Journal of Young Scientist, Volume VII, 2019
ISSN 2344 - 1283; ISSN CD-ROM 2344 - 1291; ISSN Online 2344 - 1305; ISSN-L 2344 – 1283

ANTIMICROBIAL RESISTANCE- RESISTING THE CURE

Alexandru Cristian Nicolae MANOLACHE

Scientific Coordinator: Assoc. Prof. PhD Biotech. Irina GREBENIŞAN

University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Mărăşti Blvd, District 1, 011464, Bucharest, Romania, Phone: +4021.318.25.64, Fax: + 4021.318.25.67

Corresponding author email: alex.mano622.winxp@gmail.com

Abstract

Living organisms are known for their ability to adapt to environmental changes, to evolve to overcome the challenges these new environments they encounter, and microbes are no different. They have an outstanding ability to adapt to differences in environments, to different antimicrobial substances which are synthesised in order to counter them, or to quickly multiply to overcome their inevitable fate if they chose to not adapt. And that is extermination. Another factor that plays nicely with them is the chaotic usage of antibiotics, which leads to these microbes' natural ability of developing an anti-antibiotic behaviour.

Keywords*: DNA, drugs, salmonella, antibiotics, genes.*

INTRODUCTION

The concept of antibiotics harbours a bigger range of substances which are synthesised either naturally, semi-synthetically or synthetical, and are used to a) inhibit bacterial growth (bacteriostatic) or b) annihilate/destroy them (bactericidal) (O'Neill, 2016; Pourmand et al., 2017; World, 2015). These are categorised by the way they go about dealing with the bacteria, either bacteriostatic or bactericidal, and by how little or how strong are the effects of said antibiotic, or even by how many different species of bacteria they can deal damage to. In addition, in agriculture, global-wide class antibiotics drugs have been used with no regard to: what kind of potential side-effects they have, or what risk managements steps have to be taken in the worst-case scenario. The antibiotics which are used in agriculture come from the following classes: tetracyclines, aminoglycosides, β-lactams, lincosamides, macrolides, pleuromutilins and sulphonamides (Klein et al., 2018; World, 2018; Dar et al., 2016; Manyi et al., 2018).

Of course, the antibiotic resistance pattern varies between regions and countries due to differences in how and what antibiotics are used, and also in what quantity. All activity with regard to antibiotic consumption is regulated by said country's antibiotic policies. In recent years, China has led both production and consumption of antibiotics for both human and animal use. Antibiotic crisis is generally caused by misuse of antibiotics which are ultimately released to the environment, in the presence of residues (parent antibiotic or the metabolites from said parent, livestock waste and animal derived products), livestock products, and finally because of the lack of effective supervision and control over the production, usage and disposal of antibiotics.

In a nutshell, antibiotic resistance is an "One Health subject", both in cause and solution, because it addresses the interactions between human, animals and the environment. In an attempt to control this whole ordeal, the WHO (World Health Organisation) instituted a GAP (Global Action Plan) which demands each and every country develop national plans to align themselves with the GAP, within their limits, especially the developing countries due to their lack of capacity and integration which affects their surveillance systems.

MATERIALS AND METHODS

A study in Nairobi, Kenya, sampled fresh chicken droppings from 150 chicken Households at random and tested them for: *Salmonella, Escherichia coli*, and antimicrobial

susceptibility tests were carried out in the scientific papers I have studied. This test illustrates the current situation in a developing country, in part because it is estimated that by 2050 that drug resistant infections are likely to be the cause of death of 10.000.000 people annually if the current trend of antimicrobial resistance persists. Global efforts have generally been slow and inadequate, while antibiotic consumption has been on the rise. A report of the WHO indicated that several bacteria, including *Escherichia coli and Salmonella*, have mutated and have high resistance to current antibiotics.

In developing countries, such as Kenya, poultry farming is a flowering livestock enterprise, with surveys revealing that Kenya has ~37.000.000 birds, with about 65% of Kenyan households keeping chicken, and out of all places, Nairobi has the biggest CPP (chicken per capita) in Kenya (Omiti and Okuthe, 2009). These chickens are drugged with antibiotics to prevent and treat diseases, while previous studies have shown that bacteria swabbed from chicken rectums has shown that antimicrobial resistance levels were high.

Back to the study in Kenya. There have been 6 steps to be addressed to complete the study:

1. Sampling. Between 2017.09 and 2017.12, 150 chicken households were randomly selected from slum settings of: Kawangware, Kiberia, Mukuru, Kariobangi, Dandora and Mathare in Nairobi. From each household, fresh chicken dropping samples were collected, based on previous studies, to ensure isolation of bacteria like *Salmonella* and *Escherichia coli*.
2. Isolation and identification. ~10g of each sample was enriched in selenite F broth and buffered peptone broth overnight. Then, selenite enriched samples were subcultured onto XLD (Xylose Lysine Deoxycholate) while buffered peptone enriched samples were inoculated onto EMB agar media (Eosin Methylene Blue agar) and then were incubated at 37°C for 24 h.
3. Antimicrobial susceptibility tests. Disc diffusion methods were used to determine the antimicrobial susceptibility of isolated samples to antibiotics. Some antimicrobial agents were used, including gentamicin (10 µg) and ciprofloxacin (30 µg), and *Escherichia coli* ATCC 25922 was used as control, with results interpreted in accordance with CLSI (Clinical and Laboratory Standards Institute) guidelines.
4. Bacterial DNA extraction. For the organisms confirmed resistant to amoxicillin, samples were further subjected to molecular characterisation for the identification of resistance genes which are associated to amoxicillin resistance. The bacterial DNA was extracted with the use of boiling-centrifugal processes, with a slight change. A loop of overnight culture was suspended in 1000 µl of sterile distilled water, boiled for 15’ at 95°C in a heating block, then the resulting suspension centrifuged for 5’ at 14.000 RPM. The supernatant was used as a DNA template.
5. Characterisation of resistance genes. Resistance genes that encode resistance to: beta-lactams, TEM (temoneira), SHV (sulfhydryl variable enzyme), oxacillin and CTX-M (cefotaximiase-Munich) were screened, and polymerase chain reaction amplification was confirmed by visualisation with ethidium bromide staining of the gel.
6. Genotyping of sample carriers of TEM genes. Samples carrying TEM were analysed with (GTG) 5 genotyping methods, amplicons electrophoresed and fingerprint banding patterns recorded and analysed with GelCompar II software with UPFG arithmetic mean and dice correlation.

A few examples of aminoglycoside resistance genes discovered as of 2011, related to *Escherichia coli* and *Salmonella* are present in Table 1.

Table 1 Examples of genes which determine resistance to different bacteria

Gene name	Mechanism	Length (nt)	Accession number	Coding region	Genera
aac(3)-Ia	ACT	534	X15852	1250..1783	*Acinetobacter, Escherichia, Klebsiella, Salmonella, etc.*
aac(3)-Id	ACT	477	AB114632	104..580	*Proteus, Pseudomonas, Salmonella*
ant(2'')-Ia	NUT	543	X04555	1296.1829	*Acinetobacter, Enterobacter, Escherichia, Salmonella, etc.*

The most important bacteria which have mutated and developed resistance to antibiotics are:
-*Staphylococcus aureus*, which is methicillin resistant (MRSA);
-*Enterococcus*, which is resistant to vancomycin (VRE);
-*Mycobacterium tuberculosis*, which is multi drug resistant (MDR-TB);
-*Enterobacteriaceae gut bacteria*, which is carbapenem resistant (CRE).

RESULTS AND DISCUSSIONS

Of the 150 different dropping samples, over 57% of the samples were found to contain *Escherichia coli*, but just 12% were positive for *Salmonella*.

Escherichia coli isolates exhibited resistance to all but gentamicin and ciprofloxacin-based antibiotics. Out of 85 *Escherichia coli* samples, the highest resistance was observed to amoxicillin (54%) and the least resistance for nalidixic acid (2%) and for chloramphenicol (2%). Intermediate results were common in all antibiotics tested with a range of 1 through 24%.
Salmonella isolates differed in percentage of resistance, ½ of them being resistant to amoxicillin. Co-trimoxazole, tetracycline and streptomycin were 28%, 11% and 6% respectively, while absolutely no sample was resistant to gentamicin, nalidixic acid, ciprofloxacin and chloramphenicol.
Susceptibility profiles for both bacteria are presented in Tables 2 and 3.

Table 2. Antimicrobial susceptibility of *Escherichia coli*

Antibiotics	Susceptible No. (%)	Intermediate No. (%)	Resistant No. (%)
Streptomycin	64 (75)	13 (15)	8 (9)
Gentamicin	83 (98)	2 (2)	0 (0)
Chloramphenicol	69 (81)	14 (17)	2 (2)
Nalidixic acid	71 (84)	12 (14)	2 (2)
Ciprofloxacin	84 (99)	1 (1)	0 (0)
Tetracycline	55 (65)	20 (24)	10 (12)
Amoxicillin	23 (27)	16 (19)	46 (54)
Co-trimoxazole	52 (61)	11 (13)	22 (26)

Table 3. Antimicrobial susceptibility of Salmonella

Antibiotics	Susceptible No. (%)	Intermediate No. (%)	Resistant No. (%)
Streptomycin	12 (67)	5 (28)	1 (6)
Gentamicin	18 (100)	0 (0)	0 (0)
Chloramphenicol	18 (100)	0 (0)	0 (0)
Nalidixic acid	17 (94)	1 (6)	0 (0)
Ciprofloxacin	18 (100)	0 (0)	0 (0)
Tetracycline	11 (61)	5 (28)	2 (11)
Amoxicillin	2 (11)	7 (39)	9 (50)
Co-trimoxazole	8 (44)	5 (28)	5 (28)

The results illustrated a 57% prevalence of *E. coli* in chicken droppings from broilers and layers, which is different from other studies. In Kenya whole, a prevalence of 67 to 100% was reported in rectal swabs of indigenous and faecal samples of chicken (Wesonga et al., 2010; Adelaide et al., 2008). In Grenada, a prevalence of 99% was reported (Amadi et al., 2015). As far as *Salmonella* goes, the 12% the study revealed in Nairobi is higher than studies from the entirety of Kenya, where there is a prevalence of 3,6% from chicken rectal swabs. The difference can be attributed to many factors, including differences in hygiene practices between chicken farmers. In addition, direct transmission from humans and differences in contamination levels of different poultry feeds can be used to justify. In neighbouring Uganda, a prevalence of 21% was observed (Odoch et al., 2017).

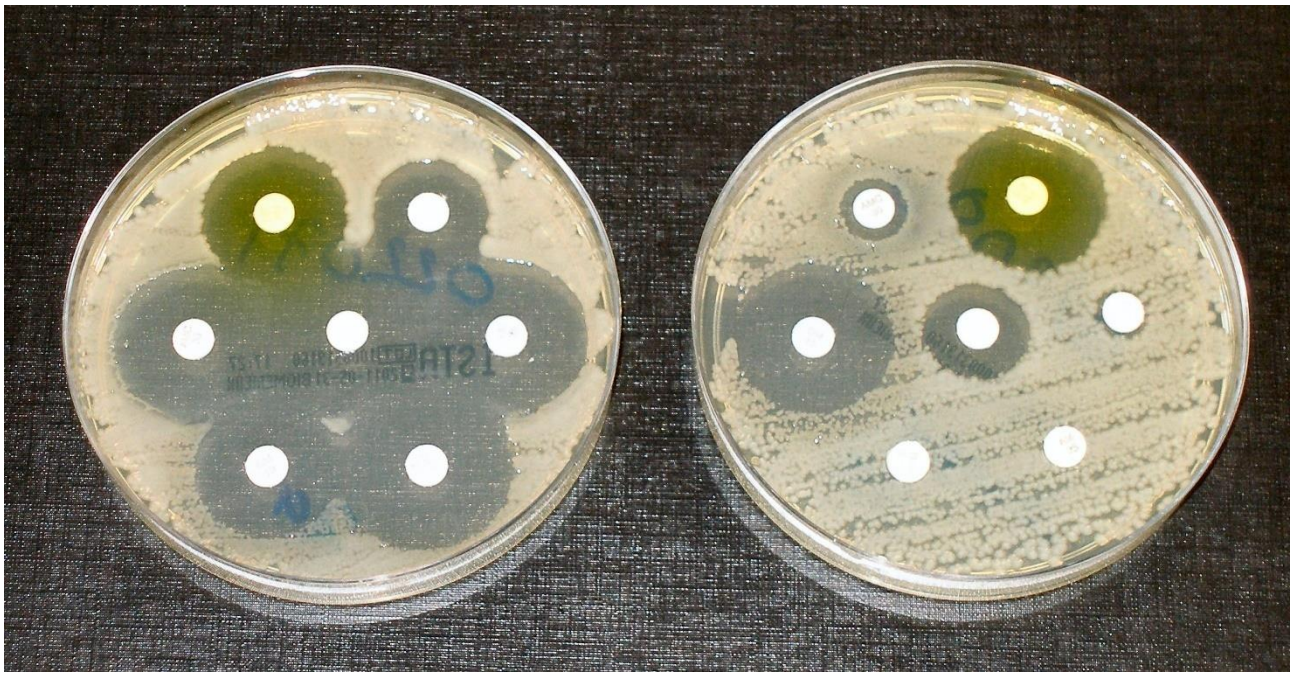

Figure 1. Antibiotic resistance tests; the bacteria in the culture on the left is sensitive to the antibiotics contained in the white paper discs. The bacteria on the right are resistant to most of the antibiotics.

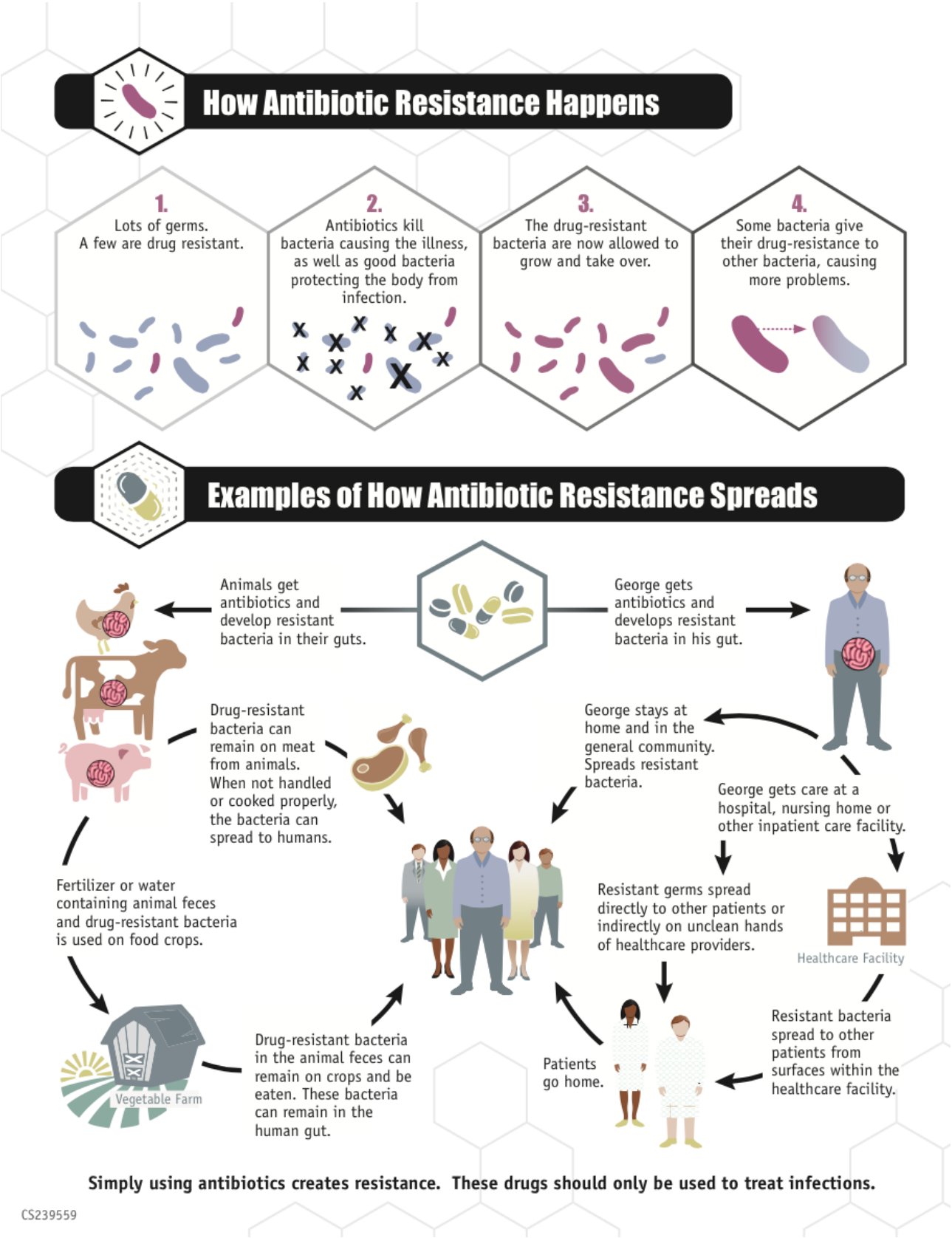

Figure 2. How antibiotic resistance evolves and spreads.

CONCLUSION

Antibiotics: naturally, semi-synthetically or synthetically produced substances, are vital in the agricultural, veterinary, and clinical environments. These substances are endowed with bacteriostatic (inhibitory) or bactericidal activities necessary for prophylactic and therapeutic purposes in the lives of humans and animals. All for one purpose: to prevent and

treat diseases. Regardless of these beneficial roles, associated with antibiotics use are also side-effects; in addition to their implementation at a sub-therapeutic level as growth promoters in feed and water consumed by livestock over an extended period. These may lead to antibiotic pollution, resulting in antibiotic residues in animal-derived products, including meat, milk, eggs, and edible tissues, and when consumed by humans, can cause direct toxicity, the development and emergence of antibiotic-resistant strains of bacteria, as well as therapeutic failure in clinical cases (Vishnuraj et al., 2016).

Of great concern are the food/waterborne pathogens responsible for life-threatening and difficult-to-treat gastro-intestinal infections in humans thus, of great concern to public healthcare systems worldwide. Nevertheless, following the chronicles of multidrug resistance of these pathogens in the developing countries, the conclusion is that continuous surveillance of the antibiotic resistance profiles of bacterial pathogens (obtained from humans, animals, food, and other environmental sites) of public and environmental significance must be implemented because of the high burden of infectious diseases plaguing or soon-to-be-plaguing humans, their habitat, and ultimately altering their day to day life.

REFERENCES

Adelaide O. A., Bii C., Okemo P., 2008. Antibiotic resistance and virulence factors in Escherichia coli from broiler chicken slaughtered at Tigoni processing plant in Limuru, Kenya. East Afr Med J.; 85(12):597–606.

Amadi V. A., Watson N., Onyegbule O. A., Matthew-Belmar V., Avendano E., Tiwari K., Sharma R., Hariharan H., 2015. Antimicrobial resistance profiles of Escherichia coli recovered from feces of healthy free-range chickens in Grenada, West Indies. Int J Curr Appl Sci., 4(6):168–75.

Clinical Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing: 16th informational supplement, M100-S24, 2014, Wayne, PA, USA

Dar O. A., Hasan R., Schlundt J., Harbarth S., Caleo G., Dar F.K., Littmann J., Rweyemamu M., Buckley E.J., Shahid M., Kock R., 2016. Exploring the evidence base for national and regional policy interventions to combat resistance. Lancet ;387(10015):285–95.

Klein E. Y., Van Boeckel T. P., Martinez E. M., Pant S., Gandra S., Levin S. A., Goossens H., Laxminarayan R., 2018. Global increase and geographic convergence in antibiotic consumption between 2000 and 2015. Proc Natl Acad Sci., (115)15, doi: 10.1073/pnas.1717295115.

Manyi-Loh C., Mamphweli S., Meyer E., Okoh A., 2018. Antibiotic use in agriculture and its consequential resistance in environmental sources: potential public health implications. Molecules, 23(4):795.

Odoch T, Wasteson Y, L'Abée-Lund T., Muwonge A., Kankya C., Nyakarahuka L., Tegule S, Skjerve E., 2017. Prevalence, antimicrobial susce.ptibility and risk factors associated with non-typhoidal Salmonella on Ugandan layer hen farms. BMC Veterinary Research.;13(1):365.

Omiti J. M, Okuthe S. O., 2009. An overview of the poultry sector and status of Highly Pathogenic Avian Influenza (HPAI) in Kenya: Background Paper. International Food Policy Research Institute (IFPRI).

O'Neill J., 2016. Tackling drug-resistant infections globally: final report and recommendations. In: Review of antimicrobial resistance. London: HM Government and Wellcome trust.

Pourmand A., Mazer-Amirshahi M., Jasani G., May L., 2017. Emerging trends in antibiotic resistance: implications for emergency medicine. Am J Emerg Med. 35(8):1172–6.

Vishnuraj M. R., Kandeepan G., Rao K. H., Chand S., Kumbhar V., 2016. Occurrence, public health hazards and detection methods of antibiotic residues in foods of animal origin: A comprehensive review. Cogent Food Agric.;2:1235458. doi: 10.1080/23311932.2016.1235458

Wesonga S. M., Muluvi G. M., Okemo P.O., Kariuki S., 2010. Antibiotic resistant Salmonella and Escherichia coli isolated from indiginous Gallus domesticus in Nairobi, Kenya. East Afr. Med J., 87(5):205–10.

World Health Organization. Worldwide country situation analysis: response to antimicrobial resistance. Geneva: World Health Organization; 2015.

World Health Organisation. Global antimicrobial resistance surveillance system (GLASS) report. http://www.who.int/glass/resources/publications/early-implementation-report/en/. Accessed 29 Jan 2018.

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3202223/

https://en.m.wikipedia.org/wiki/File:Antibiotic_sensitvity_and_resistance.JPG, licensed under the Creative Commons Attribution-Share Alike 4.0 International license.

https://en.m.wikipedia.org/wiki/Antimicrobial_resistance#/media/File%3ACDCinfographicANTIBIORESISTANCE.pngThis image is a work of the Centers for Disease Control and Prevention, part of the United States Department of Health and Human Services, taken or made as part of an employee's official duties. As a work of the U.S. federal government, the image is in the public domain.