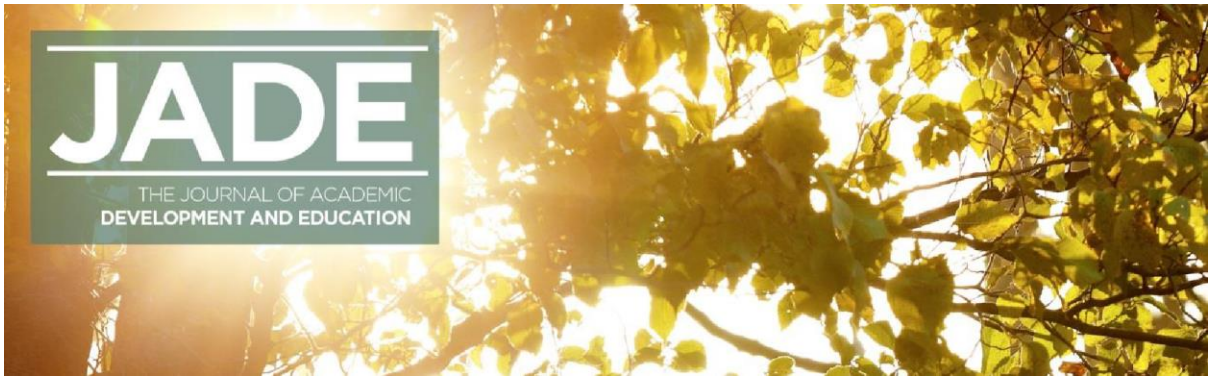

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Article:

Emergence of highly antibiotic resistant ‘Superbugs’ and the implications of antibiotic resistance

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Key words: Antibiotic resistance, ‘Superbugs’, Methicillin-Resistant Staphylococcus aureus, Penicillin-Binding Protein 2a

Abstract

Many types of bacteria are now insensitive to antibiotics, the drugs which were designed to kill them; this insensitivity is known as antibiotic resistance (AR). Horizontal gene transfer (HGT) is responsible for widely distributing antibiotic resistance among bacteria. ‘Superbugs’, such as Methicillin-Resistant *Staphylococcus aureus* (MRSA), are particularly evasive, with resistance to several different antibiotics. The expression of high levels of resistance in these bacteria is due to novel, non-standard, proteins, including Penicillin-Binding Protein 2a (PBP2a), which allows MRSA to survive in the presence of antibiotics,

even when other kinds of *Staphylococcus aureus* (SA) cannot. Unnecessary antibiotic use is mainly to blame for the development of AR, with the Centers for Disease Control and Prevention reporting that nearly 50% of all US antibiotic use in outpatients is inappropriate. Other factors include ease of availability, and public unawareness; in a concerning recent study of around 55,000 members of the public, around 15,000 said they had never heard of antibiotic resistance. AR in the bacterium *Heliobacter pylori (H. pylori)* has resulted in antibiotic-based therapy success rates dropping by 17% for nitroimidazole therapies, and 36% for bismuth therapies. The Organisation for Economic Co-operation and Development (OECD) says that hospitals may be required to spend up to an extra US$40,000 to treat resistant infections. Current predictions show the resistance crisis worsening significantly by 2050; changes must be made now to contain antibiotic resistance before it is too late.

Context

Introduction to antibiotic resistance

Antibiotics are drugs that are designed to kill bacteria. In recent years, many bacteria have become immune to these drugs, meaning that they can no longer be killed by them – this is what is known as antibiotic resistance.

When an antibiotic is used on a population of bacteria, a small number of bacteria in the population may not be killed, due to a random genetic change, or mutation, emerging in their DNA that allows them to survive. These surviving bacteria are now the only ones that can reproduce, so the genetic change will be passed on to the next generation. An entire new population of bacteria which is immune to the antibiotic is therefore created.

The processes which take place due to the genetic changes responsible for antibiotic resistance are extremely complex; they are therefore not understood well, and very little progress has been made in trying to find ways to prevent it (Davies and Davies, 2010).

What are ‘Superbugs’?

Bacterial Superbugs are bacteria with very high levels of antibiotic resistance, such as MRSA. They can quickly infect patients and spread rapidly as there is little that can be done to stop them (ScienceNews, 2013); this is especially so because an important characteristic of bacterial Superbugs is resistance to several different antibiotics.

Infections caused by Superbugs can be very dangerous, making them the cause of an increasing number of deaths and disabilities throughout the world. Therefore, it is extremely important to control resistance to stop a superbug which is immune to all existing antibiotics from emerging (NHS, 2019).

Superbug bacteria show resistance by the same fundamental processes as other bacteria, but the processes are better adapted for resistance in Superbugs, with some modifications that are advantageous to survival against antibiotics (Benveniste and Davies, 1973; Foster, 2004; Chang et al., 2014).

Discussion

Causes of resistance

A major cause of antibiotic resistance has been the overuse of antibiotics. As mentioned previously, the use of an antibiotic on a population of bacteria has the potential to create a new, resistant population of the bacteria. More frequent use of an antibiotic therefore means a greater number of opportunities for a resistant population to be created.

Figure 1 shows the extent of antibiotic use in the United States in 2017; it can be seen that a significant proportion of states are on the higher end of the scale. A few states are at the maximum level, with more than 900 antibiotic prescriptions per 1000 people in one year.

[Insert figure 1 here]

It is concerning that, in the US, at least 30% of antibiotics that are prescribed in the outpatient setting (where around 80-90% of human antibiotic use occurs) are done so unnecessarily, and that nearly 50% of antibiotic use in the outpatient setting is inappropriate (Centers for Disease Control and Prevention, 2019).

Another issue is self-medication, which is when someone takes antibiotics without first consulting a certified medical professional. Unless a patient has expert knowledge, it is likely that when patients self-medicate, they do not know exactly what they are doing, and when it is appropriate or not to take antibiotics. Not consulting a medical professional increases the chances of an antibiotic being taken when it is not necessary.

Also, some antibiotics are available over the counter, without the need for a prescription. This encourages people to use these drugs as the fact that they are so easily available gives the impression that professional guidance is not required.

The general public does not fully understand antibiotic resistance and how to use antibiotics properly; in a recent study involving around 55,000 members of the public, only around 70% of participants said they had heard about antibiotic resistance in the past. This means that a large number of people do not know about resistance, which means they are not aware of the most significant negative effect of inappropriate antibiotic use (Bajpai et al., 2017).

Resistance mechanisms

There are several mechanisms by which bacteria can show resistance to antibiotics, such as those shown in figure 2. The main categories of mechanism are modification of a bacterium’s own structure, and modification of the structure of the antibiotic.

[Insert figure 2 here]

A bacterium can modify parts of its structure in a way which stops the antibiotic reaching its target, or which stops it from functioning normally at its target. The antibiotic can be stopped from reaching its target by, for example, modifying the cell wall of the bacterium to make it impermeable to the antibiotic, and therefore stop it from being able to get inside the bacterium, as seen in figure 2.

Modification of the drug target is an example of a way to stop the normal function of an antibiotic; if the antibiotic carries out its function by binding to a specific shaped region in the bacterium, then changing the shape of this region will stop the normal function of the antibiotic. This can be better understood with a lock and key model, where the antibiotic is a key and the binding region is the lock; if the lock is changed then the key will no longer work as it is the wrong shape to open the lock.

Figure 2 shows one method of inactivating an antibiotic, which is to change its structure by adding a phosphate group. This impairs the normal function of the antibiotic, which would be to bind to the ribosomes of the bacterium, and affect the bacterium's ability to produce proteins, which it needs in order to survive.

The mechanisms described above are all possible due to alterations in the DNA of the bacterium, known as mutations (Benveniste and Davies, 1973). The DNA is the code to make proteins. For example, to modify the cell wall as in figure 2, the DNA mutates so that it codes for the new, modified cell wall protein, which causes its impermeability, instead of the normal cell wall protein, which is permeable.

There are different types of mutation, but figure 3 shows an insertion mutation, where extra DNA is inserted into the bacterium's existing DNA (U.S. National Library of Medicine, 2020). In figure 3 the DNA is shown to code for a sequence of amino acids, which are the the building blocks of a protein.

[Insert figure 3 here]

In the lower DNA strand the insertion of the nucleotide has clearly changed the amino acid sequence, where the alterations are shown in orange. From this it can be seen how changes in the bacterial DNA can indirectly lead to the development of antibiotic resistance in bacteria.

What makes 'Superbugs' 'super'?

Penicillins are a group of antibiotics that impair the ability of bacteria to repair their cell wall, the outer covering that protects all the internal structures of the bacteria. Penicillins, which include methicillin, are part of a wider group of antibiotics known as β-lactams. The example of MRSA is better adapted for resistance to β-lactams than non-superbug types of SA.

In SA and MRSA, transpeptidases, also known as penicillin-binding proteins (PBPs), are either partly or fully responsible for cell wall formation. For β-lactams, PBPs are good targets as they are essential for cell wall repair, and for bacterial survival. SA has four PBPs; MRSA has the same four PBPs, but it also has a fifth, PBP2a. Figure 4 shows the difference between PBPs in SA, and PBP2a; in A, PBP2 is seen to have a binding region for the β-lactam,which PBP2a does not, preventing the antibiotic from functioning normally. MRSA can then proceed to inactivate the β-lactam, as in B.

[Insert figure 4 here]

PBP2a is still able to continue functioning close to normally in the presence of β-lactams, where the functions of the other four PBPs would be severely impaired. This means that MRSA can sustain cell wall repair when β-lactams are used, while SA cannot. The capability to produce PBP2a is unique to MRSA, and the production gene was acquired from another, non-SA organism (Chang et al., 2014).

Superbugs have a particularly high ability to infect a patient and to spread rapidly. Of hospital based SA infections, many are caused by MRSA, which is antibiotic resistant, meaning it has resistance to more than one antibiotic; a number of MRSA kinds are resistant to most commonly used antibiotics (Foster, 2004). Several other bacteria have also been classified as being resistant to multiple antibiotics (Bajpai et al., 2017).

Until recently, MRSA had remained sensitive to the antibiotic vancomycin, but subsequently the bacteria developed low levels of resistance to the drug; MRSA now has, however, developed high levels of vancomycin resistance due to the transfer of resistance genes to SA from another kind of bacteria known as *Enterococcus* (Foster, 2004).

Gene transfer

When humans reproduce, some of the DNA of both parents is replicated and then passed on to their children. Figure 5 shows the stages in meiosis, the production of sex cells (eggs and sperm). In the first phase all the DNA is duplicated; the parent cell then divides into four cells, which are the sex cells, shown at the bottom of the diagram, each with some of the parent cell's DNA. At fertilisation a sex cell from the mother (egg) joins with a sex cell from the father (sperm), creating a new parent cell with some genes identical to those of the mother and father.

[Insert figure 5 here]

In addition to the standard kind of gene acquisition, such as that in humans, where genes are passed to new generations through reproduction, bacteria can transfer genes between each other in the same generation, by what is known as HGT, which is responsible for widely distributing antibiotic resistance genes among bacteria (de la Cruz and Davies, 2000).

Figures 6a-6c show one method of HGT between bacteria. First a bacteriophage carrying DNA from a donor bacterium binds to the surface of the recipient bacterium (6a). The bacteriophage then inserts the donor DNA into the recipient bacterium (6b), and this DNA then becomes integrated into the recipients own DNA (6c).

[Insert figures 6a, 6b and 6c here]

In HGT, DNA mutations, as discussed earlier, are not required in an organism for a transferred gene to be acquired by it; instead, the resistance gene is acquired from another organism. The newly acquired gene can become part of the DNA of the bacterium, or it can be introduced via a plasmid, which is kept separate and does not become part of the bacterium's DNA.

The new gene can either be acquired from a living organism, or a dead organism. A big issue with HGT is that resistance can not only be distributed within a single bacterial population, but unlike in gene transfer through reproduction, it can also be distributed to another species of bacteria (Kaiser, 2012)

Implications

HGT means that bacteria can become resistant to an antibiotic without ever being exposed to that antibiotic, but by merely being in the presence of bacteria which are already resistant to it. An example of a horizontally transferred gene is CTnDOT, which gives resistance to the antibiotic tetracycline (Gardner et al., 2005; Gui-Rong et al., 2009).

Although its presence is not necessary for HGT, tetracycline use stimulates HGT of CTnDOT from tetracycline resistant bacteria. Overuse of antibiotics not only increases frequency of resistance by mutation, but also frequency of resistance by HGT (Sapkota et al., 2011).

The development of antibiotic resistance has meant that there are very few antibiotics available now that are effective against bacteria. In the example bacterium *H. pylori,* rates of antibiotic resistance are in some places as high as 95%. There have been many studies which show the negative effects of resistance on the success rates of antibiotic based therapies; for example, in nitroimidazole therapies, success rates have been recorded as 90% vs 73%; in bismuth therapies, 89% vs 53% and in macrolide therapies, 68% vs 33%, in non-resistant vs resistant kinds of *H. pylori.*

The clear lack of effectiveness means that treating bacterial infections often requires combinations of drugs rather than just a single antibiotic, known as combination therapy, as a single antibiotic drug is not effective enough alone. The components of the drug combination are one or more antibiotics and, for example, an acid suppressive drug, which is a drug that weakens stomach acid (Gerrits, 2006).

Antibiotic resistance is responsible for large numbers of deaths around the world; figures 7a and 7b illustrate these numbers. Antimicrobial resistance (AMR) is resistance of all microorganisms, such as bacteria and fungi, to drugs that are designed to kill them; antibiotics are a type of antimicrobial.

[Insert figures 7a and 7b here]

Figure 7a shows that although there are around 700,000 deaths per year in 2017, this is still relatively low when compared to cancer and traffic accidents. The darker blue portion clearly shows a huge, very worrying, predicted rise in death rates by AMR by 2050. The figure indicates that although death rates are still relatively small, AMR is increasingly becoming a much larger problem.

In figure 7b it is apparent that the majority of predicted AMR deaths in 2050 are in Africa and Asia. This can be explained by points mentioned previously; in developing countries health care is very expensive leading to a lot of self-medication as it avoids the need for a professional. Also, prescription drugs in these countries can be easily purchased without the need for a prescription (Bajpai et al., 2017). This means AMR is much more widespread in less developed countries.

The World Bank believes that due to AMR, by 2050 annual global gross domestic product (GDP), the value of all goods and services produced, will fall by between 1.1% and 3.8%, and in low income countries the fall could exceed 5% of their annual GDP. Also, there may be up to an additional 28.3 million people falling into extreme poverty in 2050 due to AMR.

Other impacts of AMR by 2050 are increases in global healthcare costs that may be in excess of US$300 billion per year, and a decrease in global livestock production which may be between 2.6% and 7.5% per year. According to the OECD, more intensive and also more expensive healthcare will be needed by patients due to resistance, with hospitals being required to spend up to an extra US$40,000 to treat a resistant bacterial infection. (Ahmad and Khan, 2019).

Conclusions

To avoid the current resistance crisis becoming much worse in the future, antibiotic use needs to be controlled. In many developing countries there are poor laws regarding the dispensing of antibiotics, making it too easy for antibiotics to be misused. It needs to be harder to get antibiotics, so they are only used when necessary.

Bacterial infections must be diagnosed properly so that antibiotics are not used unless they will have an effect. For example, viral infections cannot be treated using antibiotics. The issue of self-medication means people without expert knowledge are attempting to diagnose themselves with bacterial infections which can be treated using antibiotics. Infections should instead only be diagnosed by a professional, as self-diagnosis is much more likely to be wrong.

The public needs to be educated on how to use antibiotics properly, and on antibiotic resistance as well as its causes (Bajpai et al., 2017). Many people do not know they are using antibiotics wrongly, and that they could be adding to the resistance crisis. Also, the development of antibiotic resistance is not something that would be immediately obvious to someone without a certain level of scientific knowledge, which not everyone has.

Antibiotic resistance can be controlled easily right now, but if the current situation continues to worsen, we will reach a point in the future where the spread of some bacterial infections becomes uncontrollable, and we will be able to do nothing about it. Changes must be made urgently before this point is reached.

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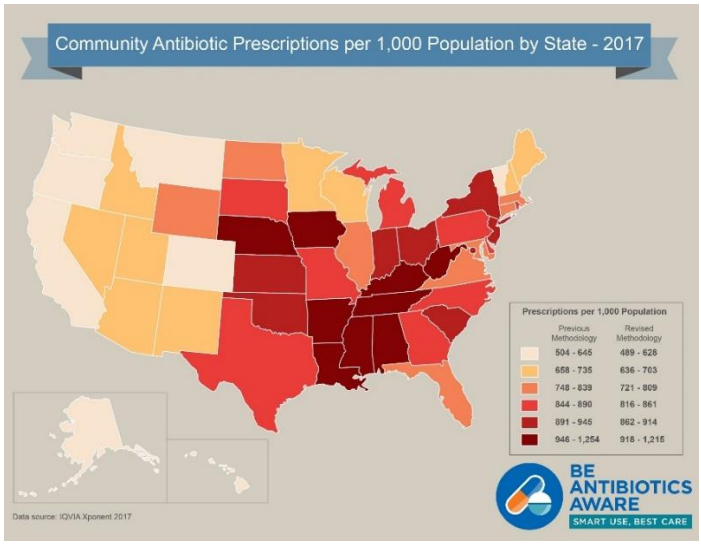
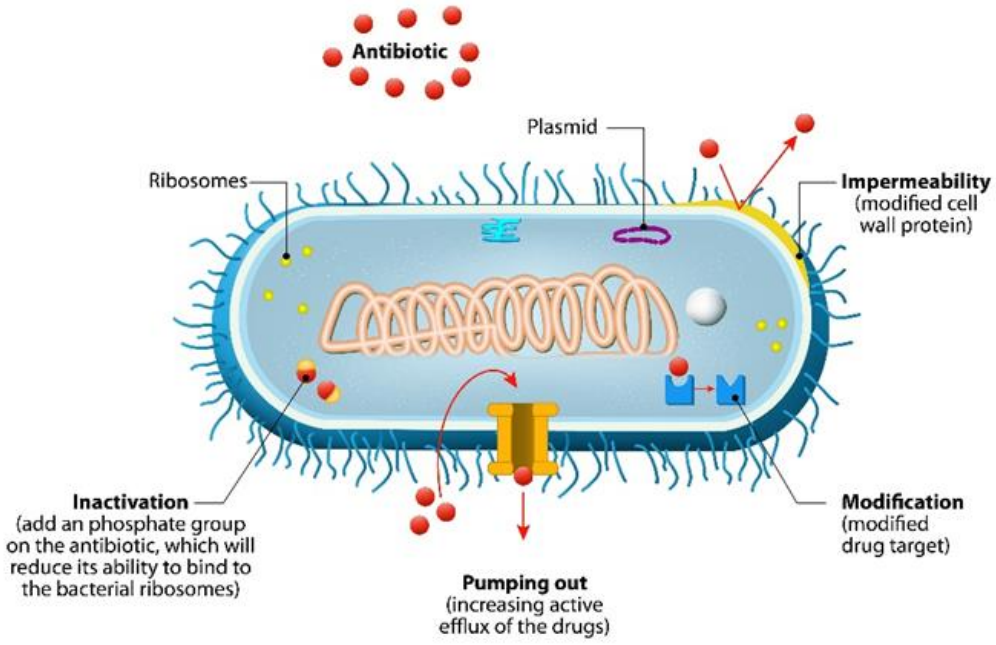
I

Figure 1: US antibiotic prescription rates by state (Centers for Disease Control and Prevention, 2019)



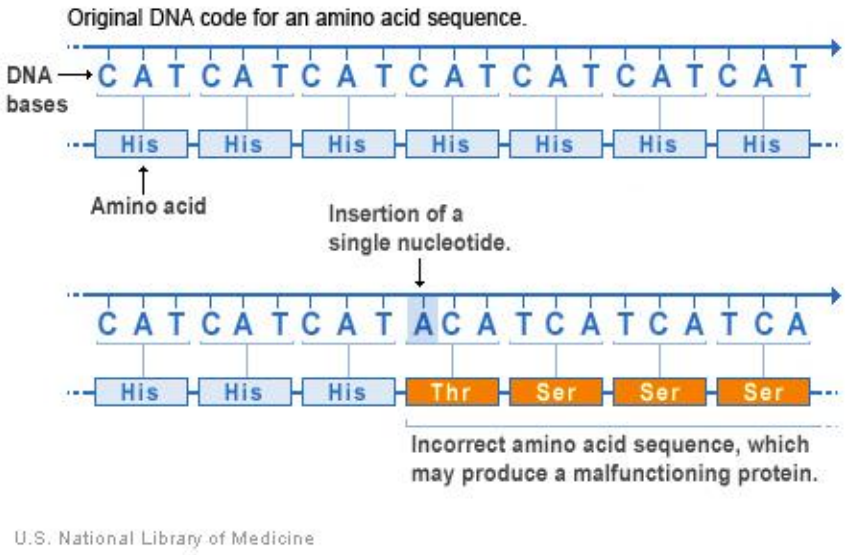
II

Figure 2: Mechanisms of antibiotic resistance (*Adapted from* Thermofisher Scientific, 2016)



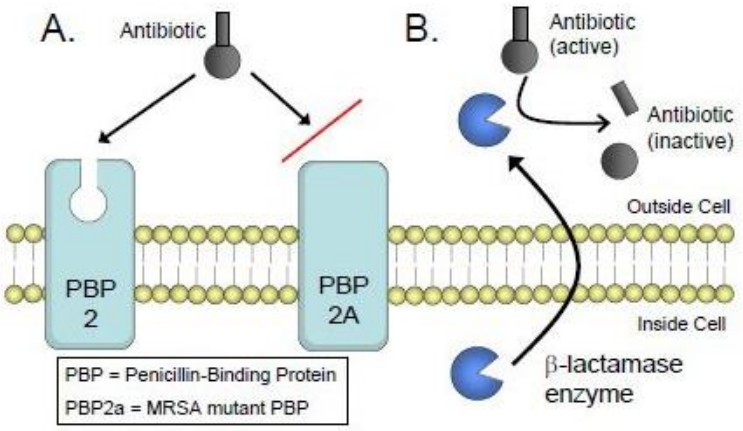
III

Figure 3: Insertion mutation (*Adapted from* U.S. national library of medicine, 2020)



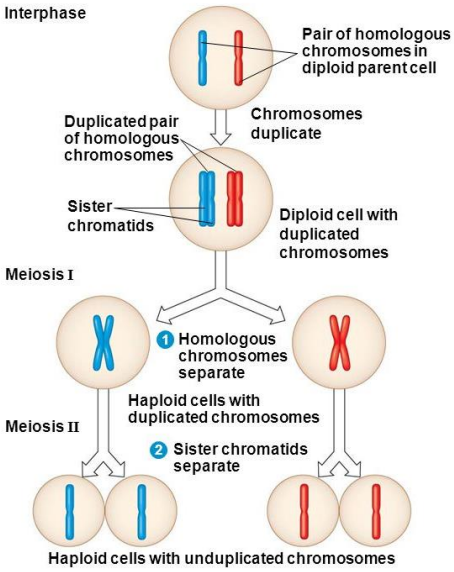
IV

Figure 4: PBP2a vs PBP2 in the presence of β-lactams (Los Cazadores de Microbios, 2012)



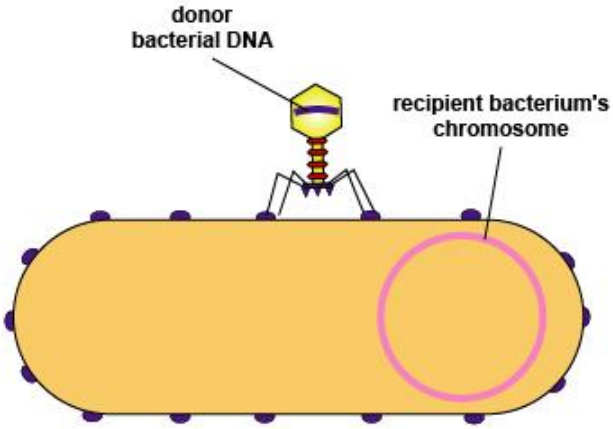
V

Figure 5: Stages of meiosis (*Adapted from* Fitzpatrick and Tunbridge, 2014)



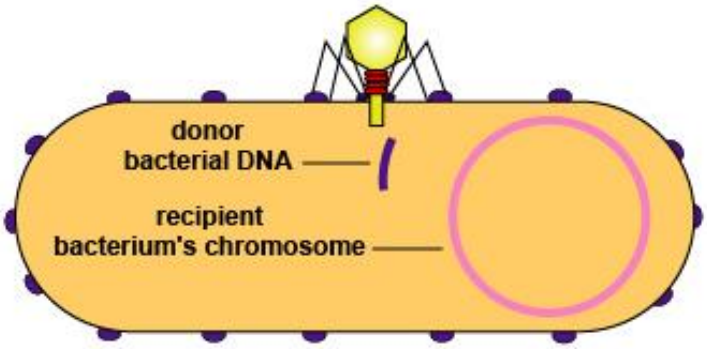
VI

Figure 6a: Binding of bacteriophage to recipient bacterium (*Adapted from* Kaiser, 2019)



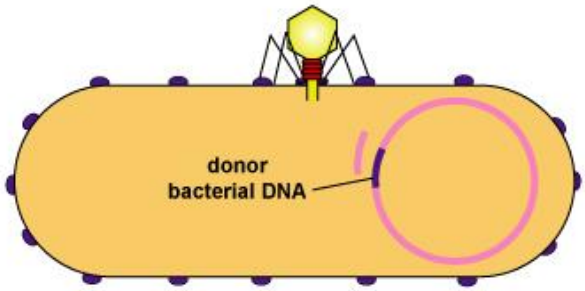
VII

Figure 6b: Insertion of donor DNA into recipient bacterium (*Adapted from* Kaiser, 2019)



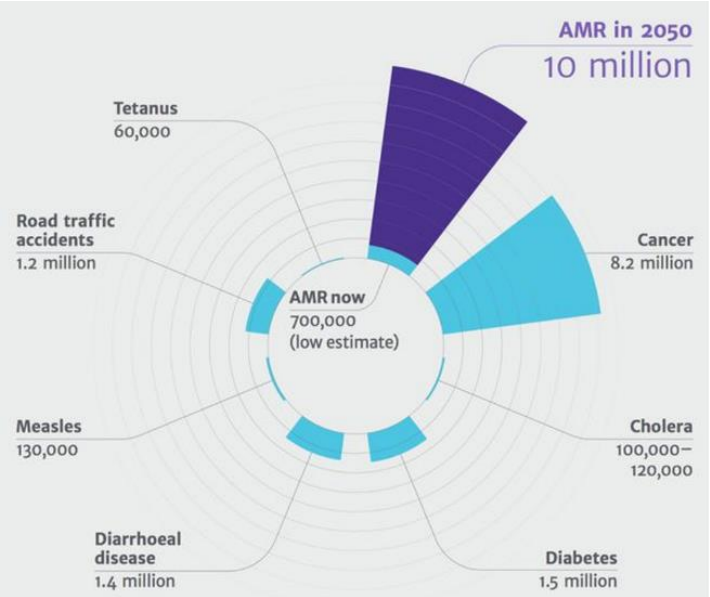
VIII

Figure 6c: Incorporation of donor DNA into recipient bacterial DNA strand (*Adapted from* Kaiser, 2019)



IX

Figure 7a: Deaths per year due to AMR vs other causes (*Adapted from* iGEM, 2017)



X

Figure 7b: Predicted death rates due to AMR in 2050 (*Adapted from* iGEM, 2017)