

Treatment of apple trees with streptomycin and potential risk to human health

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Following the recent Biosecurity Australia advice recommending that export quality apples produced using New Zealand's standard commercial practices be permitted access to Australia, concerns have been raised about the possible health and safety risks due to the use of the antimicrobial streptomycin on apple orchards to control the plant disease fire blight.

FSANZ has evaluated this potential issue and has concluded it is of negligible food safety concern. This view has been confirmed by internationally recognised experts in the field of antimicrobial resistance, who have peer-reviewed the FSANZ assessment.

Below is a summary of the key points:

- Only a small proportion of apple orchards in New Zealand are treated with streptomycin during each growing season (less than 5% in 2009-10). When used, it is applied to trees during blossom, or three to six months prior to harvesting of mature fruit.
- The possibility of residues of streptomycin being present on apples is minimal. This is due to the time period between treatment of the orchard and harvesting of mature fruit, as well as practices undertaken by growers which limit the potential retention of residues on fruit.
- Like many antimicrobials, streptomycin is produced by a bacterium commonly found in soil (*Streptomyces griseus*). There is a background level of bacteria naturally resistant to streptomycin in the environment.
- The use of streptomycin in human medicine is extremely limited, with alternative classes of antimicrobials available to treat most infections.
- The consumption of apples imported from New Zealand poses a negligible increased risk to Australian consumers from potential exposure to antimicrobial resistant organisms.

Executive Summary

This report describes a qualitative evaluation of the risk to human health from consumption of apples sourced from orchards treated with streptomycin. Streptomycin is an antimicrobial that is applied directly to apple and pear blossom to control the plant pathogen *Erwinia amylovora*, the causative agent of fire blight, in countries where the disease is present.

Streptomycin is naturally produced by the soil bacterium, *Streptomyces griseus*. Acquired resistance to this antimicrobial compound has been frequently observed in other soil-based bacteria. As a consequence, humans are exposed to naturally occurring streptomycin resistant bacteria on food, even where streptomycin has not been used during food production.

Resistance has, however, been increasingly observed in bacteria in the clinical setting since the therapeutic use of antimicrobials commenced. In humans, exposure to bacterial pathogens (disease-causing microorganisms) that are resistant to antimicrobials can result in treatment failure, loss of treatment options and increased likelihood and severity of disease. Use of streptomycin in human medicine is now extremely limited, with alternative classes of antimicrobials available to treat most infections.

For antimicrobial resistance to cause clinical harm in humans, the following conditions need to be met:

- The resistance must be present, or develop, in the bacterium
- People need to be exposed to the resistant bacterium
- The bacterium must be able to cause disease in humans, or transfer its resistance to bacteria that can cause disease in humans
- The disease requires treatment with antimicrobials for which the bacterium is resistant, leading to treatment failure.

Streptomycin treatment of apple orchards in New Zealand is tightly controlled, with a small proportion (less than 5% in 2009–10) of orchards treated during a growing season. Industry programs exist to assist growers determine the disease potential of orchards based on the prevailing environmental conditions (average temperatures, moisture levels etc), and provide guidance on Good Agricultural Practice for the application of streptomycin to susceptible trees.

In New Zealand, apple trees flower from September to December, with mature apples harvested in late summer and autumn (between February and early May)—three to six months after the application of streptomycin, if it is used.

Due to the nature of application (surface treatment of blossom) and the separation in time between treatment and fruit development, the possibility of residue retention on mature fruit is minimal. This is further controlled by implementation of NZ maximum residue limits (MRL) of 0.1 mg/kg in pome and stone fruit.

Dietary exposure to streptomycin was modelled on a worst-case scenario assuming that all foods consumed had levels of streptomycin at existing Australian MRLs (mammalian meat and edible offal, milk), plus NZ MRL levels in pome and stone fruit. Even using these extreme assumptions the mean exposure to streptomycin was estimated to be 6% of the Acceptable Daily Intake¹ (ADI; 0–0.05 mg/kg body weight), with a 90th percentile of exposure at 13%. The overall contribution from apples to total streptomycin exposure was <5%. The potential for residues from the consumption of apples sourced from trees treated with streptomycin exerting increased selection pressure (exposure to an antimicrobial at concentrations that inhibit growth of sensitive bacteria) on gut microflora is therefore negligible.

There is no scientific evidence linking the consumption of whole, intact apples with the transmission of pathogenic microorganisms to humans. Apple producers in New Zealand follow Good Agricultural Practices (GAP) as well as Good Manufacturing Practices (GMP) during processing/packing, which further limits microbiological contamination of product. It is therefore extremely unlikely that pathogenic microorganisms would be present on apples imported from NZ. It is similarly unlikely that there would be measurable increase in potential transfer of streptomycin resistance from non-pathogenic microorganisms carried on these apples.

¹ The Acceptable Daily Intake (ADI) for humans is defined as an estimate of the amount of a chemical that can be ingested daily over a lifetime without appreciable risk to health

In conclusion, the consumption of apples sourced from New Zealand orchards potentially treated with streptomycin for the control of fire blight poses a negligible increased risk to Australian consumers from exposure to antimicrobial resistant organisms above natural background levels.

Treatment of apple trees with streptomycin and potential risk to human health

1 Scope

This report addresses the development of antimicrobial resistant (AMR) bacteria and possible risks to human health associated with consumption of apples from trees treated with streptomycin. It does not cover in detail risks to human health due to the exposure to AMR organisms in the community, hospital, environmental settings, direct contact with animals, or via the consumption of animal products as foods.

2 Objectives

The aim of this report is to:

- 1) Provide an overview and evaluate the possibilities of development and/or selection of AMR organisms due to the application of antimicrobial treatments during apple production;
- 2) Conduct a preliminary qualitative characterisation of the increased risks to human health due to exposure to AMR organisms through the consumption of apples from streptomycin-treated orchards.

3 Description of the antimicrobial agent

Summary

Many antimicrobial compounds are produced naturally by microorganisms. Streptomycin is produced by a soil dwelling microorganism and has been used in human and veterinary medicine, as well as for the control of plant diseases. Streptomycin has been rated as of “low importance” based on its limited use for humans in Australia

Antimicrobials are a chemically diverse group of compounds naturally produced by microorganisms (and more recently synthetically), that have microstatic or microcidal activity. They function by a variety of mechanisms that disrupt microbial metabolism. Antimicrobials are used in humans, animals and to a very limited extent in plants, to control bacterial diseases.

Streptomycin is an aminoglycoside antimicrobial, which consists of aminocyclitol groups with a variable number of amino-sugars attached. This group of antimicrobials act rapidly by blocking bacterial protein synthesis, and the effects are concentration dependent.

Streptomycin is derived from *Streptomyces griseus*, a Gram-positive filamentous bacterium readily isolated from soil (Nwosu 2001). Streptomycin has been widely applied for prophylactic and therapeutic use in human and veterinary medicine and, to a limited degree, to control plant diseases in the horticultural industry.

3.1 Use of streptomycin in humans

Streptomycin was the first broad spectrum antimicrobial to be discovered, and has been used for the treatment of tuberculosis and Gram-negative bacterial infections in humans, although its use has declined more recently (Han et al. 2003).

Streptomycin is very rarely used in Australia. Its role in treatment of tuberculosis and other mycobacterial diseases has been superseded by alternative agents, including other aminoglycosides (e.g. amikacin), which are not affected by streptomycin resistance mechanisms. Streptomycin is very rarely required for enterococcal endocarditis, where it is used in combination with other agents to control bacterial strains that are resistant to high levels of gentamycin. Additionally, streptomycin is no longer marketed in Australia and can only be accessed through the Therapeutic Goods Administration (TGA) Special Access Scheme.

In 2006, the then Expert Advisory Group on Antimicrobial Resistance (EAGAR) rated streptomycin as of 'low' importance for use in humans in Australia.

4 Antimicrobial resistance

Summary

Antimicrobial resistance in certain bacteria has become a major public health concern due to the resulting treatment failure, loss of treatment options and increased likelihood and severity of disease. AMR genes have existed in pathogenic bacteria before the use of antimicrobials, as these compounds are naturally produced by bacteria in the environment. However, the presence of AMR in microorganisms of human health concern has been observed to increase in line with the more widespread use of antimicrobials.

Bacteria use many pathways for the exchange of genetic material. The most important pathway for the development of AMR is through horizontal gene transfer. However, the likelihood of an organism accepting and retaining extraneous DNA is limited by many factors and must overcome several physiological and species barriers.

Antimicrobial resistance is the ability of previously sensitive bacteria to develop resistance to the effect of antimicrobial compounds. It is usually quantified as the minimum concentration (MIC) of the antimicrobial(s) under consideration which is required to exert a definable effect (such as growth inhibition) on a population of bacterial cells.

Antimicrobial resistance has become a widespread global health concern due to the apparent increase in treatment failure during the use of antibiotics in the management of human, animal and plant diseases. Acquired antimicrobial resistance appears to have developed over time in relation to the quantity of antimicrobials used.

When human pathogens become resistant to antimicrobial agents they can pose a greater health risk as a result of potential treatment failure, loss of treatment options and increased likelihood and severity of disease.

Resistance to antimicrobial treatment for bacterial pathogens was first described in the 1940's and since then, resistance has been observed for all known antimicrobials (Levy, 1998). Antimicrobials have the undesirable effect of exerting selection pressure on otherwise rare resistant microorganisms. Laboratory studies have demonstrated that exposure to antimicrobials (and other stressors) can increase the frequency of gene transfer between microorganisms and also increase their mutation rate thereby increasing the probability that new resistant strains will emerge (Heinemann, 1999).

The major factors that influence AMR are the antimicrobial itself and the genes conferring resistance. Microorganisms can acquire these genes via several mechanisms:

- Chromosomal (DNA) changes by means of genetic mutations.
- Horizontal gene transfer mediated by mobile genetic elements such as plasmids, transposons and integrons. Antimicrobial resistant genes carried on such mobile elements are transferred between bacteria that inhabit different ecological niches, and among different genera of bacteria. The AMR genes can therefore be transferred from non-pathogenic bacteria to pathogenic bacteria. Horizontal gene transfer is considered to be responsible for the highest degree of evolutionary change in bacteria, including AMR (Todd et al 2011).
- Transduction of genetic material mediated by bacteriophages (viruses that infect and replicate in bacteria), and
- Transformation, where the DNA of a host is integrated into the bacterial genome.

Evidence suggests that AMR genes have existed in bacterial populations prior to the advent and use of antimicrobials by humans. However, the presence antimicrobials in any setting can confer selection pressure in the microbial niche. It may initiate the establishment and spread of AMR by selectively amplifying the probability of survival and proliferation of the strains containing AMR genes, over and above the non-resistant microorganisms. A large reservoir of AMR microorganisms and resistant genes is therefore established.

Establishment of resistance will depend on the levels of exposure to antimicrobials, based on locality and usage, as well as the microbial population prevalent in the ecological niche under consideration. Resistance to specific antimicrobials therefore varies between and within countries (Read 2000).

Bacterial resistance to antimicrobials is affected by the following mechanisms:

- Production of enzymes that inactivate the antimicrobial
- Alteration of the cellular target
- Active removal of the antimicrobial from the bacterial cell via efflux pumps

Every organism that shares the same ecological niche can be thought of as a potential genetic donor (Hanssen and Ericson 2006). There is constant interaction of microorganisms in the environment—between humans, animals, soil and water—facilitating the spread of microorganisms and their genes. The distribution of microorganisms with antimicrobial resistant properties in the environment is widespread, particularly amongst soil organisms.

4.1 Streptomycin resistance

Summary

Streptomycin resistance is conferred through point mutations as well as horizontal gene transfer. Spread of streptomycin resistance is mainly mediated by genes carried by plasmids. The plasmids carrying these genes are likely to have evolved in soil bacteria in response to streptomycin-producing bacteria present in the environment. These resistance determinants are widespread and have been found in bacteria isolated from soil, water, plants, animals and humans.

Streptomycin resistance is conferred via two mechanisms. A chromosomal point mutation in the gene *rpsL* confers very high levels of streptomycin resistance to bacteria, and is transferred during bacterial division. Transfer of streptomycin resistance via chromosomal mutation is slow and usually not transmitted to other bacterial species (Vanneste 2008).

The other mechanism is via horizontal gene transfer. This is achieved by genes carried on mobile genetic elements such as plasmids and transposons. The gene pair which confers streptomycin resistance, *strA-strB* are carried on mobile plasmids that are transferred between bacteria during bacterial conjugation. Another gene known to confer streptomycin resistance, the *aadA1* gene cassette, is carried by a transposon termed Tn21 (Liebert et al 1999). Lower levels of streptomycin resistance are transferred across several bacterial species and genera in this manner when conditions are favourable, and lead to widespread dissemination of streptomycin resistance (Vanneste 2005).

The transferable genetic element, called plasmid Tn 5393, carrying the streptomycin resistant gene pair *strA-strB* was first discovered in streptomycin resistant *Erwinia amylovora* in 1970 (Chiou and Jones, 1991). The mobile genetic elements carrying the *strA-strB* genes are known to have evolved in a soil bacterium in response to natural streptomycin selection from streptomycin producing bacteria (Sundin and Bender 1996). They are also known to be widely prevalent in the environment, especially in plant and soil environments. Likewise, Tn21 is known to have originated in soil bacteria and widespread among clinical and environmental settings (Liebert et al 1999).

Chance associations of bacteria are thought to have led to the transposition of the mobile genetic element to a non-conjugative broad host range bacterial element indigenous to human or animal associated bacteria (Sundin and Bender 1996). It is clear that antimicrobial resistant genes and mobile genetic elements have spread between bacteria associated with humans and animals. The gene pair *strA-strB* has been found in pathogenic bacteria in plants and fish (Sundin and Bender 1995; L'Abée-Lund and Sørum 2000). Sundin et al. (1995), however, found no direct association between intensive streptomycin use in agriculture settings and the development of AMR in microorganisms, as bacteria carrying the *strA-strB* genes were detected in agricultural settings with no prior exposure to streptomycin. Similarly, Tn21 has been isolated from polluted as well as pristine sites, as well as from bacteria in the marine environment (Dahlberg et al. 1995)

In animal and human bacteria, the *strA-strB* gene pair is frequently linked to the sulphonamide resistant gene *sul111* and often multiple antimicrobial resistance is conferred. Another plasmid carrying these genes is RF 1010, first recovered in an *E. coli* strain isolated from pig faeces in Czechoslovakia in 1971 (Guerry et al. 1974). Han et al. (2004) also reported the presence of the gene pair in bacteria isolated from kiwifruit orchards in Korea and Japan. Sequence remnants of the plasmid were detected within chromosomes of clinical isolates of *Campylobacter jejuni* and *Pseudomonas aeruginosa* and on multiple antimicrobial resistant plasmids from the Gram-positive microorganism *Corynebacterium striatum*.

4.2 Antimicrobial resistant organisms in the environment and food products

Microorganisms resistant to antimicrobials have been isolated widely from the environment (e.g. soil, water), raw food products, animals and humans.

The Australian Group on Antimicrobial Resistance (AGAR) compiled data on antimicrobial resistant organisms from the National Enteric Pathogens Surveillance Scheme (NEPPS). This scheme recorded 2429 isolates of *Salmonella* Typhimurium from humans in the year 2000. Of these isolates, 2,061 (85%) were tested for resistance to antimicrobials routinely used in medical and veterinary applications and 252 were found to be resistant to one or more commonly used antimicrobials; 157 (7.6%) of *S. Typhimurium* strains were resistant to two or more commonly used antimicrobials. A group of 15 serovars (9.6% of 157) were resistant to streptomycin and sulphonamides. Isolates with similar characteristics were found from beef-producing animals in the same period (Yau et al. 2010).

Antimicrobial resistance in *Enterococcus* spp. isolates from human clinical cases was surveyed in a point-prevalence study in Australia in 2007 (AGAR 2007). Seventeen sites around Australia were sampled and a total of 1695 isolates were collected (up to a 100 per institution) and tested for susceptibility to vancomycin, high-level gentamycin and/or high-level streptomycin. High-level resistance to gentamycin was 30.8% in *E. faecalis* and 57.1% in *E. faecium* (figures for 2005 being 35.8% and 52.2% respectively). The figures for high-level streptomycin resistance were 13.9% and 58.9% respectively (survey results for 2005 being 10.3% and 60.2% respectively).

In 2008, a report was published by CSIRO on a pilot survey of antimicrobial resistant bacteria in Australian food. The survey was commissioned by the Food Regulation Standing Committee to provide data that could be used to estimate the prevalence of AMR bacteria (*Enterococcus* spp. and *E. coli*) in selected foods purchased at retail outlets. Overall, the level of AMR bacteria in the foods tested (whole poultry, pork chops, beef mince and iceberg lettuce) was low (<10 %) when compared with levels internationally. The survey found resistance to “critically important” antimicrobials to human medicine, such as the quinolone class of drugs and 3rd and 4th generation cephalosporins, non-existent or extremely low in the bacteria isolated.

The prevalence of *E. coli* on iceberg lettuce was very low, with only 7 isolates from over 70 samples. Resistance to one or more antimicrobials was observed in 5 of 7 isolates (71%). Resistance to ampicillin (57.1%) was observed most often. Resistance to amoxicillin / clavulanic acid (28.6%), cefazolin (28.6%), streptomycin (14.3%), tetracycline (28.6%) and trimethoprim / sulfamethoxazole (14.3%) was also identified. It was noted, however, that firm conclusions concerning the prevalence of AMR in *E. coli* isolates could not be made with confidence due to the extremely limited isolation of *E. coli* from this food source.

A study by Abriouel et al. (2008) compared the incidence of antimicrobial resistance amongst *Enterococcus* spp. from raw fruit and vegetables, water, soil and clinical samples in Spain. *E. faecalis* isolates from fruit and vegetables had a much lower incidence of streptomycin resistance compared to clinical samples (5.9 % versus 51.3 % respectively). Additionally, *E. faecalis* isolates from vegetables had a much lower average number of resistance traits per strain compared to clinical isolates (4.2% versus 8.7% respectively).

In Europe, van Overbeek et al. 2002 assessed the presence of specific streptomycin resistant genes in pristine as well as in polluted habitats. All habitats contained streptomycin resistant genes. Therefore, streptomycin resistance occurs widely in natural environments, and human exposure to environmental streptomycin resistant sources could be expected.

In a study of native bacteria isolated from the Yarra River, Victoria, Boon et al. (1999) found that the incidence of antimicrobial resistance in native heterotrophic bacteria was higher than that for faecal (*E. coli*) bacteria. This was most noticeable in the upper reaches of the river system that were largely unaffected by human activity. Resistance of heterotrophic bacteria to streptomycin was observed in 8 – 24 % of isolates collected at different sites of the river (0–14 % for *E. coli*). Resistance of heterotrophs to ampicillin and penicillin was 75 ± 5 % and 96 ± 1 % respectively. The authors postulated that the antimicrobial resistance of aquatic bacteria isolated from these remote sites was a function of the production of antimicrobials by the native bacteria themselves.

5 Use of antimicrobials in horticulture

Summary

Antimicrobials have been used to control plant diseases in several countries. Streptomycin is used for the control of fire blight, a disease caused by the bacterial plant pathogen Erwinia amylovora. Several conditions need to exist for outbreaks of fire blight to occur in apple orchards. In New Zealand, streptomycin application is targeted by computer models used to predict the occurrence of these conditions. Outbreaks of fire blight occur during blossoming and therefore the application of streptomycin is limited to the three months of spring (0-4 applications).

There are a number of plant pathogens which are sensitive to antimicrobials and where the application of antimicrobial preparations has proved valuable in the control of serious plant diseases. Resistance to these antimicrobials has also been observed for a number of plant pathogens, both domestically and internationally.

Streptomycin, gentamycin and oxytetracycline have been used to control the fire blight bacterium *Erwinia amylovora* in pear and apple trees. Antimicrobials have been used in all countries where outbreaks of the disease have been reported and are considered the only effective control measure available. These antimicrobials have also been employed to control bacterial speck of tomato caused by *Pseudomonas syringae* pv. *tomato*, bacterial canker (blast) *P. syringae* pv. *syringae*, and bacterial spot *Xanthomonas campestris* pv. *pruni* of stone fruit. Streptomycin as a seed dressing has been used on beans, tomatoes and cotton, but problems associated with phytotoxicity has limited its use, particularly in beans. In Queensland streptomycin sulphate had been used by the Navy Bean Marketing Board as a seed treatment to reduce the incidence of common blight (*X. campestris* pv. *phaseoli*). In addition to New Zealand, streptomycin has been registered for use for the control of fire blight in USA, Canada and Israel (Vanneste et al. 2008). Streptomycin is not permitted for use on horticulture in Europe.

In Mexico and Latin America, gentamycin (alone and in combination with other antimicrobials) is permitted for use in the control of fire blight, soft rot and other diseases in vegetables (Expert Panel on Antibiotic Resistance 2005).

Table 1 summarises the use of Streptomycin in New Zealand.

Table 1 Use of streptomycin in New Zealand horticulture (Vanesste 2005).

Pathogen	Disease	Host
<i>Erwinia amylovora</i>	Fire blight	Apple, pear and Nashi
<i>Pseudomonas syringidae</i> pv <i>syringidae</i>	Bacterial blast	Stone fruit
<i>Xanthomonas arboricola</i> pv <i>pruni</i>	Bacterial spot	Stone fruit
<i>P. syringidae</i> pv <i>tomato</i>	Tomato speck	Tomato
<i>P. syringidae</i> pv <i>syringidae</i>		Tomato
<i>X. campestris</i>	Bacterial spot	Tomato
<i>Clavibacter michiganensis</i>	Bacterial canker and wilt	Tomato

The existing maximum residue limits (MRLs) for streptomycin, gentamycin, oxytetracycline and kasugamycin on fruit and vegetables are shown in Table 2, together with the Australian and JECFA ADI's or the United States Environment Protection Agency (US EPA) chronic reference dose (kasugamycin).

Table 2 MRLs/tolerances for antimicrobials used in the production of fruit and vegetables and Australian, JECFA, or US EPA health based guidance values.

Antimicrobial	Pesticide MRL/tolerances in fruit and vegetables	Australian ADI ¹ , JECFA ADI ² or US EPA chronic RfD ³
Streptomycin	<p>Australia: Nil</p> <p>New Zealand: Pome fruit and stone fruit (0.1 mg/kg)</p> <p>United States: Tomato, potato, pepper, pome fruit (0.25 ppm); Bean succulent; bean dry seed (0.5 ppm)</p> <p>European Union: Nil</p>	<p>0.05 mg/kg bw (group ADI dihydrostreptomycin and streptomycin)¹</p> <p>0-0.05 mg/kg bw (group ADI dihydrostreptomycin and streptomycin)²</p>
Gentamycin	<p>Australia: Nil</p> <p>New Zealand: Nil</p> <p>United States: Apple (0.1 ppm) expired 31/12/10</p> <p>European Union: Nil</p>	<p>0.05 mg/kg bw¹</p> <p>0-0.02 mg/kg bw²</p>
Oxytetracycline	<p>Australia: Nil</p> <p>New Zealand: Nil</p> <p>United States: Peach, pear, apple (0.35 ppm), vegetable fruiting group 8 (0.04 ppm)</p> <p>European Union: Nil</p>	<p>0.03 mg/kg bw¹</p> <p>0-0.03 mg/kg bw (group ADI group ADI for tetracycline, oxytetracycline and chlortetracycline)²</p>
Kasugamycin	<p>Australia: Nil</p> <p>New Zealand: Nil</p> <p>United States: vegetable fruiting group 8 (0.04 ppm)</p> <p>European Union: Nil</p>	<p>0.1 mg/kg bw/day³</p>

5.1 Application of streptomycin during apple production

In countries where permitted, streptomycin is applied to apple trees during flowering (bloom) to control fire blight, with 0–4 applications per season depending on disease forecast (Expert Panel on Antibiotic Resistance 2005).

New Zealand is recognised as an established producer and exporter of apples. The major apple production areas are Hawke’s Bay (61% of total New Zealand production), Nelson (29%) and Otago (4%), with all other areas in the country (e.g. Auckland, Bay of Plenty, Gisborne, Wairarapa and Waikato) together contributing only 6% to the country’s total production (Biosecurity Australia 2006).

Apple harvest in New Zealand takes place in late summer and autumn (between February and early May). Leaf fall occurs during winter (June to August). New growth occurs in spring (September to November), with apple development occurring during summer (from December to March). See Annex 1 for an overview of the annual apple growing cycle in New Zealand.

Once harvested, apples are sorted, washed, waxed and cooled (4 °C), prior to packaging. For export markets, this is managed by New Zealand's Apple and Pear Marketing Board, which holds overall responsibility for all aspects relating to quality assurance, market access and trade.

Synchronisation of the following conditions is required for the occurrence of fire blight (Gouk et al 1996):

- Flowers open with stigmas and petals intact.
- The accumulation of at least 110 degree hours² when temperatures are greater than 18.3°C after first bloom
- Wetting caused either by dew or 0.25 mm of rain (or at least 2.5 mm of rain the previous day)
- An average daily temperature of at least 15.6°C

Validated computer models are available and utilised in New Zealand to predict the occurrence of these events, therefore enabling targeted application of streptomycin to susceptible orchards (Gouk et al 1996). The New Zealand Plant Protection Society recommends the use of no more than three applications of streptomycin over the bloom period (Vanneste, 2005). Spray applications are also subject to GROWSAFE™ controls. GROWSAFE courses are conducted through New Zealand's Agricultural Industry Training Organisation (AgITO) and commercial users of weed or pest sprays are required to hold a standard GROWSAFE certification. This course trains people to apply agrichemicals safely and accurately in accordance with the Agrichemical Users' Code of Practice.

Data from the Ministry of Agriculture and Forestry show that, during the 2009–10 growing season, 3.3% of the total 7276 apple blocks in New Zealand reported application of streptomycin to control potential fire blight outbreaks. Of these treated blocks, 162 blocks received one streptomycin application, 78 received two applications and one block received three applications.

The development and/or selection of streptomycin resistant microorganisms (pathogens and non-pathogens) could theoretically increase during September–October, coinciding with the application of streptomycin during bloom. However, the time between streptomycin application to trees and the harvesting of mature fruit is between three to six months, during which time there is no additional selection pressure.

² Degree hours are expressed as the sum of hourly values of temperature variability, computed using hourly (where available) or daily mean temperature data.

6 Potential exposure to antimicrobial resistant microorganisms via the consumption of apples from streptomycin-treated orchards

Summary

Potential health risks from consumption of apples from streptomycin-treated orchards can occur via three main mechanisms: direct exposure to AMR pathogenic microorganisms on contaminated apples; transfer of AMR genes from non-pathogenic microorganisms present on apples to human gut microflora; and amplification of AMR microorganisms in the gut from direct exposure to antimicrobial residues present on the fruit.

While AMR selection pressure may be present in the environment during treatment of fire-blight susceptible apple trees (applied to trees during bloom), the mature fruit are not harvested until late summer, resulting in three to six months without additional selection pressure. The potential for contamination with AMR microorganisms is further reduced by producers following good agricultural practice and good manufacturing practice. Based on data from the scientific literature, whole intact apples are extremely unlikely to be a vehicle of foodborne pathogens. Exposure to residues of streptomycin from these fruit is also considered to be negligible.

In assessing the risks to human health posed by the consumption of imported apples obtained from trees possibly treated with streptomycin, the pathways leading to potential adverse human health effects were considered as follows:

- 1) Direct exposure to AMR pathogenic microorganisms carried on contaminated apples

While always possible, there is no scientific evidence linking the consumption of whole, intact apples to the transmission of pathogenic microorganisms to humans. Adherence to Good Agricultural Practices (GAP) and Good Manufacturing Practices (GMP) limits the opportunity for contamination of apples by human pathogenic microorganisms (Biosecurity Australia 2006). Such practices include quality control and inspection procedures in cool stores, packing houses and during sorting as well as high pressure washing (reduces contaminating surface microorganisms by 10 to 100 fold).

AMR pathogenic bacteria are already present in the environment and on many raw food products. For example, a study by Yau et al. (2010) recently reported the presence of *S. Typhimurium* phage type 9 resistant to streptomycin and sulphonamide in Australia isolated from bovine and human sources. Therefore, potential human exposure to streptomycin resistant microorganisms has already occurred in Australia, prior to any (unlikely) exposure from imported apples.

- 2) Exposure to non-pathogenic AMR bacteria present on apples leading to possible horizontal transfer of AMR genes to human gut microflora and/or pathogens.

Although the occurrence of the *strA-strB* genes within a diverse bacterial host range is evident from recent horizontal gene transfer, the vehicles that carry *strA-strB* are often different in different bacterial groups. Bacteria from humans and animals typically carry these genes on small host range nonconjugative plasmids whereas plant pathogen isolates harbour the gene pair on large, conjugative plasmids (Sundin, 2002). Sundin and Bender (1996) investigated the spread of streptomycin resistance in 17 genera of human, animal and plant pathogens. A *S. Typhimurium* strain from New Zealand carried *strA* genes linked with sulphonamide resistant genes, but the plasmid carrying these genes was not self-transmissible, indicating the need for very specific requirements to facilitate transmission and therefore a reduced ability to disseminate antimicrobial resistance. The presence of the *aadA1* gene cassette, carried on the Tn21 transposon, is also well documented in environmental and clinical Gram-negative bacteria (Liebert et al. 1999).

While the integration and expression of AMR genes in gut microflora and colonisation of the human gut by the pathogenic microflora is possible, the increased effect from exposure to apples sourced from streptomycin-treated orchards above normal background levels is unclear. This would, however, be minimised by following good agricultural practices and food hygiene practices that minimise the presence of all food borne pathogens. Such practices are well established and followed in the apple orchards and processing centres in New Zealand.

- 3) Amplification of antimicrobial resistance due to the consumption of apples with residue levels of antimicrobials providing opportunistic selective pressure to gastrointestinal pathogens

Negligible streptomycin residues are expected on imported apples due to the following reasons:

- GAP is applied in orchards, with targeted and limited application of streptomycin.
- The time between application of streptomycin to fire blight-susceptible apple trees and harvesting of mature fruit is three to six months.
- Statutory maximum residue levels exist in New Zealand for apples.

While the assays that make up the pesticide multiscreen in the annual New Zealand MAF Food Residue Surveillance Programme and the five-yearly MAF Total Diet Survey do not analyse for residues of streptomycin, residues were not detected (limit of detection 0.05 mg/kg) in a limited number of samples taken from six apple growers in the Hastings region in 2005 (pers. com. MAF New Zealand, 2011).

The dietary exposure estimates for streptomycin were found to be below the Acceptable Daily Intake (ADI) levels of 0–0.05 mg/kg body weight when calculated using the FSANZ Dietary Modelling of Nutritional Data (DIAMOND) program. This was using a worst-case scenario that all mammalian meat and edible offal, and milk consumed have levels of streptomycin at existing Australian MRLs (0.3 mg/kg and 0.2 mg/kg respectively), along with pome and stone fruits having levels at the NZ MRL of 0.1 mg/kg. The mean dietary exposure to streptomycin from all food sources was estimated to be 6% of the ADI and the 90th percentile exposures were found to be 13% of the ADI. Overall, apples represented less than 5% of the total streptomycin exposure in comparison to exposure due to milk (73%), mammalian meat (21%) and pome and stone fruits (6%).

7 Conclusions

Microorganisms resistant to streptomycin are widely disseminated in the environment, having been isolated from soil, water, plants, animals and humans. Scientific evidence confirms that microorganisms with similar AMR characteristics are present in Australia.

AMR microorganisms (pathogenic and non-pathogenic) may be present on apples harvested from trees treated with streptomycin as contaminants. However the increased risk of exposure to streptomycin resistance genes from this commodity is considered negligible in comparison with existing exposures from clinical applications, veterinary use and animal products.

The use of streptomycin in New Zealand's apple orchards is highly targeted, with computer models used to predict the disease potential of apple trees based on a range of environmental conditions. In the 2009–10 growing season, less than 5% of apple orchards in New Zealand were treated with streptomycin.

Streptomycin application in orchards may contribute selection pressure for resistance to plant-associated and soil organisms but the significance of this is unclear. Sundin and Bender (1996), however, reported a lack of evidence for the direct transfer of *strA-strB* genes between plant and animal bacteria.

Selective pressure for AMR in the human gut microflora as a result of the exposure to streptomycin residues present on apples is low, based on the time period between treatment of apple trees and harvesting of mature fruit (three to six months), in addition to the control of maximum residue levels implemented by New Zealand.

The application of GAP and food safety practices by the exporting country as well as in Australia would reduce overall contamination by microorganisms. The New Zealand Apple and Pear Board has been established to centralise and manage all aspects associated with the export of apples by New Zealand. Stringent quality control measures stipulated at various points along the processing, packing and distribution chain including temperature control, many quality control steps and strict adherence to GMP provides added assurance that contamination by microorganisms (including AMR microorganisms) is minimised.

It is concluded that the consumption of apples sourced from New Zealand orchards treated with streptomycin for the control of fire blight poses a negligible increase in risk to Australian consumers due to exposure to antimicrobial resistant organisms above natural background levels.

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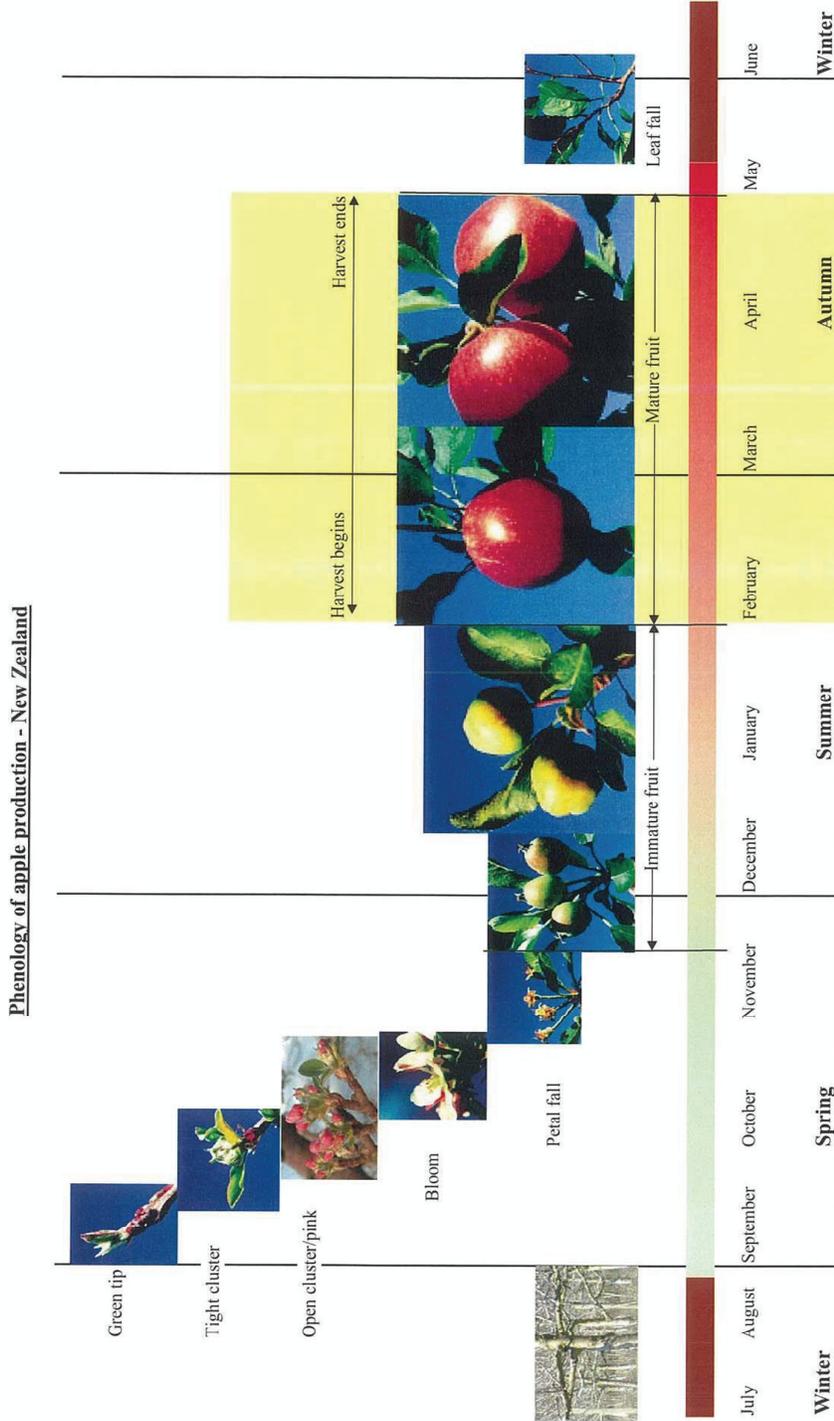
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Annex 1

Apple Production in New Zealand



Apple production in New Zealand
(Biosecurity Australia 2006)