Project Report
-Junior-

Attack of The Enemy Mutants!

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Background and Purpose

Since their discovery in the early 20th century, antibiotics have been considered the wonder drugs of the era. Antibiotics are used to treat a wide variety of bacterial infections. Therapeutic use of antibiotics increased from the 1950s until the mid 1980s[1]; it was around this time that the first antibiotic-resistant bacteria were recognised[2], and their harmful consequences were becoming apparent. Why is the development of antibiotic resistance in bacteria of concern? Firstly, infection caused by such bacteria may be very difficult, if not impossible (in some circumstances), to treat. Secondly, the cost of treating people with such infections has substantially increased[3].

The purpose of *Attack of The Enemy Mutants* was to study the rates at which a *Staphylococcus aureus* isolate acquired resistance to nine antibiotics commonly used to treat such infection, to study the rate at which this acquired antibiotic resistance recedes, and to determine possible methods which could be used to slow down the rate at which antibiotic resistance to a given antibiotic is acquired.

Hypotheses

My first hypothesis was that a *Staphylococcus aureus* isolate (from my nasal membrane) would become resistant to one or more of the following antibiotics with repeated exposure: gentamicin, clindamycin, enrofloxacin, erythromycin, amoxicillin/clavulanic acid, cephalexin,

tetracycline, methicillin and sulfamethoxazole/trimethoprim.

My second hypothesis was that the number of exposures needed for this *S. aureus* isolate to achieve resistance would vary between different antibiotics. I also hypothesized that treating with multiple antibiotics, either used concurrently, or by alternating antibiotics used, would delay the onset of resistance. My final hypothesis was that bacteria would lose resistance if they were not continually exposed to the antibiotic that they had acquired resistance to.

Procedure

I swabbed my nasal membrane and cultured this swab on blood agar at 37^{o} C for 24 hours. The catalase test, Gram stains, and the observation of â-hemolytic colonies were used to identify a *Staphylococcus aureus* culture[4]. This culture was sent to a commercial bacteriology lab to confirm that pure *Staphylococcus aureus* was isolated, and to identify which antibiotics this isolate was sensitive to.

Based on this sensitivity testing, nine antibiotics commonly used to treat *Staphylococcus aureus* infections were chosen[5]. The *S. aureus* isolate was separately exposed to each of these nine antibiotics in the form of sterile paper disks, impregnated with the drug (Antimicrobial Susceptibility Test Disks, OXOID Ltd., Hampshire, England). Susceptibility was determined using the Kirby Bauer method of measuring the zone of inhibition (the area around the antibiotic disk in which bacteria did not grow, which relates to antibiotic sensitivity)[6].Sixteen subsequent antibiotic exposures (dose simulations) were made using a swab of bacterial growth from the inner margin of the previous zone of inhibition, presumptively selecting for antibiotic resistance.

To determine the repeatability of this system, the original isolate was tested with one of the antibiotics (gentamicin) on five identical agar plates, all cultured at the same time. Zones were

measured and compared using standard deviation.

To determine if the number of exposures needed for my isolate to acquire antibiotic resistance could be increased by using different antibiotics concurrently, a modification of the original procedure was used. Enrofloxacin and cephalexin (two of the antibiotics which induced a minimum of intermediate sensitivity in the initial part of my study) antibiotic disks were used together in two separate fashions. In the first test, the two antibiotic disks were placed side-by-side on the agar plate. For the second test, these two antibiotics were used alternately for successive cultures (dose simulations). To determine whether the acquired resistance to an antibiotic was lost, I continued to subculture a cephalexin-resistant organism without the antibiotic while periodically monitoring zone size. All results were analyzed using a zone of inhibition interpretation sheet unique to the brand of antibiotic disks used in experimentation (BBL^{TM}) to determine sensitivity to each antibiotic at each exposure.

Results, Observations, and Conclusions

Following the initial exposure period of sixteen days, six of the nine antibiotics used continued to have an acceptable zone of inhibition (i.e., no development of resistance). These antibiotics were: gentamicin, clindamicyn, erythromycin, clavulanic acid/amoxicilin, tetracycline, sulfamethoxazole/trimethoprim. Three antibiotics had only an intermediate effect after multiple exposures. These were: enrofloxacin at exposure 6; cephalexin at exposure 8; methicillin at exposure 13. Only two antibiotics lost effectiveness to the point of antibiotic resistance: cephalexin at exposure 14, and gentamicin at exposure 16.

The first conclusion that I am able to make is that the system I used for experimentation

worked well and was reproducible. My first hypothesis, that my bacterial isolate will become resistant to antibiotics commonly used to treat it, was partially correct. My *S. aureus* isolate achieved resistance to gentamicin and cephalexin, and achieved intermediate sensitivity to enrofloxacin and methicillin; no change in the level of resistance to the other antibiotics used was noted.. The average duration of antibiotic therapy is usually less than 14 days[7]. This suggests that, of the antibiotics used, cephalexin would be the only one to potentially cause difficulties in initial treatment periods. My second hypothesis, that the number of exposures needed for my Staphylococcus aureus isolate to achieve resistance to different antibiotics will vary, was thus proven true. This is because antibiotics have differing effects on bacteria; a certain mechanism of resistance that bacteria use against one form of antibiotic may take longer to develop than another used against a different antibiotic. To this point, at antibiotic exposure 13, when cephalexin and enrofloxacin were used alternately, my isolate had not yet become consistently resistant. When these two antibiotics were used concurrently, resistance development was delayed by six exposures. Thus, my third hypothesis, that treating with multiple antibiotics will delay the onset of resistance appears to be true.

My final hypothesis, that bacteria will lose resistance if they are not continually exposed to the antibiotic to which they have acquired resistance is yet to be fully proved. To date, I have determined that antibiotic resistance is not lost within the first 12 days of no antibiotic exposure, but that resistance became **stronger** during the first four days, before decreasing slightly. I still feel that my hypothesis that resistance will be lost if the presence of antibiotics is lost for lengthened periods of time may be correct, however this may take months to occur.

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Appendices

Major Resources Consulted

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Endnotes (References cited)

1. Salyers A.A, White D.D. Bacterial Pathogenesis - A Molecular Approach, Second Edition, ASM Press Washington, DC., 2002.
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3. Calculations based upon quote from Pharmacist, Shoppers Drug Mart, Bullfrog Mall, Guelph Ontario
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