SUBMISSION TO SENATE INQUIRY:

Progress in the implementation of the recommendations of the 1999 Joint Expert Technical Advisory Committee on Antibiotic Resistance

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SUMMARY:

For nearly a century, we have waged a war on bacteria. We have learned to fight off these 'enemies' by using stronger and stronger weapons. As bacteria have found ways to resist the lethal effects of one antimicrobial weapon, we have unleashed another. There is now a real worry we may be running out of options to tackle antimicrobial resistant (AMR) bacteria.

In some clinical settings, the medical community has been turning to silver as an antimicrobial of last resort. New advances in nanotechnology have allowed small nanoparticles of silver (nano-silver) to deliver silver ions even more efficiently and be embedded in a wider range of clinical surfaces. Yet at the same time, many companies have seen a marketing advantage in including nano-silver as an ingredient in everyday products such as toothbrushes, towels, hairbrushes, shirts, shoes and socks.

However, as with antibiotics, the overuse of nano-silver will promote resistance to this important antimicrobial. Already, there is evidence of bacterial resistance to silver in many clinical settings. What's worse, experts have warned that nano-silver will co-select for resistance to antibiotics and other antimicrobials.

In response, industry groups claim that humans have used (bulk) silver for thousands of years without demonstrated harm. However, the quantity of nano-silver particles currently in use, the potency of their antimicrobial action, the contexts of their use, and the current antibiotic resistance crisis are unprecedented. Antimicrobial experts such as Prof. John Turnidge have warned that using such a powerful antimicrobial in these everyday products is not only unnecessary, but dangerous.

Moreover, the growing non-clinical use of nano-silver as a surface antimicrobial will compromise the microbial diversity in our immediate (e.g. skin) and wider environment, eliminating both protective and benign microbes, thus allowing the flourishing and spread of resistant bacteria.

Research into the antimicrobial - triclosan - used widely in both households and hospitals has revealed both the mechanisms for bacterial resistance and the widespread incidence of triclosan-resistant bacteria. Yet, the use of triclosan as well as nano-silver continues with few restrictions.

Experts agree that regulators need to halt the excessive and unnecessary use of powerful antimicrobials in everyday products. This kind of regulation is critical in order to maintain the effective clinical uses of those antimicrobials, as well as the continued effectiveness of antibiotics.

Friends of the Earth Australia calls on the Federal Government to:

- further restrict most human and agricultural uses of antibiotics;
- restrict the usage of antimicrobials such as nano-silver and triclosan to clinical applications; and
- establish a new independent body with statutory authority to oversee the management of AMR in Australia

a) Examination of steps taken, their timeliness and effectiveness

Background

In 1999, the JETACAR (Joint Expert Technical Advisory Committee on Antibiotic Resistance) committee of health and agriculture experts was established by the Federal Government in response to the growing concerns around antimicrobial resistance. The resulting JETACAR report '*The Use of Antibiotics in Food-Producing Animals: Antibiotic-Resistant Bacteria in Animals and Humans'* contained a broad set of 22 recommendations aimed at reducing the incidence of antimicrobial resistance (AMR) in Australia – primarily through restricting the overuse of antibiotics in humans and in animals.

In response to the JETACAR recommendations, the Government established several interdepartmental committees and pilot surveillance programs. But these efforts were never large enough nor sustained for long enough.

The problem of antimicrobial resistance is now worse than ever, with superbugs – bacteria resistant to most antibiotics – spread throughout hospitals and communities around the world. The numbers of deaths caused by bacterial resistance to antimicrobials and antibiotics in hospitals continues to rise, with experts warning of a possible return to the pre-antibiotic era.

Current Situation

There is general agreement that since 1999, successive Australian governments have failed to act accordingly, given the gravity of the situation and the enormity of the challenge.

Australians are now amongst the highest users of antibiotics in the world, with over 22 million prescriptions issued every year – more than one for each man, woman and child.

Many Australian farmers are still using antibiotics as a growth promoter or prophylaxis to improve the production of meat from animals (e.g. pigs, chickens, fish), particularly those raised in factory farms. To make matters worse, potent new antimicrobials such as nano-silver and triclosan are being widely marketed to a germ-fearing public.

Regulatory bodies have acted with little concern for the ability for non-antibiotic antimicrobial compounds to co-select for broad antimicrobial and antibiotic resistance, in spite of the large body of evidence which demonstrates this cause and effect relationship.

This submission intends to summarise some of this evidence, and asserts that all antimicrobials require stringent restrictions in order to combat a looming crisis linked to antimicrobial resistance.

b) Where and why failures have occurred

Since the release of the 1999 JETACAR recommendations the following problems have escalated:

Increased use of antibiotics in humans

Australians are amongst the highest users of antibiotics in the world, with over 22 million prescriptions issued every year – more than one for each man, woman and child.

The problems:

- In humans, most antibiotics are given for treatment of minor infectious illness, especially respiratory tract infections.

- General practitioners are placed under pressure by the public to prescribe antibiotics for common colds and the flu.

- An uninformed and demanding public shop around to find doctors who will prescribe antibiotics for the cold and flu.

The solutions:

- General practitioners should have to provide a rationale for every prescription of antibiotics

- We need better education programs to prevent use of antibiotics to treat colds and flu. The NPS 'Resistance Fighter' campaign is a good example of such an education campaign, however more funding and more diversity of information is required.

Widespread use of antibiotics in animals

Many farmers are still using antibiotics as a growth promoter or prophylaxis, to improve the production of meat from animals (e.g. pigs, chickens, fish), raised in factory farms.

Compelling new evidence that farm antibiotic use can breed bacterial antibiotic resistance which can then transfer back to humans (Price *et al*., 2012) demands the implementation of stringent restrictions regarding the agricultural and veterinary uses of antimicrobials.

The problems:

- The intensive farming of livestock places all animals under stressful conditions and encourages farmers to use sub-therapeutic doses of antibiotics to maintain animal health.

- Sub-therapeutic uses of antibiotics have been demonstrated to breed bacteria with multiple drug resistance (superbugs), which can then be passed onto humans.

- While this problem was acknowledged in the original JETACAR report, government regulations and surveillance have not been strict enough to bring about the necessary scale of changes.

The solution:

- All non-therapeutic agricultural uses of antibiotics should be immediately banned, with stringent monitoring and surveillance systems put in place to enforce this ban.

New antimicrobials in consumer goods

It is now widely accepted that the growing use of antimicrobials in everyday products can contribute to the generation and spread of antimicrobial and antibiotic resistance.

The problem:

- Products such as shoes, socks, fridges, washing machines, toothbrushes, toothpaste, shirts, mattresses (and more) containing antimicrobials such as triclosan and nano-silver are increasingly marketed and sold to Australian consumers.

The solution:

- The Federal Government needs to take immediate action to ban all non-clinical use of these powerful antimicrobials.

c) Implications of antimicrobial resistance on public health and the environment

The impacts of antibiotic resistance in humans is already enormous. The financial costs of resistance include:

- the need to use multiple courses of antibiotics
- the need for more expensive antibiotics
- increased length of hospitalisation

However, the looming disaster we now face is the steadily increasing numbers of human lives lost to multi-drug resistant bacteria.

Alarmingly, some bacteria have already shown resistance to all known antibiotics, and it is predicted that the ongoing heavy usage of antibiotics will increase further these levels of resistance (McArthur *et al*., 2012).

One of the approaches to combatting the spread of antibiotic resistant bacteria in hospitals is through the increased use of other antimicrobials, such as quaternary ammonium compounds, and broad spectrum compounds like triclosan and heavy metals.

Historical uses and properties of silver

Better known for its uses in photography and jewellery, it has also long been established that

silver can kill microorganisms. For this reason, over the last several decades, silver has become the choice of heavy metals for use as an antimicrobial in clinical settings. The release of silver ions from different silver compounds can cause damage to fungi, algae, bacteria and viruses, preventing their growth. This antimicrobial property has been exploited for thousands of years, through the use of silver in carrying vessels, cutlery, etc (Wijnhoven *et al*., 2009). As an antimicrobial, silver has offered the ability to slowly release silver ions which disinfect surfaces while seemingly presenting few, if any, short-term harmful effects to human beings, other than in large doses (Luoma, 2008; Wijnhoven *et al*., 2009). However, modern nanotechnology advances have allowed silver particles to be reduced to the nano-scale. presenting new opportunities and new challenges.

Nano-silver is an more effective antimicrobial than bulk silver

Silver nanoparticles (nano-silver) are typically used in the size range of 1-50 nm. At this very small size, the particle surface area is extremely large relative to its volume. The comparatively large surface area of nanoparticles increases their reactivity, which essentially increases their toxicity.

While studies previously suggested that nano-silver potentially exerts both ionic and particle-specific toxicity, recent research strongly suggests that toxicity and cell lysis (Figure 1) typically results from oxidative stress caused by the release of silver ions (Xiu *et al*., 2012).

Given the rate of ion release is generally proportional to the surface area of a particle, nano-silver is more efficient than bulk silver at generating silver ions (Wijnhoven *et al*., 2009).

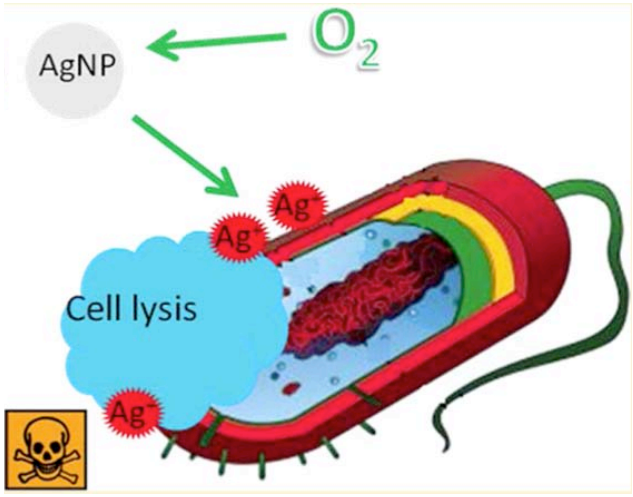

Fig. 1. The oxygen-dependent release of silver ions (Ag^+) from silver nanoparticles (AgNP) and subsequent cell lysis, demonstrating that oxidative stress caused by silver ions is the primary mode of silver toxicity to cells (Xiu *et al*., 2012).

In addition to this significantly greater release of silver ions, nano-silver presents new properties, including:

- the ability to cross many biological barriers
- increased production of reactive oxygen species
- capacity to deliver silver ions efficiently to the surface of bacteria (Marambio-Jones and Hoek, 2010)

However, in addition to the greater antimicrobial efficiencies presented by nano-silver over bulk silver, the toxicological burden of nano-silver might be up to 10,000 times as great as the same volume of bulk silver (as per calculations in Maynard, 2006)[1]. The means, the quantity of nano-silver particles, the contexts of their use, and the current antibiotic resistance crisis is unprecedented.

Clinical applications of nano-silver

Given the small size of nano-silver, it is more readily manipulated into commercial products than bulk silver. Because nano-silver can be manufactured as spheres, particles, rods, cubes, wires, film and coatings, it can be embedded into a range of substrates, such as metals, ceramics, polymers, glass and textiles (Wijnhoven *et al*., 2009).

In a medical context it has been established that approximately 80% of all human infections are caused by biofilms. This is because once bacterial biofilms become established, they are an intractable medical problem. Thus, an early application of nano-silver has been in coating clinically important surfaces, to prevent the growth of bacterial biofilms. Coatings that emit silver ions inhibit the attachment and formation of biofilms by pathogenic bacteria such as *Staphylococcus epidermis* (Ewald *et al*., 2006; Stobie *et al*., 2008). For this reason, nano-silver has important applications within a clinical setting, particularly lining wound dressings and as coatings for medical devices, such as catheters and stents (Silver *et al*., 2006). Indeed, a recent report by the Global Industry Analysts suggests that the US antimicrobial coatings market is primed for impressive growth, owing in part due to the increasing threat of hospital-acquired infections (Global Industry Analysts 2011). Moreover, given the growing resistance to other antimicrobials, nano-silver is increasingly used as an antiseptic, disinfectant and for external wound treatment. Recent industry research now promotes the effectiveness of nano-silver dressings against bacteria with NDM-1 carbapenemase enzymes (Hope *et al*., 2012).

Non-clinical applications of nano-silver

The easy manipulation of nano-silver has led to a proliferation of its use in consumer, industrial and agricultural products. Indeed, modern consumer culture can stimulate economic incentives to develop products that meet perceived needs, such as ‘odour-free’ socks or ‘ultra-hygienic’ toothbrushes.

[1] Comparing a “conventional” material made up of 2 µm diameter (bulk) particles, to a nanomaterial comprised of 20 nm particles, and assuming hazard is associated with either particle number or surface area, not mass.

Table 1: Some examples of nano-silver applications (Maillard and Hartemann, 2012).

Healthcare	Wounds dressings, antiseptics, hospital beds and furniture
Home consumer products	Fabric conditioners, baby bottles, food storage containers and salad bowls, kitchen cutting boards, bed mattress, vacuum cleaner, disposable curtains and blinds, tableware, independent Living Aids – bathroom products, furniture (chairs), kitchen gadgets and bath accessories, dishwashers, refrigerators and washing machines, toilet tank levers to sink stoppers, toilet seat, pillows, and mattresses, food storers, containers, ice trays, and other plastic kitchenware, hair brush, hair straightener, combs, brushes, rollers, shower caps Toothpaste, cosmetic deodorants, toothbrushes, tissue paper, epilator, electric shaver Pet shampoos, feeders and waters, litter pans, pet bedding and shelter, paper, pens and pencils, ATM buttons, remote control, handrails (buses), computer keyboards, hand dryers, wireless voice communicators with badge and the sleeves, yoga mat, coatings for use on laptop computers, calculators, sheet protectors, name badges and holders, shop ticket holders, media storage products, laminating film, report covers and project folders, photo holders, memory Book, office accessories, transparency film, collapsible coolers
Clothing and fabrics	Baby clothes, underwear, socks, footwear, various fabrics and vinyls, bath towels, quilts, sleeping bags, bed linens, pillows, quilts, mattress protectors and towels
Food	Packaging, nanobiotic poultry production
Construction	Powder coating (door knobs), wall paints, air conditioning, epoxy resin floor, PVC wall cladding, antimicrobial flooring, metal suspended ceiling systems, window blinds and shading systems, shelving systems, decorative wood laminates, electrical wiring accessories, notile panels (alternative to standard tiling), hygienic laminated surfaces, wallpaper, borders and murals, carpet and carpet underlay, seals (door for cooler doors and freezer cells, tank lids, mixers and kneading machines, hospital doors, for vibrating screens /vibrosieves in the pharmaceutical industry)
Disinfectants	Agricultural disinfectants, industrial disinfectants, aquaculture disinfectants, pool disinfectants

Here in Australia, nano-silver is used in a range of products, readily available on the supermarket shelves of retailers such as K-Mart, Priceline, Big W, Rebel Sport and Kathmandu. Products include toothpastes, pet shampoos, water filters, fabric softeners, bath towels, shoes, socks, cosmetics, deodorisers, baby clothes, baby bottles, baby toys, refrigerators, food containers, kitchen cutting boards, electric shavers, curling irons and washing machines. Manufacturers include big name brands such as Crocs, Samsung, LG, Remington and Vidal Sassoon.

Fig. 2. Examples of some Australia retail products promoting their use of using nano-silver.

Early examples of agricultural applications include the promotion of nano-silver as a "nanobiotic" in poultry production (Clement, 2009). Asian agricultural chemical companies have also advertised nano-silver for use as a fungicide, foliar spray and disinfectant for fish farming (Gih Hwa, 2011).

Clinical resistance to silver

Silver resistance in bacteria following the clinical use of silver has been well documented in the literature (see Table 2 summary below).

Table 2. A summary of several reports of silver resistance in bacteria.

Title	**Reference**
Salmonella typhimurium resistance to silver, chloramphenicol and ampicillin	McHugh *et al.* 1975
Silver resistant *Enterobacteriaceae* from hospital patients	Hendry and Stewart 1979
Gentamicin- and silver-resistant *Pseudomonas* in a burns unit	Bridges *et al*. 1979
Plasmid mediated resistance to silver ions in *Escherichia coli*.	Kaur *et al.* 1985
Plasmid mediated silver resistance in *Acinetobacter baumannii*.	Deshpande and Chopade 1994

In 2001, scientists identified the set of genes primarily responsible for silver resistance in bacteria - the *sil* operon (Gupta *et al.*, 2001). This information provided researchers with the ability to rapidly identify bacterial isolates with levels of resistance to silver.

Expert warnings

In its 2010 opinion document (BfR, 2010), the German Federal Institute for Risk Assessment (BfR) recommended manufacturers "*avoid the use of nanoscale silver or nanoscale silver compounds in foods and everyday products until such time that the data are comprehensive enough to allow a conclusive risk assessment which would ensure that products are safe for consumer health."*

This opinion was based in part on the unknown human toxicological effect profile associated with nanoscale silver (nano-silver) as well as concerns about the potential for nano-silver to facilitate the development and spread of bacteria resistance towards silver and antibiotics.

When interviewed for a report about the current usage of nano-silver and the potential to increase levels of AMR (Crocetti and Miller, 2011), Professor John Turnidge (Clinical Director of Microbiology and Infectious Diseases, SA Pathology; Professor of Paediatrics, Pathology and Molecular and Biomedical Sciences, University of Adelaide; and former president of the Australian Society for Microbiology) stated:

"*The usage of nano-silver is equally as frustrating, bizarre and stupid as the use of triclosan in consumer products, which is very widespread now. Antiseptics in toothpaste, washing powder, god knows what else. It's a market that created itself. In a sense, that they just use fear of bacteria as a marketing tool to introduce products that are unnecessary.*"

Such concerns are echoed in the scientific literature, with McArthur and colleagues (2012) warning: *"We predict that continued use of [antimicrobial textiles] could result in increased and widespread resistance to specific antimicrobials, especially metals, with an increased resistance to antibiotics. Such increases have the potential to find their way into bacterial populations of human pathogens leading to serious and unintended public health consequences."*

Co-selection of antibiotic and (non-antibiotic) antimicrobial resistance

The simultaneous increase in metal and antibiotic resistance is due to the phenomenon of co-selection.

Resistant bacteria and the resistance genes they carry are amplified by the exposure to antimicrobials through the process of natural selection. By selecting for resistant bacteria from the total bacterial population, large pools of resistant bacteria and resistance genes are built up where formerly they were rare.

Genes conferring antimicrobial resistance regularly travel quickly and widely due to the presence of mobile genetic (DNA) elements, such as plasmids, viruses, transposons and integrons. Stokes and Gillings (2011) explain that "*selection in stressed environments with respect to such compounds as heavy metals are enriched with antibiotic resistance genes*". Thus, the selection of bacteria with silver resistance, also simultaneously selects for other antimicrobial and antibiotic resistance genes.

Furthermore, once bacteria have already expressed resistance to these antimicrobials, it is expected that the ongoing usage of these and other antimicrobials will continuously increase levels of resistance to these antimicrobials and antibiotics.

Multiresistance Plasmids

Professor Hatch Stokes (research director, The ithree Institute, UTS) warns that the risk we face is not just silver resistance, adding *"the one thing that I'd put money on is that silver resistance is very closely linked in a genetic sense to other types of antimicrobial compounds, like antibiotic resistance genes...it's kind-of like a double whammy"* (Crocetti and Miller, 2011).

Resistance genes are most often spread between both closely and unrelated bacteria through the sharing of small circular pieces of DNA, known as plasmids. The dynamic nature of bacterial plasmids is supported by internal elements, such as transposons and integrons, which can rapidly rearrange the plasmid with new plasmids and integrate new resistance determinants (genes and cassettes). This makes plasmids an ideal platform for generating, collecting and transmitting multiple forms of antimicrobial resistance (multiresistance) between different bacterial species.

Once collected together, multiresistant plasmids can contribute to the fitness of bacteria within a given environment, often selecting for those with the greatest resistance – particularly in challenging clinical settings.

Most studies have failed to address the role of the biocidal metal with increasing the incidence of antibiotic-resistant bacteria. This is because most of the clinical microbiological analysis of the co-

selection of metal biocides (such as silver) and antibiotic resistance has relied heavily on being able to grow and identify bacterial isolates. (Baker-Austin *et al*., 2006).

Nevertheless, resistance genes to silver have been found on a range of plasmids, notorious for containing multiple antibiotic resistance genes (Gupta *et al*., 2001; Silver, 2003).

Some examples of the co-selection of silver and antibiotic resistance

A number of clinically-relevant investigations into the incidence of resistant bacteria and bacterial resistance outbreaks, particularly among Gram negative bacteria, demonstrate the connection between resistance to biocidal metals (including silver) and common antibiotics on identical mobile genetic elements such as plasmids.

1. ***Klebsiella pneumoniae*** **outbreak – Uppsala University Hospital, Sweden, 2005-2007**
   The transfer of the resistance genes from known resistance plasmids into a different plasmid backbone created a novel resistance plasmid in a different bacterial species involved in a hospital outbreak in Sweden in 2005 (Sandgreden *et al*, 2012). Here the authors describe the sitution:

   > "Beginning in May 2005, a major nosocomial outbreak of ESBL-producing *Klebsiella pneumoniae* occurred at the Uppsala University Hospital (UUH), Uppsala, Sweden, a regional hospital with 1000 beds, and 50,000 inpatient and 65,0000 outpatient visits annually. The outbreak was caused by a single, multiresistant clone of *K. pneumoniae* producing the CTX-M-15 ESBL enzyme that spread through patient-to-patient contact, mainly among elderly and immunocompromised patients at the hospital and associated community healthcare facilities. The major part of the outbreak occurred during 2005–07 and, during that time period, 248 patients were either infected or colonized with this bacterium at the hospital."
   >
   > The plasmid pUUH239.2 is a composite of the pKPN3 *K. pneumoniae* plasmid backbone and the $bla_{CTX\text{-}M\text{-}15}$-encoding multiresistance cassette associated with the internationally recognized outbreak strain *E. coli* ST131."

   With the assistance conferred by the plasmid pUUH239.2 (Figure 3), K. pneumoniae DA15000 was resistant to the metals silver, arsenic and copper, ampicillin, first-, second-, third- and fourth-generation cephalosporins, quinolones, kanamycin, spectinomycin, erythromycin, tetracycline, trimethoprim and sulfamethoxazole, and also showed reduced susceptibility to streptomycin, amikacin, gentamicin and tigecycline.

   This research clearly demonstrates the presence of silver resistance genes alongside resistance genes to most clinically-relevant antibiotics. Thus, the selection for silver resistance, simultaneously selects for resistance to multiple forms of antibiotics.

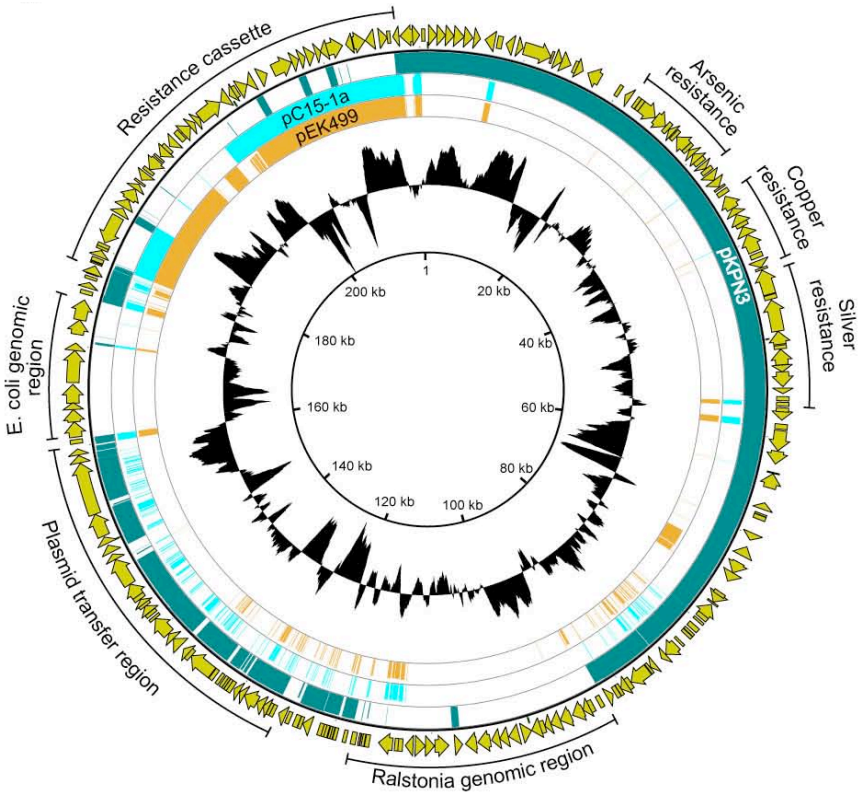

Figure 3. Plasmid pUUH239.2, isolated from the outbreak of *Klebsiella pneumoniae* at the Uppsala University Hospital (UUH). This plasmid contains genes encoding resistance to the metals arsenic, copper and silver, macrolides [*mphR*(A), *mrx* and *mph*(A)], chromate (*chrA*), trimethoprim (*dhfrXII*), aminoglycosides (*aadA2*), sulphonamides (*sul1*), β-lactams (bla_{TEM-1} and bla_{OXA-1}), the ESBL gene $bla_{CTX-M-15}$ and genes encoding resistance to aminoglycosides/fluoroquinolones [*aac(6')-1b-cr*] and tetracycline (Reprinted with the permission of Oxford University Press).

2. *Enterobacter* species outbreak – Hospital Universitario Son Dureta, Spain, 1995-1997

Species and subspecies referred to as "*Enterobacter cloacae*" have been isolated from plants, insects and open bodies of water, but are also commonly found blood pathogen in hospital intensive care and burns units. Kremer and Hoffman (2012) found the silver resistance genes on an IncHI plasmid as a major difference between pathogenic and nonpathogenic isolates, concluding:

> "Serving as a hygienic fitness factor, the presence of silver resistance determinants [genes] could improve survival in hospital environments and might be an explanation for the rising numbers of nosocomial infections caused by *E. cloacae*. Moreover, the distinct distribution of the *sil* genes among different (sub)species of *E. cloacae* could partially explain their unequal prevalence as nosocomial pathogens.
>
> Hence, a resistance mechanism against silver ions would serve as a potent hygienic fitness factor for bacteria, facilitating their survival in hospital environments and creating a potential infection risk for patients. This has directed our attention to the *silS* gene, a silver resistance regulator gene, which was found to be unique to the clinical outbreak strain by subtractive hybridization. We showed that the complete silver resistance determinant was present and functional in the majority of isolates of the *E. cloacae* complex" (Kremer and Hoffmann, 2012).

3. Chronic leg ulcer pathogens (comparison) – Uppsala University Hospital, Sweden, 2006-2007

A comparison of chronic leg ulcer pathogens collected from the wound treatment centre at Uppsala University Hospital in 2006 and 2007 has demonstrated a direct role of silver in reducing the effectiveness of antibiotics through the promotion of cross-resistance. Most strikingly the authors found that less than 3 weeks of treatment with silver-based wound dressings was necessary to select for a silver-resistant bacterium. They observed that:

> "Stable phenotypic silver resistance seemed to be associated with reduced susceptibility to third-generation cephalosporins. A cross-resistance to carbapenems was, in addition, observed in a derepressed *E. cloacae* strain after silver exposure *in vitro*" (Sütterlin *et al*., 2011).

4. Comparison of IncHI2 plasmids – various locations: Europe, Australia, USA, Taiwan, 1969–2008

The IncHI2 plasmid is one of the most frequently encountered plasmids in clinical enterobacterial strains and is associated with the spread of relevant antimicrobial resistance genes, such as extended-spectrum b-lactamase and quinolone resistance genes. A comparison of IncHI2 plasmids from humans and animals in Europe, Australia, USA and Taiwan revealed a high degree of genetic conservation between different bacterial pathogens (especially Escherichia, Enterobacter and Salmonella), with the authors concluding:

> " The stable permanence of the IncHI2 plasmids might be explained by the presence of several functions providing additional advantages, such as the mutagenesis induction system (*mucAB*), the *relE/relB* toxin–antitoxin system, bacteriophage inhibition (*phi*), and genes conferring resistance to benzylkonium chloride, sulfisoxazole, the metals tellurite, copper, silver and mercury, and the antibiotics gentamycin, tetracycline and streptomycin" (Garcia-Ferdandez and Carattoli, 2010).

5. Avian pathogenic *E. coli* (APEC) – various locations, USA, 2001-2004

Pathogenic strains of *Escherichia coli* often cause significant disease in both humans and animals. Avian pathogenic *E. coli* (APEC) strains originate from faecal flora and cause diseases in poultry. Johnson and colleagues (2006) presented the complete sequence of an IncHI2 plasmid (pAPEC-01-R) that occurs among many APEC isolates (Figure 4). While IncHI2 plasmids are typically found in different *Salmonella, Klebsiella* and *Serratia* species, the plasmid pAPEC-01-R - which encodes resistance to antibiotics and heavy metals (including silver) - is transferable to plasmidless strains of *E. coli*.

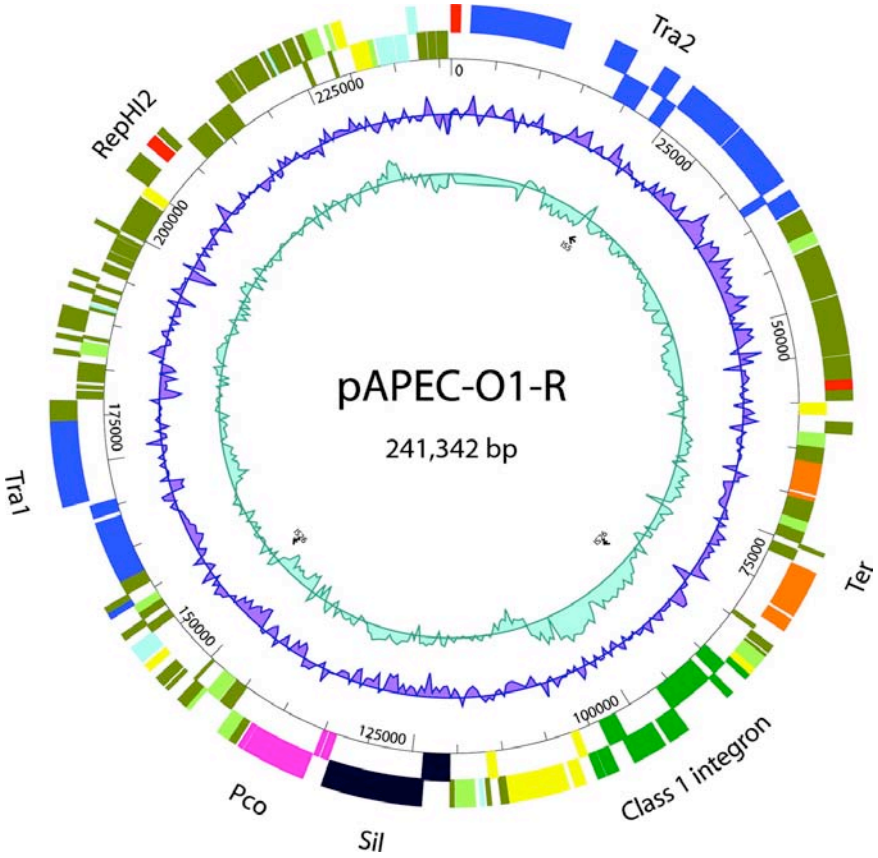

Figure 4. The plasmid pAPEC-O1-R contains genes conferring resistance to benzylkonium chloride, sulfisoxazole, the metals tellurite, copper, silver and mercury, and the antibiotics gentamycin, tetracycline and streptomycin (Johnson *et al.,* 2006).

Nanomaterials are specifically implicated in co-selection of multiresistance

Specific questions about the ability of nanomaterials to co-select for multiple forms of antimicrobial resistance are beginning to be asked by researchers. Published in the prestigious journal PNAS in 2012, Qui and coauthors compared the antimicrobial ability of different nanomaterials to promote the transfer of the multiresistance IncP plasmid RP4. While all tested nanomaterials (aluminium oxide, titanium dioxide, silicon oxide and iron oxide) promoted the conjugative transfer of the RP4 plasmid by 20-100 times, nano-aluminium increased the transfer of this plasmid from *E. coli* to *Salmonella* spp. by 200-fold. The results also showed nano-aluminium could significantly promote the conjugative transfer of plasmids RK2 and pCF10. Perhaps most alarmingly, nano-aluminium also significantly promoted the horizontal transfer of the RP4 plasmid from Gram-negative to Gram-positive bacteria (Qui *et al.*, 2012). Of specific relevance to the 'nano vs. bulk' question, the researchers found that bulk aluminium had no significant effect on the conjugative transfer of RP4 at any concentration.

Qui and coauthors also found that all tested nanomaterials strongly promoted plasmid transfer, with the authors suggesting an important role in oxidative stress damaging cell membranes, promoting the transfer of genes and nutrients (the "SOS response"). It is believed that nano-silver similarly kills bacteria primarily through this mechanism of oxidative stress damage to cell membranes (Wijnhoven *et al.*, 2009). These scientists are now posing similar questions about

nano-silver (personal communication), and it would be anticipated that similar results will be found - nano-silver will promote the transfer of plasmids in an equivalent manner.

Selection for antimicrobial resistance (AMR) by exposure to low levels of antimicrobials

The phrase '*what doesn't kill you, makes you stronger*' has possibly never been more appropriate, than when applied to antimicrobial resistance. Essentially, the concentration of an antimicrobial that comes in contact with a microorganism governs the subsequent effect on that microorganism (e.g. inhibitory, lethal, adaptation, selection).

Similar to the well-understood problem of patients not finishing a course of antibiotics, the low level usage of any antimicrobial can stimulate the spread of resistance genes to that antimicrobial (and other antimicrobials - through co-selection). The induction of bacterial resistance mechanisms following exposure to a low concentration of antimicrobials (biocides) has been reported in a number of studies for a number of antimicrobials (SCENIHR, 2009). Similarly, the sub-optimal use of therapeutic antimicrobials for animals, in particular under-dosage, can enhance the development of AMR (European Commission, 2011).

Experts recognise that to minimise development of resistant bacteria in clinical settings, wound dressings must release high levels of silver ions, in an attempt to kill all bacteria present (Chopra, 2007). Concentrations of silver ions lower than 15 µg/L have recently been reported to even boost bacterial growth instead of arresting it (Xiu *et al.*, 2012), a response that resembles suboptimal treatment with antibiotics, which creates resistant microbes.

It is therefore likely that the widespread use of nano-silver products such as dish cloths, hair brushes, baby mattresses, toothbrushes and computer keyboards, is already encouraging anti-microbial resistance in Australia and elsewhere.

Widespread use of triclosan promotes bacterial resistance

Background

The compound triclosan (2,4,4'-trichloro-2'- hydroxydiphenyl ether) was first developed and introduced as an antimicrobial and preservative in the 1960s. Since this time, triclosan has been used in clinical settings as an antiseptic, but also within a vast range of domestic products under trade names such as *Microban* and *Ultrafresh*, including hand soaps, pillows, toothpastes, cosmetics, mouthwash, deodorants, cutting boards, wound disinfectants, facial tissues, plastic utensils, socks and toys (Yazdankhah, *et al*., 2006). And like nano-silver, triclosan is a non-specific antimicrobial - it has the ability to kill good microbes as well as the bad (Saleh *et al*., 2010).

Resistance

The use of triclosan selects for resistance genes in bacteria. Several studies have demonstrated the prevalence of triclosan-resistant bacteria (Yazdankhah *et al*., 2006; Bailey *et al*., 2009; Chen *et al*., 2009). Clinical surveys have also found widespread incidence of triclosan-resistant bacteria that are also resistant to clinically important antibiotics. This has led scientists to caution against the indiscriminate use of triclosan (Yazdankhah *et al*., 2006; Mima *et al*., 2007; Chen *et al*., 2009).

Regulatory reviews of triclosan

An Australian Government review of triclosan (NICNAS, 2009) reviewed the incidence of triclosan resistance in the scientific literature between 2002-2005. This review found evidence that the use of triclosan can select for resistance *in vitro*, however concluded based on the lack of clinical evidence that the use of triclosan should not be restricted in Australia.

A review of triclosan resistance by the European Scientific Committee on Consumer Safety (SCCS, 2010) also highlighted the discrepancy between *in vitro* (laboratory) and *in situ* (clinical) findings in the scientific literature. The SCCS concluded:

> *"it is not possible to quantify the risk associated with triclosan (including its use in cosmetics) in terms of development of antimicrobial resistance (i.e. selection for less susceptible population), genetic basis for resistance and dissemination of resistance.*
>
> *Due to the limited number of in situ studies of resistance induced by triclosan to date, the SCCS can only recommend the prudent use of triclosan, for example in applications where a health benefit can be demonstrated. However, conclusions from in vitro studies cannot be ignored, notably the role of triclosan (and other biocides) in triggering resistance and in the dissemination (horizontal or vertical transfer of) resistance determinants. Research focused on triggering mechanisms of resistance, maintenance of the gene pool and the transfer of resistance and virulence determinants, and improving the translational application of laboratory results to situations in situ are needed."*

d) Implications for ensuring transparency, accountability and effectiveness in future management of antimicrobial resistance

The prudent use of antimicrobials is essential for reducing and preventing AMR. All antimicrobials should only be used if necessary and in accordance with best practices. The inappropriate use of these agents (e.g. using antimicrobials for the wrong reasons or incorrectly) is driving the emergence and selection of antimicrobial resistant microbes (European Commission, 2011).

Actions taken to date by Australian State and Federal Governments have not succeeded in containing the rising threats posed by AMR. The recommendations from various government committees reviewing AMR (such as JETACAR, EAGAR, AGAR, etc.) have not been acted upon appropriately, given the looming potentially catastrophic consequences.

A substantial reinforcement of existing regulations and recommendations, together with a new set of stringent measures are needed in order to prevent the further spread of resistance and preserve our ability to combat microbial infections in a clinical setting.

A new independent body is needed to manage AMR risks

AMR is a major Australian and global societal problem, involving many different sectors including medicine, veterinary medicine, manufacturing and trade. Isolated efforts from different sectors or regulators will not succeed in addressing the complex challenges that lay ahead. In order to

succeed, an all-inclusive approach is needed. Hence, a new independent body with statutory authority is required to oversee the management of AMR in Australia and will be best placed to coordinate alongside global efforts.

Here in Australia, a number of groups have called for such an independent body, including the National Prescribing Service (NPS), The Australasian Society for Infectious Diseases and the Australian Society for Antimicrobials.

This independent body would be empowered to implement:

- A sustainable national program which significantly lowers and monitors the use of antibiotics in both human medicine and agriculture.
- Restrictions on the use of many antimicrobials
- Regular public reporting of monitoring and surveillance of antimicrobial usage and resistance data that is made publicly available
- Regular testing of both imported and domestically produced foods for antimicrobial-resistant bacteria
- Regular testing for the presence of antimicrobial-resistant bacteria in animals and the environment
- Regular reviews identifying progress and shortfalls in promoting prudent use of antimicrobials
- Well-resourced education and training for all healthcare workers (particularly regarding the importance of increased levels of hand hygiene and the dangers associated with the over-prescription of antibiotics by GPs)
- Well-resourced and diverse education programs for all Australians
- The allocation of substantial government resources into researching new antimicrobial compounds
- Equitable access to all antibiotics in Australia and around the world
- Australian Government coordination with global bodies to facilitate the rapid improvement in the response to antimicrobial resistance by national governments (particularly in developing countries)

Regulations and restrictions can and do work

The good news is that when antimicrobial usage has been severely restricted, levels of resistance to those antimicrobials have been measurably reduced.

In Australia, restricted use of important quinolone antimicrobials has been associated with low rates of resistance (Cheng *et al*., 2012). Similarly, surveillance and education programs by the Swedish Government from 1994 to 2004 saw rates of antibiotic prescriptions fall from 536 to 410 prescriptions per 1000 inhabitants per year (Mölstad *et al*., 2008), with Sweden still boasting amongst the lowest recorded levels of MRSA (and many other problematic resistant bacteria) in the world.

The comparison of antibiotic usage versus bacterial resistance in food production also validates calls to severely restrict antimicrobial usage. A recent systematic review of the scientific literature

comparing the health effects of organic and conventional foods (Smith-Spangler *et al*., 2012) found that conventional chicken and pork have a 33% higher risk of contamination with bacteria resistant to 3 or more antibiotics than organic alternatives, with the authors suggesting "this increased prevalence of antibiotic resistance may be related to the routine use of antibiotics in conventional animal husbandry".

New restrictions on antimicrobials

Australia already has some reasonable procedures in place to regulate the use of antibiotics in human and animal use. However, very few – if any - regulations exist around the widespread use of non-antibiotic antimicrobials such as nano-silver and triclosan.

The antimicrobial applications of silver have never been adequately assessed in terms of promoting antimicrobial resistance. Due to the drastically greater release of silver ions, potent antimicrobial activity and relatively simple manipulation into substrates such as textile fibres, plastics, glass, ceramics etc., new nano-scale forms of silver (nano-silver) are increasingly being applied to a wide range of consumer goods. Similarly, the application of the broad-spectrum antimicrobial triclosan to consumer goods has been steadily increasing in Australia over recent years.

A large and growing body of scientific evidence supports the contention that the unrestricted use of these (non-antibiotic) antimicrobials will drive the further generation and spread of antibiotic resistance in human pathogens. As such, the relatively new - but already widespread - applications of these potent antimicrobials presents a novel consideration to the existing challenges faced by Australian regulators seeking to restrict antimicrobial resistance. Similar scientific and regulatory reviews of nano-silver in Europe have not yet translated into action – in spite of many recommended actions to restrict the over-use of nano-silver in consumer goods - with some commentators describing the situation of 'paralysis by analysis' (Hansen and Baun, 2012). However, given the high likelihood that these antimicrobials will further contribute to the pool of bacteria resistant to antimicrobials, the only appropriate action is to restrict these antimicrobials to their clinical applications.

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