**Exercise 3E: Antibiogram / Antibiotic Testing**

**Group 5:**

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**Introduction**

Antibiogram is a laboratory test used to determine the sensitivity pattern of a given microorganism to a range of antibiotics. The advantages of antibiogram use and the techniques involved in running these tests are well known.  An antibiogram is also the result of a laboratory testing for the sensitivity of an isolated [bacterial strain](http://en.wikipedia.org/wiki/Bacterial_strain) to different [antibiotics](http://en.wikipedia.org/wiki/Antibiotics). It is by definition an [in vitro](http://en.wikipedia.org/wiki/In_vitro)-sensitivity.

**Objectives**

* To review the antimicrobial susceptibility test and the standards behind the test
* To define a rational selection of an empirical antimicrobial therapy for treating patients with hospital-acquired infections.

**Results**

**Set A (Positive / negative result of 4 different antibiotics)**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Name** | **SXT 25** | **DA 2** | **C 30** | **S 10** |
| **A 1** | + | - | + | - |
| **A 2** | + | - | + | + |
| **A 21** | + | + | + | + |
| **A 22** | + | - | - | + |
| **S-CR 8** | - | + | + | + |
| **S-CR 9** | - | + | + | + |
| **S-CR 12** | + | + | + | + |
| **S-CR 13** | + | + | + | + |

**Set B (Positive / negative result of 5 different antibiotics)**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Name** | **AK** | **RA** | **KF** | **OX** | **P** |
| **A 1** | + | + | - | - | - |
| **A 2** | + | + | - | - | + |
| **A 21** | + | + | + | - | + |
| **A 22** | + | + | + | - | - |
| **S-CR 8** | + | + | - | - | - |
| **S-CR 9** | + | + | + | - | + |
| **S-CR 12** | + | + | + | + | Slightly +/- |
| **S-CR 13** | + | + | - | - | - |

**Set A Zone of Inhibition in millimeters (mm)**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Name** | **SXT 25** | **DA 2** | **C 30** | **S 10** |
| **A 1** | 27.31 | - | 20.87 | - |
| **A 2** | 26.68 | - | 16.73 | 17.85 |
| **A 21** | 45.96 | 21.44 | 19.37 | 21.64 |
| **A 22** | 20.93 | - | - | 19.87 |
| **S-CR 8** | - | 22.91 | 33.47 | 19.97 |
| **S-CR 9** | - | 25.58 | 33.73 | 22.01 |
| **S-CR 12** | 26.10 | 27.5 | 26.61 | 28.33 |
| **S-CR 13** | 24.31 | 18.82 | 29.11 | 22.97 |

**Set B Zone of Inhibition in millimeters (mm)**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Name** | **AK** | **RA** | **KF** | **OX** | **P** |
| **A 1** | 23.70 | 16.05 | - | - | - |
| **A 2** | 34.60 | 13.28 | - | - | 14.36 |
| **A 21** | 24.22 | 11.58 | 14.42 | - | 26.30 |
| **A 22** | 22.58 | 13.62 | 13.52 | - | - |
| **S-CR 8** | 26.62 | 15.59 | - | - | - |
| **S-CR 9** | 34.89 | 11.20 | 31.63 | - | 48.19 |
| **S-CR 12** | 31.78 | 15.62 | 20.81 | 14.59 | Slightly +/- (10.71) |
| **S-CR 13** | 22.66 | 13.70 | - | - | - |

**Discussion**

* **Sulfamethoxazole-trimethoprim (SXT) –** This medication is a combination of two antibiotics used to treat a wide variety of bacterial infections (e.g., middle ear, urine, respiratory and intestinal infections). It is also used to prevent and treat a certain type of [pneumonia](http://www.medicinenet.com/pneumonia_facts/article.htm) (pneumocystis-type).This medication should not be used in children less than 2 months of age due to the risk of serious side effects. This medication treats only certain types of infections. It will not work for viral infections (like [flu](http://www.medicinenet.com/influenza/article.htm)). Unnecessary use or misuse of any antibiotic can lead to its decreased effectiveness.
* **Clindamycin (DA) –** It is an antibiotic that fights bacteria in the body. Clindamycin is used to treat serious infections caused by bacteria and also used for other purposes.
* **Chloramphenicol (C) –** It is used in severe, potentially life-threatening infections, such as blood poisoning or [meningitis](http://www.netdoctor.co.uk/infections/meningitis.htm), particularly those caused by *Haemophilus influenza* and [Typhoid](http://www.netdoctor.co.uk/infections/typhoid.htm). It is known as a broad-spectrum antibiotic, which means it is effective against infections caused by a wide variety of bacteria.
* **Streptomycin (S) –** Streptomycin sulfate is a bactericidal antibiotic. It acts by interfering with normal protein synthesis. This [medication](http://www.webmd.com/drugs/index-drugs.aspx) can sometimes cause serious [nerve damage](http://www.webmd.com/brain/nerve-pain-and-nerve-damage-symptoms-and-causes), possibly resulting in permanent [hearing loss](http://www.webmd.com/a-to-z-guides/hearing-loss-causes-symptoms-treatment) and balance problems.
* **Amikacin (AK)** – It is an [aminoglycoside](http://en.wikipedia.org/wiki/Aminoglycoside) [antibiotic](http://en.wikipedia.org/wiki/Antibiotic) used to treat different types of [bacterial](http://en.wikipedia.org/wiki/Bacteria" \o "Bacteria)[infections](http://en.wikipedia.org/wiki/Infection). Amikacin works by binding to the bacterial [30S](http://en.wikipedia.org/wiki/30S) [ribosomal](http://en.wikipedia.org/wiki/Ribosome) subunit, causing misreading of [mRNA](http://en.wikipedia.org/wiki/MRNA) and leaving the bacterium unable to synthesize [proteins](http://en.wikipedia.org/wiki/Protein) vital to its growth. This [medication](http://www.webmd.com/drugs/index-drugs.aspx) can cause serious [kidney](http://www.webmd.com/urinary-incontinence-oab/picture-of-the-kidneys) problems and [nerve damage](http://www.webmd.com/brain/nerve-pain-and-nerve-damage-symptoms-and-causes), resulting in permanent [hearing loss](http://www.webmd.com/a-to-z-guides/hearing-loss-causes-symptoms-treatment) (including deafness or decreased hearing) and balance problems.
* **Rifampin (RA)** – It is a [bactericidal](http://en.wikipedia.org/wiki/Bactericidal) [antibiotic](http://en.wikipedia.org/wiki/Antibiotic) drug of the [rifamycin](http://en.wikipedia.org/wiki/Rifamycin" \o "Rifamycin) group. It is also used to treat infections caused by other types of bacteria and to prevent infection in people who have been in close contact with a person who has certain serious bacterial infections.
* **Cephalothin (KF)** – Is a cephalosporin antibiotic usedl in preventing infection during surgery and treating many kinds of infections of the blood, bone or joints, respiratory tract, skin, and urinary tract.
* **Oxacillin (OX) –** It is used to treat a wide variety of [bacterial infections](http://www.webmd.com/a-to-z-guides/bacterial-and-viral-infections) such as a staphylococcal (also called "staph") infection. This medication is known as a [penicillin](http://www.webmd.com/drugs/drug-3781-penicillin+g+benzathine+im.aspx)-type antibiotic. It works by stopping the growth of bacteria.
* **Penicillin (P) –** Penicillins are a certain collection of [antibiotics](http://www.medicalnewstoday.com/articles/10278.php) that eliminate infection causing bacteria. They originate from a type of fungi called *Penicillium* fungi. They are used in the treatment or prevention of many different bacterial infections, usually caused by Gram-positive organisms. They are well known in medicine as they are one of the first types of antibiotic used for major infections and diseases, and are still used regularly in modern medicine. Penicillins are all*β-Lactam*(Beta-Lactam) antibiotics, which are antibiotic molecules with a β-Lactam nucleus.

**Answer to Questions**

1. **Tabulate the following:**

|  |  |  |
| --- | --- | --- |
| **Name of Antibiotic** | **Mechanism of Action** | **Effectivity against which microorganisms** |
| 1. **Sulfamethoxazole-trimethoprim (SXT)** | **~** Sulfamethoxazole inhibits bacterial synthesis of dihydrofolic acid by competing with para-aminobenzoic acid (PABA). Trimethoprim blocks the production of tetrahydrofolic acid from dihydrofolic acid by binding to and reversibly inhibiting the required enzyme, dihydrofolate reductase. Thus, sulfamethoxazole and trimethoprim blocks two consecutive steps in the biosynthesis of nucleic acids and proteins essential to many bacteria. | ~ Bacterias |
| 1. **Clindamycin (DA)** | **~** Clindamycin has a primarily [bacteriostatic](http://en.wikipedia.org/wiki/Bacteriostatic) effect. It is a bacterial [protein synthesis inhibitor](http://en.wikipedia.org/wiki/Protein_synthesis_inhibitor) by inhibiting ribosomal translocation, in a similar way to [macrolides](http://en.wikipedia.org/wiki/Macrolide). It does so by binding to the [50S](http://en.wikipedia.org/wiki/50S) rRNA of the large bacterial [ribosome](http://en.wikipedia.org/wiki/Ribosome) subunit. | ~ It is most effective against infections involving the following types of organisms:   * Aerobic [Gram-positive](http://en.wikipedia.org/wiki/Gram-positive) [cocci](http://en.wikipedia.org/wiki/Cocci" \o "Cocci), including some members of the [*Staphylococcus*](http://en.wikipedia.org/wiki/Staphylococcus) and [*Streptococcus*](http://en.wikipedia.org/wiki/Streptococcus) * Anaerobic, [Gram-negative](http://en.wikipedia.org/wiki/Gram-negative) [rod-shaped bacteria](http://en.wikipedia.org/wiki/Bacteria_Morphology), including some [*Bacteroides*](http://en.wikipedia.org/wiki/Bacteroides), *[Fusobacterium](http://en.wikipedia.org/wiki/Fusobacterium" \o "Fusobacterium)*, and *[Prevotella](http://en.wikipedia.org/wiki/Prevotella" \o "Prevotella)*. |
| 1. **Chloramphenicol (C)** | **~** Chloramphenicol is a [bacteriostatic](http://en.wikipedia.org/wiki/Bacteriostatic_agent) drug that stops bacterial growth by [inhibiting protein synthesis](http://en.wikipedia.org/wiki/Protein_synthesis_inhibitor). Chloramphenicol prevents [protein chain elongation](http://en.wikipedia.org/wiki/Protein_synthesis) by inhibiting the [peptidyl transferase](http://en.wikipedia.org/wiki/Peptidyl_transferase" \o "Peptidyl transferase) activity of the bacterial [ribosome](http://en.wikipedia.org/wiki/Ribosome). It specifically binds to A2451 and A2452 residues in the [23S rRNA](http://en.wikipedia.org/wiki/23S_ribosomal_RNA) of the 50S ribosomal subunit, preventing peptide bond formation. While chloramphenicol and the [macrolide](http://en.wikipedia.org/wiki/Macrolide) class of antibiotics both interact with ribosomes, chloramphenicol is not a macrolide. It directly interferes with substrate binding, whereas macrolides sterically block the progression of the growing peptide. | ~ It is effective against a wide variety of [Gram-positive](http://en.wikipedia.org/wiki/Gram-positive_bacteria) and [Gram-negative](http://en.wikipedia.org/wiki/Gram-negative_bacteria)[bacteria](http://en.wikipedia.org/wiki/Bacteria), including most [anaerobic organisms](http://en.wikipedia.org/wiki/Anaerobic_organism). |
| 1. **Streptomycin (S)** | **~** Streptomycin is a [protein synthesis inhibitor](http://en.wikipedia.org/wiki/Protein_synthesis_inhibitor). It binds to the small 16S rRNA of the 30S subunit of the bacterial ribosome, interfering with the binding of [formyl-methionyl-tRNA](http://en.wikipedia.org/wiki/Formyl-methionyl-tRNA" \o "Formyl-methionyl-tRNA) to the 30S subunit.This leads to codon misreading, eventual inhibition of protein synthesis and ultimately death of microbial cells through mechanisms that are still not understood. Speculation on this mechanism indicates that the binding of the molecule to the 30S subunit interferes with 50S subunit association with the [mRNA](http://en.wikipedia.org/wiki/MRNA) strand. | ~ It is effective against gram-negative bacteria, although it is also used in the treatment of tuberculosis. |
| 1. **Amikacin (AK)** | **~** Inhibition of protein biosynthesis by irreversible binding of the aminoglycoside to the bacterial ribosome 30S subunit. | ~ Amikacin is highly effective against aerobic gram-negative microorganisms and some gram-positive microorganisms. Amikacin is moderately active against Streptococcus spp., it does not act on anaerobic bacteria. |
| 1. **Rifampin (RA)** | **~** Rifampicin inhibits bacterial DNA-dependent RNA synthesis by inhibiting bacterial DNA-dependent [RNA polymerase](http://en.wikipedia.org/wiki/RNA_polymerase).  Crystal structure data and biochemical data indicate that rifampicin binds to RNA polymerase at a site adjacent to the RNA polymerase active center and blocks RNA synthesis by physically preventing extension of RNA products beyond a length of 2-3 nucleotides ("steric-occlusion" mechanism).  Resistance to rifampicin arises from mutations that alter residues of the rifampicin binding site on RNA polymerase, resulting in decreased affinity for rifampicin. Resistant mutations map to the *[rpoB](http://en.wikipedia.org/wiki/RpoB" \o "RpoB)* gene, encoding RNA polymerase beta subunit. | ~ It has some effectiveness in vaccinia virus to inhibit the synthesis of host bacterial proteins during recombinant protein expression in bacteria. |
| 1. **Cephalothin (KF)** | **~** Cephalosporins are [bactericidal](http://en.wikipedia.org/wiki/Bactericidal) and have the same mode of action as other [beta-lactam antibiotics](http://en.wikipedia.org/wiki/Beta-lactam_antibiotic) (such as [penicillins](http://en.wikipedia.org/wiki/Penicillin" \o "Penicillin)) but are less susceptible to [penicillinases](http://en.wikipedia.org/wiki/Beta-lactamase" \o "Beta-lactamase). Cephalosporins disrupt the synthesis of the [peptidoglycan](http://en.wikipedia.org/wiki/Peptidoglycan) layer of bacterial [cell walls](http://en.wikipedia.org/wiki/Cell_wall). The peptidoglycan layer is important for cell wall structural integrity. The final transpeptidation step in the synthesis of the peptidoglycan is facilitated by [transpeptidases](http://en.wikipedia.org/wiki/Transpeptidase" \o "Transpeptidase) known as [penicillin-binding proteins](http://en.wikipedia.org/wiki/Penicillin-binding_protein) (PBPs). PBPs bind to the D-Ala-D-Ala at the end of muropeptides (peptidoglycan precursors) to crosslink the peptidoglycan. [Beta-lactam antibiotics](http://en.wikipedia.org/wiki/Beta-lactam_antibiotic) mimic the D-Ala-D-Ala site, thereby irreversibly inhibiting PBP crosslinking of peptidoglycan. | ~ It is effective against most gram-positive strains, including Staphylococcus aureus, S. epidermidis, micrococci, and all streptococci except enterococci. |
| 1. **Oxacillin (OX)** | **~** Exerts bactericidal activity via inhibition of bacterial cell wall synthesis by binding one or more of the penicillin binding proteins (PBPs).  Exerts bacterial autolytic effect by inhibition of certain PBPs related to the activation of a bacterial autolytic process. | ~ Effective against bacterial infections |
| 1. **Penicillin (P)** | **~** Bacteria constantly remodel their [peptidoglycan](http://en.wikipedia.org/wiki/Peptidoglycan) cell walls, simultaneously building and breaking down portions of the cell wall as they grow and divide. [*β*-Lactam antibiotics](http://en.wikipedia.org/wiki/Beta-lactam_antibiotic) inhibit the formation of peptidoglycan [cross-links](http://en.wikipedia.org/wiki/Cross-link) in the bacterial [cell wall](http://en.wikipedia.org/wiki/Cell_wall); this is achieved through binding of the four-membered *β*-lactam[ring](http://en.wikipedia.org/wiki/Cycloalkane) of penicillin to the [enzyme](http://en.wikipedia.org/wiki/Enzyme) [DD-transpeptidase](http://en.wikipedia.org/wiki/DD-transpeptidase). | ~ Effective against Gram-positive bacteria. |

1. **Tabulate (unknown bacteria code and zone of inhibition)**

**Set A Zone of Inhibition in millimeters (mm)**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Name** | **SXT 25** | **DA 2** | **C 30** | **S 10** |
| **A 1** | 27.31 | - | 20.87 | - |
| **A 2** | 26.68 | - | 16.73 | 17.85 |
| **A 21** | 45.96 | 21.44 | 19.37 | 21.64 |
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| **S-CR 9** | - | 25.58 | 33.73 | 22.01 |
| **S-CR 12** | 26.10 | 27.5 | 26.61 | 28.33 |
| **S-CR 13** | 24.31 | 18.82 | 29.11 | 22.97 |

**Set B Zone of Inhibition in millimeters (mm)**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Name** | **AK** | **RA** | **KF** | **OX** | **P** |
| **A 1** | 23.70 | 16.05 | - | - | - |
| **A 2** | 34.60 | 13.28 | - | - | 14.36 |
| **A 21** | 24.22 | 11.58 | 14.42 | - | 26.30 |
| **A 22** | 22.58 | 13.62 | 13.52 | - | - |
| **S-CR 8** | 26.62 | 15.59 | - | - | - |
| **S-CR 9** | 34.89 | 11.20 | 31.63 | - | 48.19 |
| **S-CR 12** | 31.78 | 15.62 | 20.81 | 14.59 | Slightly +/- (10.71) |
| **S-CR 13** | 22.66 | 13.70 | - | - | - |

1. **Based on the test results, what is the best antibiotic?**

**~** Based on our group experiment, the best antibiotic in Set A is Sulfamethoxazole-trimethoprim (SXT) having 45.96 mm. and in Set B is Penicillin (P) having 48.19 mm.

1. **Based on the test results, which bacteria is most resistant to the different antibiotics?**

**~** Based on our group experiment, the most resistant bacteria in Set A is A 22 and in Set B is S-CR 13

**Conclusion and Recommendation**

The group can therefore conclude that the use of Antibiogram or Antibiotic Testing is helpful and it is a tool for clinicians, pharmacists and public health officials to:

* Track susceptibility patterns
* Raise awareness of antimicrobial resistance
* Educate people on antimicrobial usage

We also recommend the use of antibiotic testing to know if such antibiotic is going to work on the different kinds of bacterial infections.

**References**

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