

Effect of the antibiotics gentamicin, penicillin and tetracycline on four species of *Lactobacillus* probiotics*

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Humans typically host more than 1.5 kg of microflora in their gastrointestinal tracts some of which are probiotics which aid food digestion and inhibit pathogenic bacterial growth. However, when antibiotics which are intended to target pathogenic bacteria are taken, they could also have an adverse effect on beneficial probiotics. I investigated the effects of the antibiotics gentamicin, penicillin and tetracycline on four different species of *Lactobacillus* probiotics.

I used the Kirby-Bauer method to test the sensitivity of the probiotics to the four antibiotics. I swabbed eight *Lactobacillus*-MRS Agar plates with each probiotic and placed five gentamicin, penicillin and tetracycline antibiotic sensitivity disks on them. If the probiotic grew uninhibited, this indicated that it was resistant to the antibiotic. If zones of no bacterial growth appeared around the sensitivity disks the probiotic was sensitive. The larger the zone of inhibition, the more sensitive is the probiotic to the antibiotic; the smaller the zone, the more resistant is the probiotic.

My results showed that the four *Lactobacillus* probiotics were each variably affected by two of the three antibiotics. Tetracycline affected all the *Lactobacillus* species. Penicillin affected all the species except *L. reuteri*. Gentamicin affected only *L. reuteri* (to a minor extent). As these were *in vitro* results, they provide only an indication of what may happen *in vivo*, in the human body. My experiment has demonstrated differing combinations of resistant probiotics that may be used with antibiotic therapies, where the probiotic may still offer benefit to the host.

Introduction

Humans typically host more than 1.5 kg of microflora in their gastrointestinal tracts (Natural History NZ 2006). A proportion of these are probiotics, which are complex organisms that produce vitamins, lipids, and help to digest food such as amino acids. Probiotic bacteria inhibit growth of or kill other bacteria by producing natural antibiotics called bacteriocins (Brewer 2002, p. 257).

Not only are probiotics promoted to boost the immune system, but also to offer benefit during or after courses of antibiotics (Science Daily 2008). Antibiotics inhibit the growth of or kill pathogenic bacteria, such as *Streptococcus pyogenes*,

which causes tonsillitis and rheumatic fever (Hingston, pers. comm. 2012). However, they can also destroy some of the essential microflora in the gastrointestinal tract. This creates opportunity for other pathogenic bacteria to flourish, for example *Clostridium difficile*, which produces toxins that cause the intestinal linings to secrete fluid and shed their epithelial cells, causing diarrhoea and other side-effects (Hingston, pers. comm. 2012). When a course of antibiotics is taken, the balance in the bowel is often disturbed, causing antibiotic-induced diarrhoea (*British Medical Journal* 2002), which affects 30% of people taking antibiotics (*Science Daily* 2012). Diarrhoea side-effects often prompt people to discontinue their antibiotic treatments. While the drug-sensitive bacteria have been killed, drug-resistant bacteria can continue multiplying and thriving (Nash 2001), and discontinuing an antibiotic treatment is postulated to contribute to antibiotic resistance: a major concern in contemporary medicine (Chetley 1995, p. 79). Literature suggests that taking supplementary probiotics helps to reduce diarrhoea by repopulating necessary and beneficial bacteria in the gut (*Science Daily* 2012).

In this investigation I measure the effects of the antibiotics penicillin, gentamicin, and tetracycline on the *Lactobacillus* probiotic species *L. acidophilus*, *L. plantarum*, *L. reuteri*, and *L. rhamnosus* and explore whether probiotics may play a beneficial role during antibiotic therapy.

Review of literature

Probiotic bacteria live in niches in the gastrointestinal tract and compete with other pathogenic bacteria for nutrients and space (Natural History NZ 2006). *Lactobacillus* species are gram-positive anaerobes; many are probiotic and work in symbiosis with the human body (Lang 2004, p. 292). Many probiotic *Lactobacillus* bacteria in the intestines ferment glucose to produce lactic and acetic acids (Brewer 2002, p. 257). This not only decreases the pH in the intestines (creating an ideal environment for gastrointestinal bacteria) but also discourages the growth of pathogenic bacteria, such as *Salmonella*, *Escherichia coli* and *Helicobacter pylori* (Natural History NZ 2006). *L. reuteri* aids the human body by producing the biofilm 'reuterin', an anti-pathogenic compound which helps inhibit the growth of other

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Lydia Hingston undertook this science study during years 12 and 13 at Queen Margaret College, Wellington, as her Extended Essay towards the International Baccalaureate diploma. She submitted it to the NIWA Wellington Regional Science Fair, winning the Royal Society of NZ Wellington Branch Prize for best overall exhibit and a place in the week-long 'Realise the Dream' tour of science institutions from Auckland to Wellington, where she especially enjoyed the challenge of investigating the effects of stress on the erythrocyte count in fish, as well as broadening her network of science colleagues. Lydia was recognised by her school as 2012 IB Dux, and by the Governor-General as an IB top scholar. She loves art and painting, and has competed in rhythmic gymnastics for the past 12 years at a national and international level. Lydia has started her tertiary study of Biomedical Science at the University of Auckland.

bacteria, both gram-positive and gram-negative (Versalovic 2009). Probiotics are promoted to consumers as fermentation agents used to create yogurts, or as probiotic supplements which ‘aid in maintaining a normal healthy immune system’ (Inner Health Plus 2008).

I chose to investigate three antibiotics – penicillin, gentamicin, and tetracycline – for the following reasons. Penicillin was the first antibiotic discovered and is arguably the most well-known (Damon *et al.* 2007, p. 541). Tetracycline is a broad-spectrum antibiotic inhibiting the growth of many organisms. Unlike most penicillins and tetracyclines, gentamicin is primarily administered parenterally, and used in more severe infections within a hospital environment (Hingston, pers. comm. 2012).

Bactericidal penicillin kills bacteria by inhibiting the synthesis of peptidoglycan (cross-linking) molecules in bacterial cell walls. It does this by binding to transpeptidase enzymes. Holes then form in the peptidoglycan wall, causing the previously turgid bacterial cells to burst (lyse). Penicillins are most effective in killing gram-positive bacteria, because they have two thick layers of peptidoglycan (Jeremy 2008a).

Penicillin is used to treat a range of bacterial infections including septicaemia, meningitis (Novartis NZ 2009), gonorrhoea and particularly streptococcal infections in the throat (Hingston, pers. comm. 2012). Penicillin can be administered orally or parenterally (Hingston, pers. comm. 2012).

Gentamicin is an aminoglycoside antibiotic. Gentamicin molecules kill bacteria by binding to the 30S subunits of ribosomes, inhibiting the synthesis of the bacteria’s essential proteins (Jeremy 2008b). Gentamicin is primarily effective against gram-negative bacteria. It has a lesser effect on anaerobic bacteria, as the transport of the gentamicin molecules over the bacteria’s membrane is dependent on oxygen molecules, and this can limit penetration into the bacterial cell (Lang 2004, p. 34).

Because gentamicin is relatively toxic, causing nephrotoxicity and ototoxicity (Jeremy 2008b), it is mainly used in hospitals to treat more severe respiratory tract infections (particularly *Staphylococcus* and *Pseudomonas*), urinary tract infections, and bacteraemia (Pfizer NZ 2005).

Like gentamicin, tetracycline affects protein synthesis at the ribosomes. It can inhibit the growth of both gram-negative and gram-positive bacteria, by binding to the 30S subunits of the

ribosome, thereby inhibiting the synthesis of essential proteins (Lang 2004, p. 42). Tetracycline is an old broad-spectrum drug, used orally to treat respiratory infections such as bronchitis, but is also used to treat acne, chlamydia, and urethritis (Hingston, pers. comm. 2012).

Materials and methods

The materials used are shown in Table 1.

There were two independent variables:

- Antibiotic sensitivity disks (Gentamicin (10 µg), Penicillin (10 µg), Tetracycline (30 µg))
- *Lactobacillus* species (*Lactobacillus acidophilus*, *Lactobacillus plantarum*, *Lactobacillus reuteri*, *Lactobacillus rhamnosus*)

The diameters of the zones of inhibition surrounding each antibiotic disk, measured in cm, formed the dependent variable (± 0.1 cm).

To ensure a fair test, I controlled the following factors:

- Incubation temperature (30.0°C), measured with a thermometer (°C, $\pm 0.5^\circ\text{C}$)
- Incubation duration (48 hours for the primary suspensions, and a further 48 hours for the sensitivity disk testing)
- Nutrients (Lactobacillus-MRS (LMRS) Agar plates)
- Equivalent doses of antibiotics sensitivity disks. The antibiotic sensitivity disks contained different concentrations: 30 µg of tetracycline and 10 µg in the gentamicin and penicillin disks. These concentrations were understood to be equivalent doses (Fort Richard Laboratories 2009), and are commonly used on laboratory specimens.
- Anaerobic conditions (Use of the ‘GasPak’ carbon dioxide envelopes and indicators).

I incubated all of the agar plates in the same incubator for the same time periods, at a constant 30°C and in anaerobic conditions in zip-lock bags, with one ‘GasPak’ kit per bag. The LMRS Agar plates were ordered from an Auckland laboratory ensuring that they were all of the same quality with the same expiry date (Fort Richard Laboratories 2009). The antibiotic sensitivity disks were commercially made and supplied by Fort Richard Laboratories.

When incubating the primary suspension plates and the final plates with the antibiotic sensitivity disks, I placed one control plate (containing no bacteria) in the incubator, to show that

Table 1. Materials used.

Micro-organisms (single doses as recommended by the manufacturers)	<i>Lactobacillus acidophilus</i> (‘Thompson’s Acidophilus Plus’ 500 million organisms per 0.5 g capsule) <i>Lactobacillus plantarum</i> (‘Ethical Nutrients – Professional Natural Medicines’ 20 billion organisms per 0.3 g capsule) <i>Lactobacillus reuteri</i> (‘Blackmore’s Digestive Health’ 200 million organisms per 0.4 g tablet) <i>Lactobacillus rhamnosus</i> (‘Ethical Nutrients – Professional Natural Medicines’ 20 billion organisms per 4 g powder)
Antibiotic sensitivity disks	Gentamicin (10 µg) Penicillin (10 µg) Tetracycline (30 µg)
Other	Prepared Culture Media: Forty-two Lactobacillus-MRS Agar Plates (90 cm) (D. L. Milicich, pers. comm. 2012) Five ‘GasPak’ disposable carbon dioxide generator envelopes Five ‘GasPak’ disposable anaerobic indicators

the plates were sterile at the start of the experiment. For each antibiotic I ensured that I had at least half of one plate without an antibiotic disk, to ensure that no zones of inhibition due to other causes occurred.

I used the Kirby-Bauer disk diffusion method (Thompson 1996) to test the effects of the antibiotics. I placed antibiotic sensitivity disks on the LMRS Agar, which were swabbed with the respective probiotics. Following incubation I measured the zone diameters, and used these to show which antibiotics inhibited the growth of the respective bacteria most effectively.

If a probiotic was resistant to an antibiotic, bacterial growth would be uninhibited. The probiotic would flourish in the presence of its desired nutrients and no zone of inhibition would appear. Effective antibiotics cause zones of inhibition to appear around the sensitivity disks, as the probiotic's growth is inhibited. The larger the zone of inhibition, the more effective the antibiotic is against the bacteria. Conversely, the smaller the zone of inhibition, the less effective is the antibiotic and the more resistant the bacteria (Thompson 1996).

I did two preliminary trials before I worked out my final method. As I had found that *Lactobacillus* species could grow aerobically (Lang 2004, p. 292), and would produce ATP if oxygen were present, I assumed that probiotics would grow in an aerobic incubator. However, none of the probiotic species grew on the nutrient agar during the first two trials in an aerobic incubator.

Following the first two unsuccessful trials I conducted a third trial in which I grew the bacteria anaerobically. Because Queen Margaret College does not have an anaerobic incubator, I borrowed a glass canister from Victoria University, and used several disposable 'GasPak' carbon dioxide generator envelopes to create an anaerobic incubator. When setting up the primary suspension plates the canister worked well. It could be seen that the probiotics were growing well, as the disposable 'GasPak' indicators turned green. However, the glass canister could only accommodate 15 plates at a time, not the total 32 plates required for the final stage of the experiment. My creative solution was to construct four anaerobic environments sealed with duct tape inside four zip-lock plastic bags (Figure 1). Each contained one disposable 'GasPak' carbon dioxide generator envelope and one 'GasPak' carbon dioxide indicator.

The nutrient agar also proved a problem. From discussion with Fort Richard Laboratories staff, I established that I needed 'Lactobacillus-MRS Agar' (D.L. Milicich, pers. comm. 2012). LMRS Agar plates are designed specifically for the growth of *Lactobacillus* bacteria and offer the nutrients necessary for their fermentation and growth:



Figure 1. Zip-lock bag method for keeping agar plates in an anaerobic environment.

pH of 6.5, acetate, glucose, DL-lactic acid from lactose, and other carbohydrates (Enrik 1993). *Lactobacillus* reproduction is most efficient at 35–37°C (Hill 2004), and incubation needs to be for approximately 24 to 48 hours. New Zealand high school laboratory standards forbid the incubation of agar plates at temperatures above 30°C. This did not pose a problem since all four species thrived in the final trial.

My original goal was to compare strains of *Lactobacillus* with *Bifido* probiotics. However, the range of probiotics on the New Zealand market is limited. Products tend to include cocktails of probiotic bacteria. Hence I decided to use pure probiotic supplements containing a single species, to clearly distinguish the bacteria type, and to limit the sub-culturing required. I tested the four most readily available species of *Lactobacillus* separately: *L. acidophilus*, *L. plantarum*, *L. reuteri*, and *L. rhamnosus*. I used probiotic supplements instead of yogurt, to minimise contaminants such as fungi and other bacteria which grow in yogurt. The supplement capsules also contained a greater concentration of organisms than yogurt (Inner Health Plus 2008).

Each day put on face mask and latex gloves and carried out the following procedures.

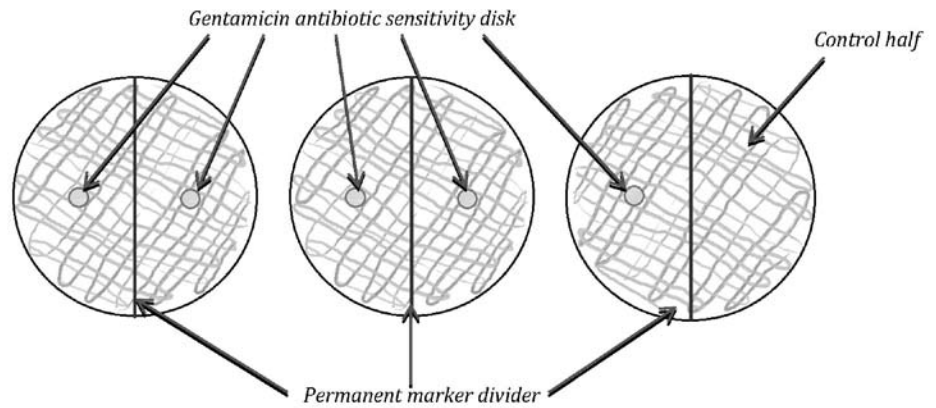
Day 1

1. Set incubator to 30°C, using a thermometer to monitor the temperature.
2. Prepare suspensions of the four *Lactobacillus* species. Take one glass test tube, empty the contents of the *Lactobacillus acidophilus* capsule into it using a funnel. Pour 5 ml distilled water into the funnel, ensuring all the contents go into the test-tube. Stir using the glass rod to suspend the probiotic particles. Repeat with the other three probiotics. (Note *L. reuteri* comes in sugar tablets which require grinding to a powder with a mortar and pestle.)
3. Once the four suspensions are complete, set the Bunsen burner to a blue flame. Holding the first LMRS Agar plate approximately 10 cm away from the Bunsen burner (to create a sterile environment), soak a cotton bud in the *L. acidophilus* suspension. Swab the bud over the plate in one direction. Repeat with a second cotton bud but swab in the second direction. With a third cotton bud, swab the plate in a third direction, passing over the second swab. Put used cotton buds into a beaker of ethanol. Repeat this step with a second (spare) agar plate and the same probiotic.
4. Repeat step 3 with the other three remaining probiotic suspensions, creating a total of eight primary suspension plates.
5. Place the eight plates into the CO₂ canister, with an additional LMRS Agar plate containing no bacteria as the control. Place one GasPak anaerobic indicator upright inside the canister. Fill one disposable carbon dioxide generator pack with 10 ml of water, and place upright, down the inside of the canister. (The indicator will change from yellow to green when CO₂ is present.) Place the CO₂ canister in the incubator and leave for 42 hours.

Day 3

6. Take the CO₂ canister out of the incubator, and take out the eight primary suspension plates.
7. Choose the one plate for each probiotic that has a pure culture with the best growth.
8. Sub-culturing: First for *L. acidophilus*, scrape off a couple

Figure 2: Day 3, Step 11, showing the three *L. acidophilus* plates, with the five trials with the gentamicin antibiotic disks in the centre of each half. The zigzagging represents the *L. acidophilus* lawn. The sixth half is the control and has no antibiotic sensitivity disk.



of the bacterial colonies using a cotton bud; dissolve with 25 ml of distilled water in a 50 ml beaker. Repeat this step for the other three probiotics.

9. Take the 32 LMRS Agar plates and divide each in half by drawing a line in permanent marker down the middle on the underside of the plates.
10. Cover eight agar plates with the *L. acidophilus* solution using a fresh cotton bud. (Brush zigzag patterns in one direction, then repeat in the perpendicular direction. Cover eight plates with *L. reuteri*, eight plates with *L. plantarum*, and eight plates with *L. rhamnosus* – thirty-two in total.
11. Placing the antibiotic disks: Take three LMRS Agar plates with *L. acidophilus* lawns. Place five gentamicin sensitivity disks, one in the centre of each half. The sixth half will not have an antibiotic disk, because it is the control used to ensure no zones of inhibition grow without the presence of an antibiotic (Figure 2).
12. Repeat step 11, using remaining LMRS Agar plates with *L. acidophilus* lawns with the other two antibiotics: penicillin and tetracycline.
13. Repeat steps 11 and 12 with the three remaining probiotics: *L. plantarum*, *L. reuteri*, *L. rhamnosus*. There will be a total of 32 plates, eight with each probiotic and five of each antibiotic per probiotic (constituting sixty trials).
14. Ensure that each half is labelled on the underside of the plate with the correct antibiotic and probiotic. Do not seal the plates with selotape, since they require an airflow of carbon dioxide.
15. Set up four anaerobic environments in four plastic bags (one for each probiotic). Put one anaerobic indicator and one carbon dioxide generating pack with 10 ml water in each bag. Quickly place the probiotic plates in the appropriate bags, so that minimal carbon dioxide escapes, making sure that they are upside down. Add one uncontaminated LMRS Agar plate to one of the bags, to act as a second control. Zip-lock each bag and place an extra seal of thick tape over the top.
16. Place the four plastic bags back into the incubator (30°C) and incubate for 48 hours.

Day 5

17. Take all plates out of their respective anaerobic plastic bags. Measure the diameter of each zone of inhibition of the bacteria with a 30 cm ruler. Because the diameter is difficult to measure precisely, measure the diameter from three different angles and record the mean measurement.
18. Once all data have been collected, soak every agar plate in bleach overnight to kill the populations, for subsequent disposal.

Once the measurements had been made, I calculated one standard deviation for each of the four *Lactobacillus* species, using the formula: $\sqrt{\{(\sum[x - \bar{x}]^2)/n\}}$

Results

No bacteria grew on the control plates, indicating that all the LMRS Agar plates were sterile at the start. No zones of inhibition occurred on the halves of the plates without antibiotic disks, indicating that there were no other antimicrobial substances present on the plates.

All four of the *Lactobacillus* species created lawns (diffuse colonies) that were white in colour. *L. acidophilus* (shown as an example in Figure 3) and *L. rhamnosus* appeared to have the thickest lawns, suggesting these species performed binary fission most rapidly, or that their starting populations were higher. It possibly indicates that 30°C was closer to their optimum temperature than for *L. reuteri* and *L. plantarum*.

The edges of the zones of inhibition of the tetracycline were the most distinct, and therefore the diameters were the easiest to measure. In comparison, the zones of inhibition around the penicillin and gentamicin disks were more difficult to measure, since the bacteria-free zones faded gradually from the full bacteria lawn.

The *L. reuteri* plates appeared to contain contaminants. All eight grew mini white colonies, some of which appeared in

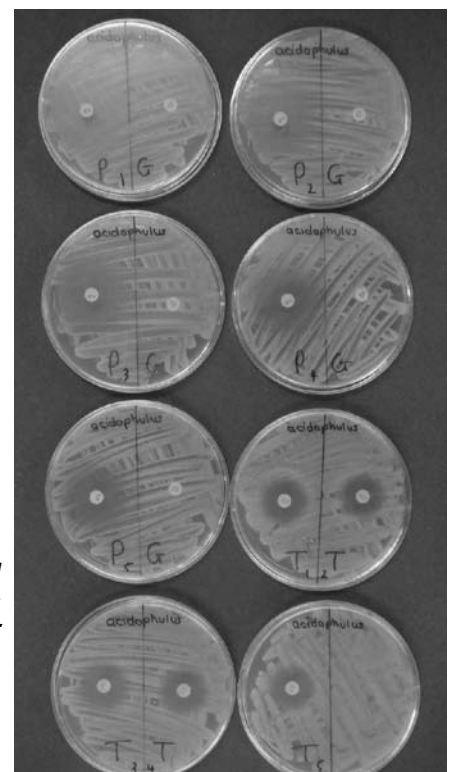
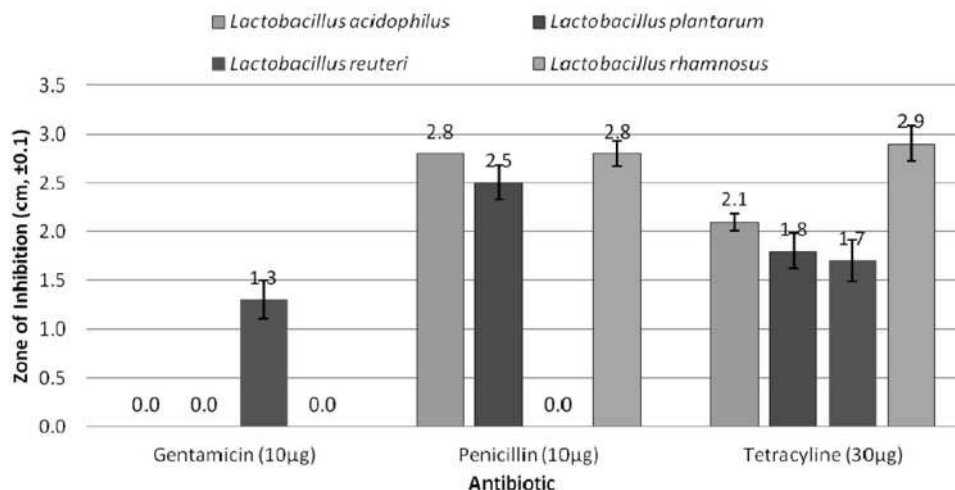


Figure 3. The final *Lactobacillus acidophilus* agar plates, where P = penicillin, G = gentamicin, T = tetracycline.

Figure 4. Final results for all four *Lactobacillus* species with the three antibiotics. The error bars represent one standard deviation.



the zones of inhibition. These minority colonies also appeared in the *L. reuteri* primary suspension plates. When making up the water suspensions I swabbed areas unaffected by these colonies; however, it appears some of these minority colonies contaminated my *L. reuteri* antibiotic plates. This demonstrates the difficulties of isolating bacteria.

The full results are shown in Figure 4.

Discussion

My experiment supports the notion that some probiotics are resistant to particular antibiotics.

Gentamicin was the least effective antibiotic, as three probiotics grew uninhibited and the fourth probiotic's growth (*L. reuteri*) was inhibited only minimally (with the smallest zone diameter of 1.3 cm). As gentamicin primarily inhibits the growth of gram-negative aerobes (Lang 2004, p. 33), and all four *Lactobacillus* species are gram-positive anaerobes, my results are expected, apart from the effect on *L. reuteri*.

With penicillin, *L. reuteri* grew uninhibited, whereas growth of the other three probiotics was inhibited. Several references assert that *L. reuteri* is a gram-positive anaerobe (e.g. Tovar 2007), and culture contamination is the most plausible explanation for these differing growth patterns. (Note *L. reuteri* grew similarly to the other probiotics in the presence of tetracycline, which is to be expected due to tetracycline's broad-spectrum nature.) Contamination could be possible, because *L. reuteri* was supplied unrefrigerated in a pill with a sugar coating that may have harboured other bacteria. Another time I would obtain *L. reuteri* from a number of different sources, in a powder form like the three other probiotics.

Tetracycline inhibited the growth of all four *Lactobacillus* species, causing large zones of inhibition, with diameters of at least 1.7 cm. This was expected, given its broad-spectrum nature (Lang 2004, p. 42). It appeared to be the strongest antibiotic, since it also caused the largest zones of inhibition for *L. reuteri* and *L. rhamnosus*. (In contrast, penicil-

lin seemed to be most potent towards the *L. acidophilus* and *L. plantarum* species, with zones of inhibition at 2.8 cm and 2.5 cm, respectively.)

The small standard deviations indicate that my investigation produced precise results. *L. acidophilus* with penicillin produced a significant average zone of inhibition of 2.8 cm, yet the standard deviation was 0.0 cm. The maximum standard deviation was small, at only ± 0.2 cm for *L. reuteri* with tetracycline.

Antibiotic sensitivity testing aims to indicate whether a bacterium is sensitive (effective) to an antibiotic, or whether it is resistant. In Table 2, I have categorised the probiotics as resistant, sensitive, or intermediate to gentamicin and tetracycline according to criteria given in Larkin (undated). The probiotics' sensitivity to penicillin has been categorised according to the 'Antimicrobial Susceptibility Testing' chart (Biolabs undated).

Conclusions

My experiment has suggested some combinations in which the body's natural flora may be adversely affected by antibiotics, for example the sensitive combinations (see Table 2) such as the use of penicillin with *L. acidophilus*. Taking a supplementary probiotic during antibiotic treatment is unlikely to be beneficial

Table 2. Degree of sensitivity of probiotics tested to three antibiotics.

Antibiotic	Probiotic species	Sensitivity to antibiotic
Gentamicin (10µg)	<i>Lactobacillus acidophilus</i>	Resistant
Gentamicin (10µg)	<i>Lactobacillus plantarum</i>	Resistant
Gentamicin (10µg)	<i>Lactobacillus rhamnosus</i>	Resistant
Gentamicin (10µg)	<i>Lactobacillus reuteri</i>	Intermediate
Tetracycline (30µg)	<i>Lactobacillus reuteri</i>	Intermediate
Tetracycline (30µg)	<i>Lactobacillus plantarum</i>	Intermediate
Tetracycline (30µg)	<i>Lactobacillus acidophilus</i>	Sensitive
Tetracycline (30µg)	<i>Lactobacillus rhamnosus</i>	Sensitive
Penicillin (10µg)	<i>Lactobacillus reuteri</i>	Resistant
Penicillin (10µg)	<i>Lactobacillus plantarum</i>	Sensitive
Penicillin (10µg)	<i>Lactobacillus acidophilus</i>	Sensitive
Penicillin (10µg)	<i>Lactobacillus rhamnosus</i>	Sensitive

for these sensitive combinations, as the antibiotic may kill the probiotic, negating any potential benefit. However, taking these supplementary probiotics after treatment may help repopulate the depleted probiotics. For resistant antibiotic–probiotic combinations, probiotic supplementation may be a plausible method to reduce antibiotic side-effects such as diarrhoea.

My investigation only explored the effects of three antibiotics on four *Lactobacillus* species, which is unlikely to fairly represent the probiotic populations in the human body, and it does not cover all the antibiotics used in medical practice. Other probiotics should be studied to further investigate the effects of antibiotics on them. Identifying the probiotic species which live in the human gastrointestinal tract would be a valuable area of further study. This could be conducted by testing a large sample of different stool samples. Gaining a deeper insight would give doctors more information as to which probiotics to prescribe in combination with particular antibiotics. It is likely that most of these bacteria would be anaerobes.

Given that three out of four probiotics showed resistance to gentamicin, this antibiotic with the three probiotics may be the best combination to study. The hospital environment in which gentamicin is primarily used (Hingston, pers. comm. 2012) would make it easier to study in more controlled settings. During antibiotic therapy with gentamicin, taking the resistant probiotics may still have a benefit, because they should survive to benefit the host. However, for penicillin and tetracycline, both have antimicrobial activity against the *Lactobacillus* (except for penicillin with *L. reuteri*); therefore there is no benefit in taking the corresponding probiotic, during a course of penicillin or tetracycline.

Another direction may be to study how these four *Lactobacillus* species become resistant over time. This could be carried out by sub-culturing the bacteria repeatedly, and examining whether the zones of inhibition change over time. If the zones decreased, this may give insight into the ever-increasing medical concern about antibiotic resistance.

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