

# Lab report: antibiotic evaluation by the kirby-bauer method

Business



## Lab Report: Antibiotic Evaluation by the Kirby-Bauer Method Introduction

Chemical antimicrobial agents are chemical compounds capable of either inhibiting the growth of microorganisms or killing them outright. Those which are taken internally to alleviate the symptoms of or promote healing from disease are called chemotherapeutic drugs, and among these is a class of compounds called antibiotics. In order for a chemotherapeutic drug to be classed as an antibiotic, it must be produced by a microorganism such as bacterium or fungus or at least derived from a chemical produced by one.

It must also be capable of killing or inhibiting the growth of other microorganisms and of doing so when taken in very small quantities.

To study whether a microbial product qualifies as an effective antibiotic, a standard procedure called the Kirby-Bauer method is employed. This method, which is the procedure recommended by the US Food and Drug Administration, was devised by William Kirby and A. W. Bauer in 1966. In the current protocols involved in the Kirby-Bauer method, Mueller-Hinton standard agar is used as the medium for bacterial culture.

The pH of the standard agar is 7.

2 to 7.4 and it is poured exclusively to a depth of 4 mm. The medium is heavily inoculated with bacteria and paper disks containing enough of the antibiotic under study to create an optical density of 1 (the McFarland standard) are placed on top of the cultures. By examining the results of incubation in the form of a zone of inhibition around each disk after incubation, it can be determined how effective each antibiotic is against any given bacterium. A minimum inhibitory concentration can then be deduced

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for the given antibiotic vs. the specific bacterium tested so that appropriate dosage may be determined.

Resistant bacteria cultures will show a small or no zone of inhibition if their growth is not sufficiently inhibited for the antibiotic to be a viable candidate in treating infection by that organism. Sensitive cultures, on the other hand, will be appreciably inhibited in their growth or, ideally, eliminated entirely in a relatively large radius around the McFarland standard disk. In this case, the antibiotic under study might be prescribed as a useful counter to illness brought on by that particular bacterium.

In the experiment discussed here, we tested eight antibiotics against four common opportunistic pathogens, namely *Streptococcus faecalis*, *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa*. Of these, the first two are Gram positive and the latter pair are Gram negative. The eight antibiotics tested were: Ampicillin, a beta-lactam antibiotic that inhibits the final stage of bacterial cell wall synthesis by binding to receptors within the cell wall.

The result is a porous cell wall and subsequent lysis carried out by the bacteria's own enzymes.

It is effective against many bacteria, both Gram positive and Gram negative, and is particularly used in treating infection by *E. coli*, *Salmonella typhosa* and *Enterococcus faecalis*, among others. (DrugBank) This antibiotic is a semi-synthetic derivative of penicillin, which is itself an antibiotic produced by the fungus *Penicillium notatum*. Bacitracin, a mixture of polypeptides obtained from *Bacillus subtilis* var Tracy.

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It inhibits synthesis of the peptidoglycan layer in Gram positive bacteria by preventing the function of a molecule that transports components to synthesis sites.

Bacitracin has a low threshold of toxicity when taken orally or injected, but it has found application as a topical ointment in the prevention of wound infection by Staphylococci. (DrugBank) Chloramphenicol, a broad spectrum antibiotic that is produced synthetically but which was originally discovered in a Streptomyces bacterium. It can be employed against several types of infection but most notably has found application in combating typhoid fever cholera. This antibiotic inhibits protein synthesis by suppressing the function of the 50S subunit in bacterial ribosomes.

Chloramphenicol is bacteriostatic but does not kill bacteria.

It also has a low toxicity threshold when ingested, and so it is now used almost exclusively to combat life-threatening illness or infection. (DrugBank) Erythromycin, which is produced by a Streptomyces and functions as a protein synthesis inhibitor in much the same way as Chloramphenicol. It is much less toxic than Chloramphenicol and is used to combat such diseases as whooping cough, diphtheria, and pelvic inflammation due to syphilis. (DrugBank) Novobiocin, one of the aminoglycoside antibiotics.

This class of antibiotics works by binding to the bacterial 16S rRNA and causing the misreading of tRNA. Because of this, the bacteria synthesizes incomplete or toxic polypeptides, resulting in the death of the bacterial cell.

Novobiocin can be used to treat infection by Gram negative bacteria and

Mycobacteria, including Mycobacterium tuberculosis. It is not effective  
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against anaerobic bacteria, however, and is not often used against Gram positive infections because other antibiotics that are less toxic to the patient are available for this purpose. (DrugBank) Moreover, Novobiocin is known to bind to and alter the function of DNA gyrase, effectively stopping proper replication in the bacterial cell and thus bactericidal. Penicillin G, another antibiotic of the beta lactam class. It is used primarily against Gram positive bacteria such as the Streptococci but is also effective against some Gram negatives such as *Neisseria gonorrhoeae* and the spirochete *Treponema pallidum*, which is responsible for syphilis.

Penicillin G inhibits synthesis of peptidoglycan by the same mechanism as in Ampicillin. (DrugBank) Polymyxin B, a mixture of polypeptides derived from *Bacillus polymyxa*. It can be used bactericidally against most Gram negative bacteria and is applied most often against urinary tract, blood, and meningeal infections of *Pseudomonas aeruginosa*. It has no effect upon Gram positive bacteria. It kills bacterial cells by binding to and removing lipids in the cell membrane.

Due to this mechanism, however, Polymyxin B also damages eukaryotic cells and thus sometimes proves to be a neuro- and nephrotoxic in humans. (DrugBank) Tetracycline, synthesized from chlortetracycline, a compound produced by a *Streptomyces*. It works by binding to the bacterial ribosome and interfering with protein synthesis and is effective against a wide range of Gram positive and negative bacteria, including the *Mycoplasma* and the bacteria responsible for Rocky Mountain Spotted Fever and nongonococcal urinary tract infections. (DrugBank) Material and Methods Cultures:

*Streptococcus faecalis* *Staphylococcus aureus* *Escherichia coli* 1  
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*Pseudomonas aeruginosa* One person each in a team of four heavily inoculated two Mueller-Hinton agar plates with one of the cultures listed by aseptic transfer from a broth culture using a sterile cotton swab. Each plate was marked off into four segments, a total of eight sectors. One McFarland standard disk containing one of the eight antibiotics tested was placed, using alcohol-flame sterilized forceps, in the center of a sector.

After incubating for 18 hours at 37°C, the diameters of the clear zones (zones of inhibition) around each McFarland disk was measured with a standard ruler to the nearest millimeter. The measurements obtained were matched against a chart (Claus 407) to determine whether the bacterium was resistant, sensitive, or intermediate in susceptibility to the antibiotic used. The numbers against which these measurements are matched take into account the difference in zone sizes caused by variations in diffusion rates through agar in the antibiotics tested. Results

Bacterium: *Streptococcus faecalis* | | Antibiotic used | Inhibition zone size (mm)| Culture response | | Ampicillin | 28 | S | | Bacitracin | 20 | S | | Chloramphenicol | 22 | S | | Erythromycin | 21 | S | | Novobiocin | 20 | S | | Penicillin G | 20 | S | | Polymyxin B | 0 | R | | Tetracycline | 24 | S | Bacterium: *Staphylococcus aureus* | | Antibiotic used | Inhibition zone size (mm)| Culture response | | Ampicillin | 48 | S | | Bacitracin | 22 | S | | Chloramphenicol | 24 | S | | Erythromycin | 25 | S | | Novobiocin | 39 | S | | Penicillin G | 43 | S | | Polymyxin B | 0 | R | | Tetracycline | 32 | S | Bacterium: *Escherichia coli* | | Antibiotic used | Inhibition zone size (mm)| Culture response | | Ampicillin | 20 | S | | Bacitracin | 11 | R | | Chloramphenicol | 0 | R | | Erythromycin | 25 | S | | Novobiocin | 21 | S | | Penicillin G | 8 | R | | Polymyxin B | 6 | R | |

Tetracycline | 12 | R | Bacterium: Streptococcus faecalis | | Antibiotic used |  
Inhibition zone size (mm)| Culture response | | Ampicillin | 0 | R | | Bacitracin |  
0 | R | | Chloramphenicol | 21 | S | | Erythromycin | 22 | S | | Novobiocin | 10 |  
R | | Penicillin G | 0 | R | | Polymyxin B | 18 | S | | Tetracycline | 25 | S |

Discussion *S. faecalis* was sensitive to all of the antibiotics tested except for Polymyxin B. Since that antibiotic is known to be effective only against Gram negative bacteria, this observation is in keeping with expected results.

As a Gram positive, one would expect, as we observed, that it would be sensitive to polypeptide inhibitors, such as Penicillin G and Ampicillin, as well as protein synthesis inhibitors, such as Tetracycline and Erythromycin. Novobiocin, while not often used against Gram positives due to concerns about its toxicity, is known to be effective against Gram positives as well, which is borne out by these observations. Like *S. faecalis*, *S. aureus* is a Gram positive, and so would be expected to be sensitive to the same antibiotics and resistant to Polymyxin B.

Our observations verify this as well. *E. coli* is Gram negative, and our observations show it to be sensitive only to Ampicillin, Erythromycin, Novobiocin.

It was, however, resistant to Penicillin G, demonstrating that there is some chemical factor which allows one beta lactam antibiotic to inhibit this bacterium (Ampicillin) while another (Penicillin G) does not. This is likely due to the difference in chemical structure of the two. Penicillin G lacks an amino group, which is present on Ampicillin.

It may well be that the presence of a partially-charged amino group on Ampicillin allows it to be uptaken by the bacterium more readily than Penicillin G. (Deacon) More unexpectedly, *E. coli* was resistant to Chloramphenicol, even though this antibiotic is useful in inhibiting other Gram negative bacteria. It may be that the *E. coli* strain used in this experiment has developed a resistance to this particular antibiotic. *P.*

*aeruginosa* also proved to be resistant to Ampicillin, Bacitracin, Novobiocin, and Penicillin G. This suggests that the chemical structure which allows Ampicillin to inhibit *E. coli* is not effective in the case of this bacterium, which is sensitive to neither of these beta lactams. Bacitracin is only useful against Gram positives as well, so it had no noticeable affect upon *P. aeruginosa*. On the other hand, this culture was sensitive to Polymyxin B, Tetracycline, Chloramphenicol, and Erythromycin, as one would predict for a Gram negative.

Its resistance to Novobiocin is due to its having a modified DNA gyrase (Miller 674).

It is worth noting in this case that while the bacterium is resistant, Novobiocin does have some effect at a standard concentration, and higher concentrations can be used to kill the organism. Such concentrations, however, would also be toxic to the patient if taken as a chemotherapeutic dosage. The emergence of antibiotic-resistant strains of bacteria has been an ongoing phenomenon since shortly after the widespread use of penicillin, the first antibiotic, began. The rate at which this occurs has increased as the availability and employment has increased since then, and it has now

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become a significant medical problem. The major reason for this is that the use of antibiotics acts as a selective pressure.

Those bacteria which carry a mutation on their plasmids that make them able to survive treatment give them access to a niche which non-resistant strains cannot exploit, leading to their proliferation both in infected individuals and ultimately in the environment in general. The use of antibiotics kills not only pathogens, but also normal microflora which might otherwise prevent virulent bacteria from establishing a foothold in the human body. Thanks to the phenomenon of transformation, in which living bacteria can incorporate naked genetic material left over when other cells are lysed for reasons other than antibiotic sensitivity, resistance genes can cross between genera. For example, the Staphylococci can incorporate resistance genes from Bacilli and Streptococci.

Added to this are resistance genes that exist as transposons found in the main bacterial genome and which can be transmitted to other members of the same species by temperate phages via the process of transduction. These mechanisms have resulted in the arising of such things as Vancomycin-resistant strains of *S.*

*aureus*, responsible for a usually-lethal nosocomial infection that, ironically, is carried by the very bacteria that Alexander Fleming first observed as being susceptible to penicillin, the original antibiotic. (Deacon) Literature cited Bauer AW, Kirby WMM, Sherris JC, Tenckhoff M. 1966. Antibiotic susceptibility testing by a standardized single disk method. *Am J Clin Pathol* 45(4): 493-6.

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