With some pain, I entered a pharmacy in Bangkok, asking for 'diazepam' pills. I explained to the pharmacist, that my chest pain is caused by stress and not by heart disease. Diazepam might relieve the stress and pain. 'By no means', he will sell me diazepam without a prescription, the pharmacist was telling me. So, I left, knowing that the German card identifying me as a physician, will not help here in Thailand. Some days later, I got a cold. Against better wisdom, I asked a pharmacist for an antibiotic. I got a small box which she just snatched from under the counter, and the next customer got another one. He seemed to suffer from a cold as well. Because of the antibiotic, I developed a serious allergic reaction the next day. I accepted that as a punishment, due to my professional misdemeanor. The pharmacist in Bangkok was certainly right to follow the regulations, not selling diazepam without prescription. Contrary, to not freely giving away drugs like diazepam, there seems to be no regulation forbidding to sell common antibiotics to customers. Maybe there is such a regulation, but it is not strictly enforced. Anyway, I should have known better in not taking an antibiotic without good reason, because this is one of the causes, why 'antimicrobial resistance' (AMR) turned out to be a global health problem.

Taking an antibiotic or better not?

The term 'antimicrobial' refers to the antibiotic-, antiviral-, antifungal- and antiprotozoal therapies to fight against various infectious agents. How 'resistance' develops is best explained by the ability of bacteria to be less or totally unaffected by antibiotics. Once a bacterium enters an animal or human, a complex reaction of the organism to fight the invasion is initiated. Details of this response are not to be discussed here. Fortunately, evolution provided us to fight infections, and that's why we are saved from dying readily from all sorts of invading harmful bugs. After the end of the Second World War, antibiotics became common therapeutics to help to fight off bacteria. (Please note, that this is just 75 years ago.) By nature, the intention of the 'microbe' is to multiply. Basically, the antibiotic interferes with the metabolism of the microorganism, which ultimately dies. But evolution also works for bacteria, enabling them to fight back. They change the course of metabolic steps through mutation. So, despite administering the once effective antibiotic, the germs keep multiply and spreading through the organism. Luckily for the host, the immune system might get the upper hand, and eventually, the infection is gone. Facing a mild common cold, probably triggered by a virus for which antibiotics don't work, I should have hung on for a while, relying on my immune system. Usually 'symptomatic treatment' provided at home by a caring wife or husband, might work. Only, if the infection gets more severe because of a 'concomitant' infection through bacteria, antibiotic treatment might be indicated. Such an infection might have been caused by Streptococcus pneumonia. The drug the pharmacist was freely giving away, was a penicillin derivate, against which I turned out to be allergic. (Note, I am definitely not the only one being allergic against the medication, and the reaction can be life-threatening!)

How AMR became a serious public health problem

Why I shouldn't have taken the antibiotic without need? One often neglected fact is, that medicine which works also has side effects. In my case, the side effect was the allergic reaction, which caused me to stop the medication immediately. In case I would have had a bacterial infection, one dose of some sort of penicillin, might not have killed many germs. The danger is that those which survived are now resistant against the antibiotic I took. I also might have killed quite some germs, which are protective of health, such as those within the gut. In case the therapy with an antibiotic is 'indicated' the decision at best is with a medical doctor. The medicine should be taken as long as advised to make sure, that all the infective agents are eliminated. A very common mistake is, to stop taking the medicine once the acute symptoms are overcome. Having no longer any symptoms doesn't mean that all the pathological bacteria are gone. Those remaining might turn to be resistant against the antibiotic used for the therapy. They even might transfer the ability to survive the antibiotic to other germs as well. The person which harbors resistant microorganisms' spreads those into the environment, such as family, home for elderly and hospitals.

Another dangerous source for AMR is the agricultural sector. So, for instance, preventing infections in poultry farms, the animals are fed antibiotics. Other life stock gets antibiotics to ensure healthy and quick growth. Drug-resistant bacteria are spread than through animal feces and contaminate meat. This then will be ingested together with the resistant germs, if not properly fright or cooked. Animal feces containing resistant bacteria can be found in fertilizers and water used for vegetable gardening. Nowadays, the increase in resistant and multi-resistance bacteria strains has reached an alarming level starting to be a serious public health problem.

Example of resistance, mortality estimates, and countermeasures

The problem India has with AMR illustrates a situation existing in many countries (1). Main aspects are 'a poor public health infrastructure and a high burden of diseases going along with an unregulated sale of cheap antibiotics, which are also used frequently in animal farming'. Additional examples for the severity of AMR are reports from the Congo in Africa, where a multidrug resistance of E. coli was observed (2), and in Greece, the antibiotic carbapenem was found not to work any longer against Klebsiella pneumonia in a nationwide hospital survey (3). For the group of Enterobacteriaceae the drug carbapenem is used when any other treatment no longer helps. It is estimated that 50.000 individuals die because of antibiotic-resistance infections yearly in Europe and the USA, and all over the world 700.000 deaths occur annually, while it is expected to increase in the middle of this decennium to be 10 million (Jim O'Neill report).

There are a number of antibiotic targets interfering with the multiplication of bacteria. One of the most common ways if one of the treatments fails, another antibiotic is given instead. In the meantime however, bacteria became resistant to quite a number of different classes of antibiotics, and treatment with antibiotics is approaching a limit in substituting one antibiotic drug with another one. Since 1984 no new classes of antibiotics were found (The Washington Post, 28 July 2016).

Antibiotic therapy nowadays is an indispensable necessity for modern medicine to fight morbidity and mortality, and AMR is threatening health and survival around the world. The seriousness of the situation has been addressed in national and international forums. The Jim O'Neill report from England and the Executive Order from the White House are examples for the awareness of national governments about AMR. The topic also has been selected for a high-level meeting of the United Nations. This meeting was only one of four occasions the United Nations selected a health topic as the agenda of these special sessions. The other three topics covered HIV, non-communicable diseases and Ebola. Additional activities included an 'Global action plan on antimicrobial resistance' from the World Health Organization (WHO), and initiatives to develop new antibiotics (4).

Testing and detecting AMR in clinical settings

The ultimate aim of all these efforts is to prevent patients suffering from microbial infections, while antibiotics administered won't work. In clinical settings, it is necessary to identify which antibiotic is resistant and which another one might function. The conventual testing method in the laboratory is to apply bacteria on dishes impregnated with different antibiotics. Bacteria growing around or not around the spots of antibiotics show, that they are resistant or not resistant against the drugs. These sorts of methods are quite expensive, time-consuming and labor-intensive.

Genomic diagnostic systems to detect those genes in bacteria to be resistant against certain types of antibiotics are more recent methods. 'A rapid cost-effective' test for clinical laboratories based on Recombinase Polymerase Amplification (RPA) has been introduced recently (5). RPA is an advanced development of the well-known Polymerase Chain Reaction (PCR), being very sensitive, fast and suitable for the amplification of various targets including RNA, miRNA, ssDNA from various organisms and samples (6). The usefulness of the RPA technique has been demonstrated in detecting the gene which allows *Streptococcus pyogenes* to be resistant to an important group of antibiotics, including erythromycin A and azithromycin. This class of antibiotics belong to the macrolides, produced by strains of Streptomyces, a kind of bacteria, from which antibiotics are produced, interfering with the protein synthesis of the pathogen. The pathogen here the *Streptococcus pyogenes,* being resistant to macrolides because of its *mef*(A) gene. The abbreviation stands for 'Macrolide Efflux A', which basically is a pump which allows the pathogen to regulate its internal environment through the cell wall, but also eliminates toxic substances including 'antimicrobial agents' (7-11). This is only one example of the multitude of the complex interrelationship between bacteria and antibiotics and how resistance towards the antibiotic substance might work.

The immediate effect of resistance against an antibiotic is observed in hospital settings. A patient with high fever and already in a very frail condition, in need of antibiotic treatment, doesn't respond to the medicament and might succumb before finally a medication is identified which works. Even more unfortunate is a situation where resistant pathogens are responsible for, 'nosocomial' infections, which are those unfortunate events, when an infection is acquired within the hospital. This might be an otherwise healthy young patient, just having a routine operation because of acute appendicitis. To minimize the occurrence of these events, a comprehensive approach in antimicrobial resistance research and surveillance, not only on a local, but also national, regional, and global level, is necessary.

AMR surveillance on population basis from sewage inlets

So far there are a number of formal and informal initiatives to assess the overall expansion of AMR. A WHO report on surveillance underlined the' alarming level of resistance to common bacteria and only a few available treatment options for common infections remain to be effective.' The information available are restricted to a number of different pathogens, and usually focus on hospitalized patients and last resort therapies such as carbapenems. The main emphasis is laid on patient care and less on AMR surveillance. (12).

A recent editorial of the on-line journal 'BMC Infectious Diseases' summarized steps necessary to improve surveillance and concluded, that there is a need for more information about the emergence and prevalence of AMR, and the identification of populations at risk (13). Such data are required for policy development and to assess the impact of an intervention. A supplementary surveillance method, especially beneficial for public health, would be an advantage. Such a system, which might be appropriate globally, could be in examining samples taken from sewage inlets into treatment plants (14). Currently, samples from patients are tested in isolating bacteria in cultures. In this way, resistance against a few antibiotics can be determined. A remarkable additional increase in surveillance would be to determine the presence of AMR genes from a large and healthy population especially from urban settings.

The significance of the suggestion to examine a sample of smelly inflow into a municipality sewage plant for enlarging information about AMR, might not be apparent straight away. More and more people globally live in urban areas and about 40% are connected to sewer systems (15). Without any ethical concerns, a sample can be taken to assess the AMR genes from a predominantly healthy population. Through the 'Next-Generation Sequencing Primer' technology millions of random DNA fragments can be determined, and bacterial genes identified enabling the microbe to resist antibiotics (16).

'Next-Generation Sequencing Primer' technology used to test for AMR genes

Just how, thousands of known AMR genes can be detected by a so-called 'metagenomic-based' technique? As explained above, the genetic background of resistance against antibiotics can be transferred from bacteria to bacteria and from sick individuals with an infection to healthy individuals and from healthy individuals to other healthy persons as well. This mechanism is known as 'horizontal gene transfer'. A particular role in this chain of events the 'commensal' bacteria flora plays an important role. The meaning of 'commensal' being of no harm but also no benefit, is no longer valid for the vast amount of this sort of bacteria in the human body, especially for those in the gut. It is now known that this microbiome has an important role in homeostasis and diseases (17). It also includes bacteria strains with AMR genes, excreted with the feces.

The Global Sewage Surveillance project consortium

What assessing of antimicrobial resistance through sewage collection might accomplish, has been demonstrated on a global basis (18). A consortium conducted a global sewage surveillance project including 79 sites in 60 countries from Europe, North America, Africa, Asia, and South

America. Samples were sequenced through Illumina HiSeq and data processed by MGmapper (19). On average a sample provided 120 million reads. AMR reads on average accounted for 0.03%. Most abundant AMR genes encoded to macrolides, tetracyclines, aminoglycosides, beta lactams and sulfonamides. Europe and North America had a high proportion of macrolide resistance genes while the samples of Asia showed resistance against sulfonamides and phenicols, the latter group also includes chloramphenicol. From the dominant AMR genes, none was restricted to a specific bacterial genera. The majority of reads, with 68% could not be assigned to a reference sequence. Human genomic material was determined from 29% of reads, microorganisms such as bacteria and protozoa to 1%, and plants to 0.4%.

The usefulness of sewage surveillance

All in all, it was found that 'AMR gene abundance' correlated with health, environmental- and socio-economic factors taken from a limited number of World Bank variables. The lowest level of AMR was measured from the Netherlands, New Zealand, and Sweden, while the highest levels were found for Tanzania, Vietnam, and Nigeria. The finding that AMR only slightly correlated with the use of antibiotics was explained by the difficulties to assess antimicrobial use (AMU). Sewage-based surveillance might be adopted, as a complementary system to monitor AMR on the basis of community populations. Culture- and PCR based methods, however, cannot be abandoned. Samples from patients needed to be tested by bacterial isolation and culture so that resistance to particular antibiotics can be identified for clinical use.

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