International Journal of Pharmacy Teaching & Practices 2013, Vol.4, Issue 4, 768-774.

Glimpse of Usage of Antimicrobial Agents and its Unwanted Effects

Zubair Khalid Labu, Khurshid Jahan, Afsana Afrin
Department of Pharmacy, World University of Bangladesh (WUB)

Review Article

Please cite this paper as: Zubair Khalid Labu, Khurshid Jahan, Afsana Afrin. Glimpse of Usage of Antimicrobial Agents and its Unwanted Effects. IJPTP, 2013, 4(4), 786-774.

Corresponding Author:

Zubair Khalid Labu
Department of Pharmacy, World University of Bangladesh (WUB)
Zubair.labu@yahoo.com
Mobile#01558388956

Abstract

An antibiotic is given for the treatment of an infection caused by bacteria, fungi and parasites. However, they are not effective against viruses. Antibiotics are usually taken by mouth (orally); they can also be administered by injection, or applied directly to the affected part of the body. They are used for the treatment of bacterial infection, protozoan infection, periodontal inflammation, autoimmune disease. The most common side-effects of antibiotics are diarrhea, Feeling and being sick, fungal infections of the mouth, digestive tract and vagina. Due to misuse and overuse of antibiotics may cause antibiotic resistance and also produce super infection.

Keywords: *Antibiotic, side effects, antibiotic resistance, super infection.*

Introduction

Antibiotics, also known as antibacterial, are types of medications that destroy or slow down the growth of bacteria. The Greek word *anti* means "against", and the Greek word *bios* means "life" (bacteria are life forms).Antibiotics are used to treat infections caused by bacteria. Bacteria are microscopic organisms, some of which may cause illness. Such illnesses as syphilis, tuberculosis, salmonella, and some forms of meningitis are caused by bacteria [2]. Some bacteria are harmless, while others are good for us. White blood cells that attack harmful bacteria, our immune system can usually cope and fight off the infection. The first antibiotic was penicillin. Such penicillin-related antibiotics as ampicillin, amoxicillin and benzylpenicilllin are widely used today to treat a variety of infections - these antibiotics have been around for a long time.

With advances in medicinal chemistry, most of today's antibacterial agents are chemically or semi synthetically modifications of various natural compounds [3]. These include; the beta lactam antibacterials such as Penicillins (produced by fungi in the genus Penicillium), the cephalosporins and the carbapenems. Compounds that are still isolated from living organisms are the aminoglycosides, whereas other antibacterial for example, the sulphonamides, the quinolones are produced solely by chemical synthesis. In accordance with this, many antibacterial compounds are classified on the basis of chemical origin into natural, semisynthetic, and synthetic. Another classification system is based on biological activity; in this classification, antibacterial are divided into two broad groups according to their biological effect on microorganisms.

Production: The production of antibiotics has been widespread since the pioneering efforts of Florey and Chain in 1938. The importance of antibiotics to medicine has led to much research into their discovery and production. Despite the wide variety of known antibiotics, less than 1% of antimicrobial agents have medical or commercial value. Useful antibiotics are often discovered using a screening process. To conduct such a screen, isolation of many different microorganisms are cultured and then tested for production of diffusible products that inhibit the growth of test organisms [2]. Most antibiotics identified in such a screen are already known and must therefore be disregarded. The remainder must be tested for their selective toxicities and therapeutic activities, and the best candidates can be examined and possibly modified. A more modern version of this approach is a rational design program. This involves screening directed towards finding new natural products that inhibit a specific target, such as an enzyme only found in the target pathogen, rather than tests to show general inhibition of a culture.

Industrial production techniques: Antibiotics are produced industrially by a process of fermentation, where the source microorganism is grown in large containers (100,000–150,000 liters or more) containing a liquid growth medium. Oxygen

concentration, temperature, pH and nutrient levels must be optimal, and are closely monitored and adjusted if necessary. As antibiotics are secondary metabolite, the population size must be controlled very carefully to ensure that maximum yield is obtained before the cells die. Once the process is complete, the antibiotic must be extracted and purified to a crystalline product. This is simpler to achieve if the antibiotic is soluble in organic solvent. Otherwise it must first be removed by ion exchange, adsorption or chemical precipitation.

Strains used for production: Microorganisms used in fermentation are rarely identical to the wild type. This is because species are often genetically modified to yield the maximum amounts of antibiotics. Mutations often used, and is encouraged by introducing mutagens such as ultraviolet radiation, x-rays or certain chemicals. Selection and further reproduction of the higher yielding strains over many generations can raise yields by 20-fold or more. Another technique used to increase yields is gene amplification.

What are antibiotics for?

An antibiotic is given for the treatment of an infection caused by bacteria. Antibiotics target microorganisms such as bacteria, fungi and parasites. However, they are not effective against viruses. Most upper respiratory tract infections, such as the common cold and sore throats are generally caused by viruses - antibiotics do not work against these viruses.

How to use antibiotics?

Antibiotics are usually taken by mouth (orally), however, they can also be administered by injection, or applied directly to the affected part of the body. It is important to remember to complete the whole course of the medication to prevent the infection from coming back. If the course is not completed, there is a higher chance the bacteria may become resistant to future treatments. Some antibiotics should not be consumed with certain foods and drinks. Others should not be taken with food in stomach - these would normally be taken about an hour before meals, or two hours after. It is crucial to follow the instructions correctly if we want the medication to be effective. In case of metronidazole, alcohol should not consume. Dairy products should not be consumed in case of tetracycline, as they might affect the absorption of the medication.

Medical uses: Bacterial infection, Protozoan infection e.g., metronidazole is effective against several parasites, Immunomodulation e.g., tetracycline which is effective in periodontal inflammation and dapsone which is effective in autoimmune disease such as oral mucous membrane pemphigoid, Prevention of infection ,Surgical wound, Dental antibiotic prophylaxis, Conditions of neutropenia e.g. cancer-related[3].

Rational use of antibiotics:

1. Right diagnosis should be made either clinically or by laboratory.
2. Right decision should be made whether the chemotherapy is needed or not.
3. Proper selection of drug— Specificity, Routes of administration, Cost effectiveness, Safe drug, Proper combination, Easy availability, Essential drug (drugs needed for the vast majority of the population—ORS, Paracetamol)
4. Right dose—usually we give initially loaded dose followed by maintenance dose.
5. Right duration—at least 3-5 days antibiotic should be continued.
6. Right time schedule—to maintain MIC and MBC.
7. Status of the patient—Age of the patient, Hepatic and renal function, Pregnancy, Lactating mother, immune system of the patient, Site of infection

Common side-effects of antibiotics: The most common side-effects of antibiotics are as follows- Diarrhea, feeling and being sick, fungal infections of the mouth, digestive tract and vagina [4]

The rare side-effects of antibiotics- Formation of Kidney stones (when taking sulphonamides), Abnormal blood clotting (when taking some cephalosporins), Sensitivity to sun (when taking tetracyclines), Blood disorders (when taking trimethoprim), Deafness (when taking erythromycin and the aminoglycosides*)*

Classification: The main classes of antibiotics are:

- Beta-Lactams: Penicillins, Cephalosporins
- Macrolides,
- Fluoroquinolones,
- Tetracyclines,
- Aminoglycosides

Penicillins: The penicillin's are the oldest class of antibiotics.

There are four types of penicillin:

- ***The natural penicillins*** are based on the original penicillin-G structure. Penicillin-G types are effective against gram-positive strains of streptococci, staphylococci, and some gram-negative bacteria such as meningococcus.
- ***Penicillinase-resistant penicillins***, notably methicillin and oxacillin, are active even in the presence of the bacterial enzyme that inactivates most natural penicillins.
- ***Aminopenicillins*** such as ampicillin and amoxicillin have an extended spectrum of action compared with the natural penicillins. Extended spectrum penicillins are effective against a wider range of bacteria.

Penicillins side effects: Penicillins are among the least toxic drugs known. The most common side effect of penicillin is diarrhea. Nausea, vomiting, and upset stomach are also common. In rare cases penicillins can cause immediate and delayed allergic

reactions - specifically, skin rashes, fever, and anaphylactic shock. Penicillins are classed as category B during pregnancy.

Generation	Microbial coverage	Drawback	Examples	Dosage form
First	• Good gram positive activity; cover all staphylococcal & streptococcal pathogen • Relatively modest gram negative activity; cover greatly uropathogen	Inactivated by gram negative beta lactamases	• cephalothin • cefazolin • cephapirin • cephradine • cephalexin • cefadroxil	Only oral form is available
Second	• Good gram positive & gram negative activity; cover all streptococcal pathogen & having decrease coverage against staphylococcal pathogen • Cover all uropathogen and respiratory pathogen • Better beta-lactamase enzyme resistant activity than 1st generation	Less staphylococcal activity	• cefaclor • cefamandole • cefonicid • ceforanide • cefuroxime	Both oral & Injectable form is available
Third	• wide range of coverage against only streptococcal pathogen • No coverage against staphylococcal pathogen • Much more active than 1st & 2nd generation against the Enterobacteriaceae and Pseudomonus aeruginosa • Cover all uropathogen, respiratory and typhi & paratyphi pathogen • Better beta-lactamase enzyme resistant activity than 2nd generation	Less active against gram positive cocci	• cefcapene • cefdaloxime • cefditoren • cefetamet • cefixime • cefmenoxime • cefodizime • cefoperazone • cefpimizole • cefpodoxime • ceftibuten • ceftriaxone	Both oral & Injectable form is available
Fourth	• Broadest action against the Enterobacteriaceae and Pseudomonus aeruginosa • Good gram positive activity; cover all staphylococcal & streptococcal pathogen • Better beta-lactamase enzyme resistant activity than 3rd generation • Many fourth generation cephalosporins can cross blood brain barrier and are effective in meningitis	Less anaerobic activity	• cefclidine • cefepime • cefluprenam • cefozopran • cefpirome • cefquinome	Only Injectable form is available
Fifth generation	• Activity against methicillin-resistant *Staphylococcus aureus*, penicillin-resistant *Streptococcus pneumoniae*, *Pseudomonas aeruginosa*, and enterococci. It was discovered by Basilea Pharmaceutica and was developed by Johnson & Johnson Pharmaceutical Research and Development.		Ceftobiprole Ceftaroline	Only Inject able form is available

Cephalosporins: Cephalosporins have a mechanism of action identical to that of the penicillins. However, the basic chemical structure of the penicillins and cephalosporins differs in other respects, resulting in some difference in the spectrum of antibacterial activity. Like the penicillins, cephalosporins have a beta-lactam ring structure that interferes with synthesis of the bacterial cell wall and so are bactericidal. Cephalosporins are derived from cephalosporin C which is produced from Cephalosporium acremonium. Cephalosporins are used to treat pneumonia, strep throat, staph infections, tonsillitis, bronchitis, otitis media, various types of skin infections, gonorrhea, urinary tract infections Cephalosporin antibiotics are also commonly used

for surgical prophylaxis. Cephalexin can also be used to treat bone infections. Cephalosporins are among the most diverse classes of antibiotics, they are grouped into "generations" by their antimicrobial properties. Each newer generation has a broader spectrum of activity than the one before.

Cephalosporins side effects: Cephalosporins generally cause few side effects. Common side effects associated these drugs include: diarrhoea, nausea, mild stomach cramps or upset. Approximately 5–10% of patients with allergic hypersensitivity to penicillins will also have cross-reactivity with cephalosporins. Thus, cephalosporin antibiotics are contraindicated in people with a history of allergic reactions (urticaria, anaphylaxis, interstitial nephritis, etc) to penicillins or cephalosporins. Cephalosporin antibiotics are classed as pregnancy category B.

Fluoroquinolones: Fluoroquinolones (fluoridated quinolones) are the newest class of antibiotics. Their generic name often contains the root "floxacin". They are synthetic antibiotics, and not derived from bacteria. Fluoroquinolones belong to the family of antibiotics called quinolones. The older quinolones are not well absorbed and are used to treat mostly urinary tract infections. The newer fluoroquinolones are broad-spectrum bacteriocidal drugs that are chemically unrelated to the penicillins or the cephalosporins. Because of their excellent absorption fluoroquinolones can be administered not only by intravenous but orally as well. Fluoroquinolones are used to treat most common urinary tract infections, skin infections, and respiratory infections (such as sinusitis, pneumonia, bronchitis). Fluoroquinolones inhibit bacteria by interfering with their ability to make DNA. This activity makes it difficult for bacteria to multiply.

Fluoroquinolone groupe includes: Ciprofloxacin, levofloxacin, lomefloxacin, norfloxacin, sparfloxacin, clinafloxacin, gatifloxacin, ofloxacin, trovafloxacin

Fluoroquinolones side effects: Fluoroquinolones are well tolerated and relatively safe. The most common side effects include nausea, vomiting, diarrhea, abdominal pain. Other more serious but less common side effects are central nervous system effects (headache, confusion and dizziness), phototoxicity (more common with lomefloxacin and sparfloxacin). All drugs in this class have been associated with convulsions. Fluoroquinolones are classed as pregnancy category C.

Tetracyclines: Tetracyclines got their name because they share a chemical structure that has four rings. They are derived from a species of Streptomyces bacteria. Tetracycline antibiotics are broad-spectrum bacteriostatic agents, which inhibit bacterial protein synthesis. Tetracyclines may be effective against a wide variety of microorganisms; including rickettsia and amebic parasites.Tetracyclines are used in the treatment of infections of the respiratory tract, sinuses, middle ear, urinary tract, skin, intestines. Tetracyclines also are used to treat Gonorrhea, Rocky Mountain spotted fever, Lyme disease, and Typhus. Their most common current use is in the treatment of moderately severe acne and rosacea.

Tetracycline antibiotics are: Tetracycline, Doxycycline, Minocycline, Oxytetracycline

Tetracyclines side effects: Drugs in the tetracycline class become toxic over time. Expired drugs can cause a dangerous syndrome resulting in damage to the kidneys. Common side effects associated with tetracycline include cramps or burning of the stomach, diarrhea, sore mouth or tongue. Tetracycline can cause skin photosensitivity, which increases the risk of sunburn under exposure to UV light. This may be of particular importance for those intending to take on holiday's long-term doxycycline as a malaria prophylaxis. Rarely, tetracycline may cause allergic reactions. Very rarely severe headache and vision problems may be signs of dangerous secondary intracranial hypertension. Tetracycline antibiotics should not be used in children under the age of 8, and specifically during periods of tooth development. Tetracyclines are classed as pregnancy category D. Use during pregnancy may cause alterations in bone development.

Macrolides: The macrolide antibiotics are derived from Streptomyces bacteria, and got their name because they all have a macrocyclic lactone chemical structure. The macrolides are bacteriostatic, binding with bacterial ribosomes to inhibit protein synthesis. Erythromycin, the prototype of this class, has a spectrum and use similar to penicillin. Newer members of the group, azithromycin and clarithyromycin, are particularly useful for their high level of lung penetration. Macrolide antibiotics are used to treat respiratory tract infections (such as pharyngitis, sinusitis, and bronchitis), genital, gastrointestinal tract, and skin infections. Macrolide antibiotics are: erythromycin, clarithromycin, azithromycin, dirithromycin, roxithromycin, troleandomycin.

Macrolides side effects: Side effects associated with macrolides include nausea, vomiting, and diarrhea; infrequently, there may be temporary auditory impairment. Azithromycin has been rarely associated with allergic reactions, including angioedema, anaphylaxis, and dermatologic reactions. Oral erythromycin may be highly irritating to the stomach and when given by injection may cause severe phlebitis. Macrolide antibiotics should be used with caution in patients with liver dysfunction. Pregnancy category B: Azithromycin, erythromycin. Pregnancy category C.

Aminoglycosides: Aminoglycosides may be used along with penicillins or cephalosporins to give a

two-pronged attack on the bacteria. Aminoglycosides work quite well, but bacteria can become resistant to them. Since aminoglycosides are broken down easily in the stomach, they can't be given by mouth and must be injected. Generally, aminoglycosides are given for short time periods. The amino glycosides are drugs which stop bacteria from making proteins. This effect is bactericidal.

Aminoglycoside: Amikacin, gentamicin, kanamycin, neomycin, streptomycin, tobramycin

Aminoglycosides side effects: The major irreversible toxicity of aminoglycosides is ototoxicity that is damage to the ear and hearing. Among them, streptomycin and gentamicin are primarily vestibulotoxic, whereas amikacin, neomycin, dihydrosterptomycin, and kanamicin are primarily cochleotoxic. Another important concern with aminoglycoside antibiotics is nephrotoxicity that is kidney damage [5].

Five Basic Mechanisms of Antibiotic Action against Bacterial Cells [5]:

- Inhibition of Cell Wall Synthesis (most common mechanism)
- Inhibition of Protein Synthesis (Translation) (second largest class)
- Alteration of Cell Membranes
- Inhibition of Nucleic Acid Synthesis
- Antimetabolite Activity

Concepts in antibiotic pharmacology:

Blind therapy: Initial or Blind or Umbrella or Empirical therapy refers to the treatment of an infection without knowing the causative pathogen. This will refer to the first presentation of an infected patient, where the clinician must decide which antibiotics to use prior to laboratory confirmation.

Bacteriostatic: A drug that inhibits growth or development of a bacterium, rather than directly killing it. Bacteriostatic drugs depend upon the immune system of the patient for activity, and so make poor choices where the immune system is compromised, for example patients with AIDS. Certain bacteriostatic drugs may be preferable in cases of streptococcal and clostridial gangrene, because they inhibit the production of the toxins that cause much of the morbidity.

MIC (minimum inhibitory concentration) is the minimum concentration of drug which can inhibit the growth of the microorganism.

Bactericidal: A drug that directly kills a bacterium. The **MBC** (minimum bactericidal concentration) is the minimum concentration of drug which can kill the microorganism.

Chemotherapeutic Spectrum / Antimicrobial Spectrum: The range of bacteria that an antibiotic affects, divided into Narrow Spectrum and Broad Spectrum.

- Narrow spectrum antibiotics act against a limited group of bacteria, for example sodium fusidate only acts against *staphylococcal* bacteria.
- Broad spectrum—antibiotics act against a larger group of bacteria, for example amoxicillin.

Combination therapy: The use of two or more agents simultaneously. This combination can produce [6]:

- An additive effect
- Synergistic effect, where the effect of using both agents together is greater than the sum of the effects of the drugs. This can be seen in
- Co-trimoxazole
- Co-amoxiclav
- Penicillin and an Aminoglycoside for endocarditis
- Antagonism, where the combination reduces the activity of each antibitoic. This is seen where a bacteriostatic and a bactericidal drug are used in combination.

Reasons why this may be preferred include:

- To avoid the development of resistance, particularly in bacteria
- To provide broad coverage in polymicrobial infection
- Severe infections where the cause is unknown, or in empirical therapy.
- Synergy in specific infections
- Where the pathogen cannot be easily killed and prevention of emergence of resistance. (Tuberculosis, Leprosy)

Reasons why combination therapy might not be preferred include:

- Increased toxicity
- Antagonism - the two agents interact resulting in reduced antibiotic effect
- Increased cost

Some agents are available in combination within a single pharmaceutical preparation, for example co-amoxiclav contains both the active agent "amoxicillin" and the enzyme inhibitor "clavulanic acid", which extends the spectrum of amoxicillin.

Superinfection[1]: It is a phenomenon may be defined as appearance of bacteriological and clinical evidence of new infection during the chemotherapy of a primary one.

Some causative organism of Superinfection

- Candida or fungal infection commonly
- Enterobacteriaceae (Shigella, Salmonella, Escherichia, Klebsiella)
- Pseudomonas
- Staphylococcus

Mechanism of Action of superinfection: Normal bacterial flora like *E. coli* produces vit-K. But antibiotics can destroy the flora, those who are sensitive to that antibiotic and there is imbalance of the flora. Then there is development of endogenous bacteria and overgrowth of micro-organism. So, there is another infection called Superinfection.Superinfections mostly occurs in broad spectrums. The incidence of superinfection can be reduced by using a narrow spectrum, such as benzylpenicillin, in preference to a broad spectrum antibiotic, such as cefotaxime.

Person liable to super infection: Patient less than 3 years old, those with middle ear infection lower respiratory tract infection, those treated with broad spectrum drugs or combination.

Sites involved in superinfection: Oropharynx, Intestinal, Respiratory, Gastrointestinal tract, Skin.

Clinical features of superinfection: Stomatitis, Glossitis, Cheilosis, Soft and liquid stool that may contain blood, mucous and large number of polymorphonucer leukocytes, White coating of the tongue, Whole GIT infection.

Treatment of superinfection: Discontinuation of antibiotics therapy, Culture of stool and then using proper antibiotic sensitiveto organism responsible for super infection, Vitamin B complex,

Allergic reactions to antibiotics: Some patients may develop an allergic reaction to antibiotics - especially penicillins. Side effects might include a rash, swelling of the tongue and face, and difficulty breathing. Reactions to antibiotics can be very serious and sometimes fatal - they are called anaphylactic reactions.

Resistance:

Causes:

1) Due to misuse and overuse of antibiotics.

2) In some countries, antibiotics are sold over the counter without a prescription, which also leads to the creation of resistant strains.

3) Also unsound practices in the pharmaceutical manufacturing industry can contribute towards the likelihood of creating antibiotic-resistant strains.

4) Certain antibiotic classes are highly associated with colonisation with "superbugs" (highly antibiotic resistant bacteria) compared to other antibiotic classes. The risk for colonisation increases if there is a lack of sensitivity (resistance) of the superbugs to the antibiotic used and high tissue penetration, as well as broad-spectrum activity against "good bacteria".

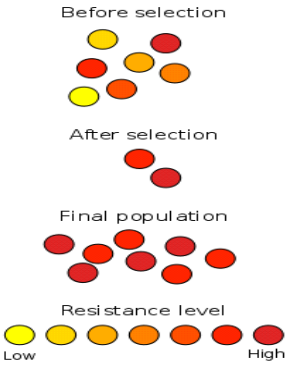
5) In the case of MRSA, increased rates of MRSA infections are seen with glycopeptides, cephalosporins and especially quinolone. In the case of colonisation with *Clostridium difficile* the high risk antibiotics include cephalosporins and in particular quinolones and clindamycin.

Mechanisms of Resistance: Antibiotic resistance can be a result of horizontal gene transfer [8] and also of unlinked point mutations in the pathogen genome at a rate of about 1 in 10^8 per chromosomal replication. The antibiotic action against the pathogen can be seen as an environmental pressure. Those bacteria with a mutation that allows them to survive live to reproduce. They then pass this trait to their offspring, which leads to the evolution of a fully resistant colony.

The four main mechanisms by which microorganisms exhibit resistance to antimicrobials are:

- Drug inactivation or modification: for example, enzymatic deactivation of *penicillin G* in some penicillin-resistant bacteria through the production of Beta-lactamases.
- Alteration of target site: for example, alteration of PBP—the binding target site of penicillins—in MRSA and other penicillin-resistant bacteria
- Alteration of metabolic pathway: for example, some Sulfonamide-resistant bacteria do not require (PABA), an important precursor for the synthesis of folic acid and nucleic acids in bacteria inhibited by sulfonamides, instead, like mammalian cells, they turn to using preformed folic acid.
- Reduced drug accumulation: by decreasing drug permeability and/or increasing active efflux (pumping out) of the drugs across the cell surface [7].

Flow chart of the Mechanisms of Resistance:



Schematic representation of how antibiotic resistance evolves via natural selection. The top section represents a population of bacteria before exposure to an antibiotic. The middle section shows the population directly after exposure, the phase in which selection took place. The last section shows the distribution of resistance in a new generation of bacteria. The legend indicates the resistance levels of individuals.

Resistant pathogens: Salmonella and E. coli,Streptococcus and Enterococcus,Pseudomonas aeruginosa,Clostridium difficile,Mycobacterium tuberculosis*:*

Alternatives: The increase in bacterial strains that are resistant to conventional antibacterial therapies has prompted the development of alternative strategies to treat bacterial diseases.

Resistance-modifying agents: One strategy to address bacterial drug resistance is the discovery and application of compounds that modify resistance to common antibacterials. For example, some resistance-modifying agents may inhibit multidrug resistance mechanisms, such as drug efflux from the cell, thus increasing the susceptibility of bacteria to an antibacterial. Targets include: The efflux inhibitor Phe-Arg-β-naphthylamide. Beta-lactamase inhibitors, such as clavulanic acid and sulbactam.Metabolic stimuli such as sugar can help eradicate a certain type of antibiotic tolerant bacteria by keeping their metabolism active.

Conclusion[8]

Antibiotics are powerful medicines that fight bacterial infections. Used properly, antibiotics can save lives. They either kill bacteria or keep them from reproducing. At the time of taking antibiotics, follow the directions carefully. It is important to finish medicine even feel better. If stop treatment too soon, some bacteria may survive and re-infect.

References

1. Apex 'Medical Pharmacology; Dr. Javed Yusuf, 6th edition
2. Bosch F, Rosich L (2008). "The contributions of Paul Ehrlich to pharmacology: a tribute on the occasion of the centenary of his Nobel Prize". *Pharmacology* **82** (3): 171–9.
3. Calderon CB, Sabundayo BP (2007). Antimicrobial Classifications: Drugs for Bugs. In Schwalbe R, Steele-Moore L, Goodwin AC. Antimicrobial Susceptibility Testing Protocols. CRC Press. Taylor & Frances group. ISBN 978-0-8247-4100-6
4. HW Florey (1945). "Use of Micro-organisms for therapeutic purposes". *Br Med J.* 2 (4427): 635–642. doi:10.1136/bmj.2.4427.635.
5. Lindblad WJ (2008). "Considerations for Determining if a Natural Product Is an Effective Wound-Healing Agent". *International Journal of Lower Extremity Wounds* **7** (2): 75–81.
6. Li, X; Nikadio, H (2009). "Efflux-Mediated Drug Resistance in Bacteria: an Update". *Drug* **69** (12): 1555–623.
7. Ochiai, K.; Yamanaka, T; Kimura, K; Sawada, O (1959). "Inheritance of drug resistance (and its transfer) between Shigella strains and Between Shigella and E.coli strains". *Hihon Iji Shimpor,* (in Japanese) **34**: 1861
8. www.Medicine plus; Centers for Disease Control and Prevention

AUTHORS' CONTRIBUTIONS

Authors contributed equally to all aspects of the study.

PEER REVIEW

Not commissioned; externally peer reviewed.

CONFLICTS OF INTEREST

The authors declare that they have no competing interests.