Bacterial antimicrobial metal ion resistance

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Metals such as mercury, arsenic, copper and silver have been used in various forms as antimicrobials for thousands of years with until recently, little understanding of their mode of action. The discovery of antibiotics and new organic antimicrobial compounds during the twentieth century saw a general decline in the clinical use of antimicrobial metal compounds, with the exception of the rediscovery of the use of silver for burns treatments and niche uses for other metal compounds. Antibiotics and new antimicrobials were regarded as being safer for the patient and more effective than the metal-based compounds they supplanted. Bacterial metal ion resistances were first discovered in the second half of the twentieth century. The detailed mechanisms of resistance have now been characterized in a wide range of bacteria. As the use of antimicrobial metals is limited, it is legitimate to ask: are antimicrobial metal resistances in pathogenic and commensal bacteria important now? This review details the new, rediscovered and ‘never went away’ uses of antimicrobial metals; examines the prevalence and linkage of antimicrobial metal resistance genes to other antimicrobial resistance genes; and examines the evidence for horizontal transfer of these genes between bacteria. Finally, we discuss the possible implications of the widespread dissemination of these resistances on re-emergent uses of antimicrobial metals and how this could impact upon the antibiotic resistance problem.

Introduction

Metals and metalloids have a long empirical history of human usage in medicine and agriculture (as reviewed below), despite problems of host toxicity or doubts about their efficacy. Even now, a few toxic metal(loid) compounds are still first-line drugs or preferred-choice chemotherapeutics or antimicrobials, although the use of most of the previously popular antimicrobial metal(loid)s, such as mercury and arsenic/antimony compounds, has been reduced or phased out in the past 50 or so years. Other metals, such as silver and copper, still have limited uses in agriculture and medicine, but are also increasingly being included in consumer products, from clothing to computer keyboards, and are being promoted as useful additions to our arsenal of antimicrobials. Against this background of their current usage, it is reasonable to ask: what is the relevance of antimicrobial metals and bacterial resistances to them to medical microbiology in the twenty-first century?

Any attempt to address this question must be set against the backdrop of widely known problems and opportunities. We are faced with new and emerging opportunistic nosocomial and community-acquired pathogens; and increasing epidemic and pandemic multidrug-resistant (MDR) pathogens. There is a recognition that the antibiotic discovery pipeline has not delivered significant quantities of new antibiotics in the past few decades, and new formulations and uses for antimicrobial metals as weapons in the antimicrobial armoury are being proposed (Department of Health, 2013; Lemire et al., 2013). The recent recommendation by the UK Chief Medical Officer that antimicrobial resistance should be added into the UK National Security Risk Assessment (https://www.gov.uk/government/news/uk-antimicrobial-resistance-strategy-published–2) provides a timely backdrop to a discussion about resistance to antimicrobials that have been in clinical, non-clinical and agricultural use for far longer than antibiotics have been.

In this review, we briefly discuss a wide range of antimicrobial metals, but will concentrate on a limited number of the historically most important and most widely used (copper, silver, mercury, arsenic and antimony), and the microbial resistances to them. We examine the past and current uses of antimicrobial metals, and the importance of the genetic legacy and dissemination of bacterial resistance to antimicrobial metals in bacteria. In particular, we discuss the genetic elements carrying multiple antimicrobial resistances, both to metals and antibiotics.

Abbreviations: ESBL, extended-spectrum β-lactamase; MDR, multidrug-resistant; RND, resistance–nodulation–cell division; ROS, reactive oxygen species; SCC, Staphylococcus cassette chromosome.
Metals in medicine and agriculture – past and present uses

Arguably the most important uses of metals and metalloids in medicine and agriculture have been as biocides and antimicrobials.

Probably the most commonly used toxic metals or metalloids in medicine and agriculture have been mercury (Hg), copper (Cu), silver (Ag), arsenic (As) and antimony (Sb), and these will be dealt with in detail in this review. Other inorganic or organic metal compounds, such as lead (Pb) (Lenihan, 1988; Trotter, 1990), tin (Sn) (Barnes & Stoner, 1959; Cooney & Wuerz, 1989), zinc (Zn) (Aarestrup & Hasman, 2004), bismuth (Bi) (Mahony et al., 1999; Ge & Sun, 2007; Yang & Sun, 2007), gold (Au) (Novelli et al., 1999; Ray et al., 2007), cerium (Ce) (Garner & Heppell, 2005), palladium (Pd) (Ray et al., 2007), tellurite (Te) (Taylor, 1999), thallium (TI) (Kazantzis, 2000) and gallium (Ga) (Chitambar, 2010), have also been investigated or seen limited use as antimicrobials. Of these less heavily used antimicrobial metals, zinc, bismuth and tin are still in common use in consumer products. Although zinc is an essential element required for life and is found in many enzymes, zinc ions can be effective as antimicrobials even at low concentration. Zinc compounds have been described since at least Roman times as an ancient ingredient in eye disease treatment, and zinc tablets were found in a small medical container dating back to 140–130 BC retrieved from a Roman shipwreck (Giachi et al., 2013). Current use of some of these metals includes the that of zinc oxide as a mild antiseptic, most often used topically to protect against diaper/nappy rash or skin irritation. Zinc compounds are also found in toothpastes (zinc chloride) and shampoos (zinc pyrithione), and used as a growth promoter/treatment for post-weaning diarrhoea in animal feeds (Hasman et al., 2006). Stannous fluoride is used in toothpastes, and bismuth subsalicylate is used to treat diarrhoea and other digestive system disturbances (Lemire et al., 2013).

In addition, compounds containing gold (Au), platinum (Pt), palladium (Pd), vanadium (V), rhodium (Rh), titanium (Ti), iridium (Ir) and other rare metals have been used recently in medical diagnostics or imaging, as radiotherapeutics, or as antiarthritis and anticancer therapeutics (Abrams & Murrer, 1993; Guo & Sadler, 1999; Zhang, Lippard, 2003; Desoize, 2004).

The medical and agricultural uses of mercury, copper, silver, arsenic and antimony as antimicrobials are discussed in detail below.

Mercury

This element has no known positive role in cellular function, and the toxicity to humans of mercury and inorganic mercury compounds has been known since the first century AD (Lenihan, 1988). The very high levels of toxicity of ethyl- and methylmercury compounds to humans have been known since they were first synthesized in the laboratory in the mid-nineteenth century, when two laboratory assistants died several weeks after helping to synthesize dimethylmercury. Even so, organic and inorganic mercury compounds have been widely used in agriculture and medicine. Organic compounds containing mercury were used in agriculture to control plant diseases from the late nineteenth century until the 1970s, with aryl, aroyl and alkyl organomercurials becoming widely used in the 1950s, particularly as antifungal seed dressings, but also as pesticides and fungicidal sprays (Huisingsh, 1974). Antifungal methylmercury cereal seed treatments resulted in death when treated wheat was consumed by humans in Guatemala and Iraq (brieﬂy summarized in Hobman & Silver, 2007), and mortality and reproductive failure of seed-eating birds has also been linked to organomercurial seed dressings. Use of organomercurial seed dressings was discontinued because of these problems.

Inorganic mercury compounds have been used in a variety of medicines: as laxatives, diuretics and antidepressants, but also to treat sexually transmitted diseases, skin disorders and as a topical antimicrobial since at least the fifteenth century, when inorganic salts of mercury or mercury metal were primarily used to treat syphilis, either as an ointment or fumigant (Hobman & Brown, 1997). The effects of the treatment were only slightly less unpleasant than the disease and probably futile. Mercury metal (hydrargyrum), mercuric chloride (corrosive sublimate; HgCl₂), mercurous chloride (calomel; Hg₂Cl₂) and mercury nitrate (Hg(NO₃)₂) have been used as the active ingredients in many medical treatments. Included in these uses was the nineteenth-century universal remedy, the ‘blue mass’ (or ‘blue pill’), used for treating everything from tuberculosis to parasites, but most famously in the Royal Navy during the Napoleonic Wars for treating constipation, in conjunction with the ‘black draught’. In hindsight, it seems strange that although the toxic effects of mercury on humans had been known since antiquity, mercuric chloride was commonly used in baby teething powders in the Anglo-Saxon World and in ‘Wurmschokolade’ in continental Europe in the early twentieth century. Unfortunately, the use of inorganic mercury compounds in these medicines led to Pink disease (acrodynia) in children (Black, 1999). The known toxicity of mercuric ion compounds (particularly mercuric chloride) and doubts about their efficacy meant that mercuric chloride in the primary treatment of syphilis was replaced by Salvarsan (see below) in the early twentieth century, making redundant the aphorism ‘a night with Venus, a lifetime with mercury’ (although mercury or bismuth was sometimes still used as an adjunct treatment to Salvarsan). After World War II, antibiotics became the standard treatment for syphilis, but mercury use continued in diuretics (Hall, 1970), antiseptics and organomercurial antimicrobial compounds in hospitals in the UK and America until the early 1970s (Nakahara et al., 1977; Porter et al., 1982), and until the 1990s in over-the-counter antiseptics and ointments.
(GoldenEye ointment used to contain 1–3 % mercuric oxide). Ammoniated mercury (NH₄HgCl) was used to treat psoriasis, ringworm and impetigo in the 1970s (Foye, 1977), and may still be available in some countries. A variety of organomercurial antimicrobial and antifungal agents, such as nitromersol, mercuraphene, phenylmercuric borate, phenylmercuric nitrate and o-hydroxyphenylmercuric chloride, have been used as disinfectants, preservatives and antiseptics. Even in 2014, over-the-counter 0.5 % (v/v) chloramphenicol eye drops bought in the UK contain 0.002 % (w/v) phenylmercuric nitrate as a preservative. One of the most well-known of the organomercurial preservatives is thimerosal/thiomersal (Merthiolate; sodium ethylmercurithiosalicylate), which has been widely used as a topical antiseptic or preservative and is still in use in the UK as a preservative.

Mercury-containing antimicrobial usage is in decline and is likely to be eliminated. The use of thiomersal/thiomersal as a vaccine preservative has been subject to vigorous debate and controversy, and it has been banned in some countries. Other mercury-containing disinfectants include merbromin (Mercurochrome) and nitromersol that have been superseded or withdrawn in the US or Europe.

The largest current use of mercury in a healthcare-associated role is in dental amalgam, which typically contains 43–54 % mercury, 20–35 % silver, 15 % tin, 10 % copper and 2 % zinc, depending on the formulation (Franke, 2007). There has been debate about the safety of mercury amalgam fillings and whether use of them has negative effects on human health or may select for mercuric ion-resistant bacteria, although a recent ruling by the US Food and Drug Administration stated that dental amalgam was safe. In the UK, dental amalgam can be used unrestricted, but there are limitations in its use in some other European countries and bans are in place in the Nordic countries.

**Copper**

Copper is an essential metal to aerobic forms of life, being involved in donating or accepting electrons in redox-active enzymes, or in the electron transport chain (Solioz et al., 2010). Copper is also toxic to prokaryotes and eukaryotes at higher cellular concentrations (Gaetke & Chow, 2003), and copper (and zinc) involvement in phagosomal killing of bacteria engulfed by macrophages is now recognized as an important defence mechanism (German et al., 2013).

Copper compounds are used as wood preservatives, in antifouling paints and as molluscicides (Borkow & Gabbay, 2009). In agriculture, copper compounds have been used as antimicrobial, algicidal, pesticidal and antifungal agents, and as animal feed additives. Copper sulphate solutions were used as an antifungal treatment of seed grains in the eighteenth century. In the late nineteenth century, Bordeaux mixture (copper sulphate and calcium hydroxide) and Burgundy mixture (copper sulphate and sodium carbonate) were widely used to control mildew on grape vines, and to control fungal and bacterial diseases of seeds or plants (Russell, 2005). These inorganic antifungal agents are still widely used in plant protection, even in ‘organic’ agriculture. Copper sulphate is allowed alongside zinc chloride, oxide or sulphate as an additive in animal and poultry feed. In the European Union, copper sulphate can be added at up to 250 p.p.m. in piglet feed, but also at 25 p.p.m. in feed for slaughter-weight pigs, 20 p.p.m. in broiler chickens and 2 p.p.m. in calves as a growth promoter (Barber et al., 1955), and for post-weaning control of diarrhoea (Hasman et al., 2006; Sapkota et al., 2007). Alongside copper sulphate, zinc oxide can be added at up to 2500 p.p.m. in piglet feed to control post-weaning diarrhoea.

The medical uses of copper and inorganic salts of copper go back at least 4000 years, with copper or copper compounds being used as astringents, antiseptics and antifungals, to treat wounds, to purify and sterilize drinking water, and in contraceptive intrauterine devices (Borkow & Gabbay, 2009). Inorganic and organic copper compounds have been used to treat a variety of skin diseases, syphilis, tuberculosis and anaemia, amongst other maladies (Grass et al., 2011). There is also interest in copper-containing wound/ulcer dressings that have been trialled and reported to be effective (Borkow & Gabbay, 2009; Borkow et al., 2010). Various laboratory and clinical studies have confirmed that solid copper/copper alloy surfaces promote rapid killing of Gram-negative and Gram-positive bacteria (Espirito-Santo et al., 2008, 2010; Elguindi et al., 2011). Most recently, the use of copper antimicrobial solid surfaces to reduce microbial contamination and transmission of hospital-acquired infections has progressed to clinical trials, with the installation of copper-containing surfaces and fixtures in wards and clinics. Reduction in microbial numbers, and therefore cross-contamination, has been seen (Casey et al., 2010; Marais et al., 2010; Mikolay et al., 2010). Copper usage in consumer items is perhaps less common than silver, but includes the use of copper oxide-impregnated bedding to control house dust mites and socks to treat athlete’s foot (Borkow & Gabbay, 2009). Antimicrobial copper surfaces and products may also appear in products available to the domestic market now that the US Environmental Protection Agency has registered copper and copper alloys as public health antimicrobial products.

**Silver**

There is no known beneficial role for silver in metabolism and it is highly toxic to bacteria (Nies, 1999). We have not been able to find any evidence in the literature for the use of silver compounds as antimicrobials in agriculture, except for the use of silver iodide (AgI) in cloud seeding, but the first use of silver as an antibacterial is reported to have occurred over 2000 years ago in drinking water containers (Silver et al., 2006), and silver is still widely used in water filters and in other treatments for potable water, or as an algicide for swimming pools.
Medically, silver nitrate (lunar caustic; AgNO₃) was used empirically to treat ulcers and burns in the seventeenth to nineteenth centuries, and as a cauterizing agent. It was understood in the late nineteenth century that metallic silver and silver nitrate had antibacterial properties, with metallic silver foil and silver nitrate solutions being used to treat fresh and infected burns and wounds or silver wire being used to suture surgical wounds. Following the success of Salvarsan (arsphenamine) in combination with mercury or bismuth salts as a treatment for syphilis (see above and below), spinal injection of silver arsphenamine (Neo-Silvol) was used in the 1920s as a treatment for neurosyphilis. A 2% silver nitrate solution has also been used in treating warts and eye infections, and as a prophylactic against gonorrhoeal ophthalmia neonatorum (Klasen, 2000a). Silver metal is also a major component of dental amalgam.

The introduction of sulphonamide in the 1930s and antibiotics in the 1940s appears to have led to an almost complete disappearance of interest in the use of silver and silver salts in burn and other treatments, until the 1960s, when Moyer et al. (1965) looked for antimicrobial agents that prevented invasive burns infections. Combinations of 0.5% silver nitrate and Sulphamylon became popular burns treatments in the mid-1960s, and silver sulphadiazine (Flammazine, Silvadene) was developed shortly after as a burn treatment. Silver sulphadiazine is a common treatment for serious burns (reviewed by Klasen, 2000b). More recently, silver-impregnated dressings and antimicrobial coatings have been used in infection management, stimulation of healing, wound management and treatment of infected wounds, and as antimicrobial coatings in catheters and endotracheal breathing tubes (Silver, 2003; Ip et al., 2006; Silver et al., 2006; Chopra, 2007; Mijnendonkx et al., 2013).

Silver is generally viewed as a benign metal, and the only widely reported negative health effects of silver to humans have been eschar formation on burns treated by silver, staining or destruction of skin cells when silver nitrate is directly applied for treatment of warts, sometimes elevated silver levels in blood, and the rare argyria and argyrosis in people who self-medicate colloidal silver solutions (Silver et al., 2006). There is some concern about silver and silver nanoparticle toxicity to other (particularly aquatic) organisms (Panyala et al., 2008; Chaloupka et al., 2010), initially based upon the premise that silver nanoparticles were new materials that had not been encountered in nature before, with counter-arguments that silver nanoparticles have been produced in colloidal silver preparations for over a century and the majority of approved silver biocides release nanosilver (Nowack et al., 2011). Copper/silver ionization treatments have been used in hospital water supplies, and the International Space Station has silver-coated water tanks (Van Houdt et al., 2012; Mijnendonkx et al., 2013)

One quite noticeable increase in the use of antimicrobial metal products is that of silver in consumer and ‘lifestyle’ products. In the past 20 years or so, silver-containing plasters, clothes, water filters and personal hygiene and consumer products have appeared worldwide (Silver, 2003; Silver & Phung, 2005; Edwards-Jones, 2009; Mijnendonkx et al., 2013), and the use of antimicrobial silver nanoparticles in products is also growing (Chaloupka et al., 2010), including examples where they have been integrated into household items, such as computer keyboards, washing machine drums, air conditioners and refrigerators.

**Arsenic**

Arsenic has been used for at least 2000 years as a medicine, cosmetic, tonic or poison. Arsenic trioxide (As₂O₃) (also known as Ratsbane, ‘Inheritance powder’ or *poudre de succession*) is colourless and flavourless when put in food or drink and was popular as a rat poison. Prior to the advent of sensitive and accurate chemical tests for arsenic, such as the Marsh test, it is believed that arsenic trioxide was also a popular choice for poisoning people, especially as the symptoms of arsenic poisoning somewhat resemble cholera and post-mortem toxicoology was weak/non-existent. Organic arsenic compounds such as Lewisite (2-chloroethylarsinous dichloride) and Adamsite (dibenzo-1-chloro-1,4-arsene), as well as a range of other organoarsenic halides, have also been developed as chemical warfare agents.

Agricultural and non-medical uses of arsenic compounds have included arsenical wood preservatives (particularly chromated copper arsenate), herbicides, rodenticides, defoliants (Agent Blue used in the Vietnam war was a mixture of dimethylarsenic acid and its sodium salt; Cooksey, 2012) and fungicides. Prior to the introduction of organic pesticides, arsenic compounds such as lead arsenate and Paris green (Cu(II) acetarsenate) were used as rodenticides and insecticides. Copper-arsenic and lead-arsenic compounds were used widely as insecticides in orchards from the 1930s to the 1980s, and calcium arsenate and dimethylarsenate were widely used as pesticides (Oremland & Stolz, 2003). The organic arsenic compounds carbarsone (4-carbamoylaminophenylarsonic acid), nitarson (4-nitrophenylarsonic acid) and roxarsone (3-nitro-4-hydroxyphenylarsonic acid) have been used as feed additives for poultry in the USA, acting as growth promoters and in controlling coccidiosis disease (Jones, 2007). Only very recently (June 2011) has the US Food and Drug Administration announced the voluntary suspension of the sale of roxarsone due to the presence of inorganic arsenic residues in chicken meat from chickens fed on roxarsone-supplemented feeds.

In medicine, arsenic oxide (white arsenic; As₂O₃), arsenic sulphide (red realgar; As₂S₃) and arsenic trisulphide (yellow orpiment; As₂S₃) have variously been used as antispasmodics, sedatives, haematinics, for treating skin disorders, as eye and cancer treatments, in the treatment of trichomoniasis, malaria, ulcers and syphilis, as well as a wide range of other ailments (Liu et al., 2008). Arsenic compounds were so widely used in the eighteenth century that arsenic became known as the ‘Therapeutic Mule’ (Przygoda et al., 2001).
Fowler’s solution was a very well-known inorganic arsenical medicine (1% arsenic trioxide in potassium carbonate with tincture of lavender), which was still being used even after World War II as a tonic and treatment for malaria, syphilis and chorea (Przygoda et al., 2001).

In the early part of the twentieth century, the organic arsenic compound Salvarsan (‘the arsenic that saves’) was probably the best-known arsenic compound used in medicine. Salvarsan (compound 606, arsphenamine) and subsequently Neosalvarsan (compound 914, neoarsphenamine) were developed by Ehrlich and co-workers primarily to effectively treat syphilis. Later, it was realized that once administered by injection, arsphenamine oxidized to oxophenarsine (later given the trade name Mapharsen) that was subsequently used as the drug of choice in syphilis treatment until the introduction of penicillin (Bosch & Rosich, 2008). However, programmes for the treatment of syphilis with organic arsenic compounds could last for 18 months, had serious side effects and often also required alternating with bismuth or mercury treatments. Silver arsphenamine, silver neoarsphenamine and bismuth arsenphenamine sulphonate also found therapeutic use (Gibaud & Jaouen, 2010). Tryparsamide was the first arsenical that was clinically effective in treating African sleeping sickness (trypanosomiasis), but resistance in Trypanosoma brucei was reported in the early 1930s a decade after introduction of the drug. The arsenical compounds melarsoprol (Arsobal) and melarsonyl are still used to treat sleeping sickness, and have been used to treat other diseases, including amoebic dysentery, despite serious side effects including blindness (Jolliffe, 1993; Jones, 2007; Gibaud & Jaouen, 2010), whilst others such as arsenilic acid (atoxyl, 4-aminophenylarsonic acid) have been largely discontinued as treatments due to their toxicity (Gibaud & Jaouen, 2010). Carbarsone was introduced as an antiprotozoal organoarsenical in the early 1930s, followed by diphetarsone and arsathinol in the 1950s. They were withdrawn from the market in the 1990s because of the association of arsenic exposure with a variety of abnormal growths/tumours (Gibaud & Jaouen, 2010).

Arsenic is carcinogenic in higher organisms, with a range of potential mechanisms involved, including genotoxicity, DNA methylation and cell proliferation alterations, oxidative stress, co-carcinogenesis, and tumour promotion (Hughes, 2002). Despite the reduction in use of arsenic as an antimicrobial there has been renewed interest in arsenic as an anticancer drug. In the mid-1990s, arsenic trioxide was investigated as a treatment for acute promyelocytic leukaemia and received US Food and Drug Administration approval in 2000 as a sterile injectable arsenic trioxide solution, Trisenox (Šlejkovec et al., 2012).

Antimony

Antimony may have been used as long as arsenic in medicine. In agriculture, tartar emetic (antimony potassium tartrate; \(\text{C}_3\text{H}_5\text{KO}_2\text{Sh} \cdot 0.5\text{H}_2\text{O}\) was used in the treatment of leishmaniasis, schistosomiasis/bilharziasis, trypanosomiasis and ascariasis in domestic and farm animals in the nineteenth and early twentieth centuries, and tartar emetic was also used as a pesticide spray on crops.

Antimony was used as a cosmetic or in ointments in skin treatments in biblical times, and became popular in medicine during the eighteenth century, with uses including treatments for smallpox, syphilis, dropsy and agues (McCallum, 1977). The toxic properties of antimony metal were clearly established in the sixteenth century and the powerful emetic effect of antimony was known in Roman times. This property of antimony was exploited in the seventeenth and eighteenth centuries to induce therapeutic vomiting, sweating and purging through ingestion of antimony either from drinking wine which had stood for 17–24 h in an antimony cup or through swallowing a ‘perpetual pill’ made from antimony, which soon re-emerged from the patient. Tartar emetic was also used to induce vomiting in patients, and in tropical medicine, tartar emetic has been used as a treatment for schistosomiasis. Other antimony compounds are still used as first-line treatment of visceral leishmaniasis and as treatments for schistosomiasis in humans (Guo & Sadler, 1999; Ashutosh et al., 2007; Ge & Sun, 2007; Sundar & Chakravarty, 2010; Perry et al., 2011), although resistance to antimony drugs in Leishmania donovani and Leishmania infantum in the Bihar region of the Indian subcontinent is now very high. Resistance in L. donovani to sodium stibogluconate (Pentostam) and meglumine antimonite (Glucantime) has been shown experimentally to be as a consequence of exposure of L. donovani in a mouse model to levels of arsenic equivalent to those to which humans are exposed in arsenic-contaminated drinking water from Bihar (Perry et al., 2013).

**Metal ion toxicity**

Despite the documented historical use of antimicrobial metals, our understanding of the detailed toxic effects of different metal ions and metalloids on bacteria is arguably incomplete. However, it is clear that the chemistry of the metals drives the biological effects, in terms of metal bioavailability, that a metal will have on cells and the resistance mechanisms that bacteria can use to detoxify or remove those metals.

Mechanisms of metal toxicity are generally agreed to be a consequence of metal ion affinity for cellular components and biomolecules, or the stability of metal–biomolecule complexes formed, although the consequences are varied. Metals and metalloids can exert toxic effects in a number of different ways: by binding to or blocking functional groups in biological molecules, by displacing essential metals in enzymes, by binding to the cellular thiol pool or participating in chemical reactions in the cell that are harmful. Ultimately, the deleterious effects reported include damage to proteins, DNA and biological membranes, interference in enzyme function and cellular processes.
processes, and oxidative stress (Nies, 1999; Hobman et al., 2007).

There have been various attempts to group metals based on their ligand affinity or toxicity, leading to rather vague classifications like ‘heavy metals’ or ‘toxic metals’ (Duffus, 2002; Hodson, 2004). The two classifications that are the most widely accepted descriptors of the potential for interactions of metal ions with biological ligands are the Irving–Williams series of divalent metal ion ligand affinities and the classification of metals into Lewis acids. The Irving–Williams series of ligand affinity for essential divalent metal ions clearly demonstrates the affinity of biological molecules for first-row transition metals: Ca$^{2+}$<Mg$^{2+}$<Mn$^{2+}$<Fe$^{2+}$<Co$^{2+}$<Ni$^{2+}$<Cu$^{2+}$>Zn$^{2+}$ and shows that divalent copper has a strong affinity for biological molecules, suggesting that it can displace other metals from the first-row transition metals from them (Waldron & Robinson, 2009). Another way of measuring the toxicity of metal ions is to consider their strength as Lewis acids. Hard Lewis acids (with a small, non-polarizable electron sheath) prefer ionic coordination to oxygen-containing ligands. Soft Lewis acids (with a large, polarizable electron sheath) prefer covalent coordination to soft Lewis bases, primarily sulphur and nitrogen ligands: cysteine thiols and nitrogen imidazoles. Intermediate Lewis acids will relatively stably coordinate to hard and soft donor ligands (Table 1). The metals and metalloids that are known to be toxic are largely, but not exclusively, soft Lewis acids, which are likely to be able to displace intermediate and hard Lewis acids from cysteine thiols because of their higher affinity for them. In addition to effects caused by the higher affinity of soft Lewis acids for ligands, oxidative stress is an other proposed mechanism of toxicity for some metals. Redox-active metals, such as copper, chromium, iron and vanadium, as well as redox-inactive metals and metalloids, such as arsenic, cadmium, mercury, nickel, lead and antimony, can be involved in cellular oxidative stress damage. Although arsenate and mercuric ions can be reduced intracellularly, they do not catalyse one-electron transfer reactions and consequent free radical generation as copper, iron, chromate and vanadate do. For redox-active metals, generation of hydroxyl radicals via Fenton-like reactions is believed to be the probable mechanism by which oxidative stress occurs.

For redox-inactive metals and metalloids, the potential mechanism of oxidative stress generation is that they bind to and inactivate cellular thiols, which normally quench reactive oxygen species (ROS) that are generated during normal cellular metabolism, or can be redox metal catalysed, or metal-catalysed oxidation of reduced glutathione can also generate hydrogen peroxide. Recent evidence suggests that iron–sulphur clusters in enzymes are key targets for toxic metals (Hobman et al., 2007; Macomber & Imlay, 2009; Xu & Imlay, 2012).

The broad mechanisms of toxicity for each of the commonly used antimicrobial metals are given below.

**Mercury**

Mercury is the most toxic metal to *Escherichia coli* (Nies, 1999). Mercury toxicity has been attributed to the inactivation of enzymes and interference with other protein functions by the tight binding of mercuric ions to thiol and imino nitrogen groups in these or the displacement of other metal cofactors from enzymes. Mercuric ions also bind to nucleotides and lipids, interfering with DNA function and contributing to lipid peroxidation. Mercuric ions and organomercurials have the ability to rapidly pass through biological membranes, and organomercurials are highly lipid soluble (Clarkson & Magos, 2006).

**Copper**

Copper carries out an essential role as an electron donor/acceptor in many enzymes, but copper can also take part in Fenton-like reactions leading to the generation of hydroxyl radicals, hydrogen peroxide and superoxide, which can cause cellular damage (reviewed by Grass et al., 2011). This has been generally accepted as the major mechanism for copper toxicity. However, recent experimental evidence from experiments in liquid culture shows that copper-mediated ROS generation occurs largely in the periplasm of *E. coli*, so the importance of ROS generation by copper as a cellular toxicity mechanism has been under debate (Macomber et al., 2007). Gram-positive bacteria lack a periplasm, and although many are tolerant to hydrogen peroxide (Solioz et al., 2010), recent evidence from *Staphylococcus aureus* shows oxidative stress resistance.

### Table 1. Classification of metals in terms of polarizability

| Class A (hard) metal ions | Lewis acids (electron acceptors) of small size and low polarizability (deformability of the electron sheath or hardness) |
| Class B (soft) metal ions | Lewis acids (electron acceptors) of large size and high polarizability (softness) |
| Li, Be, Na, Mg, Al, K, Ca, Sc, Ti, Fe(III), Rb, Sr, Y, Zr, Cs, Ba, La, Hf, Fr, Ra, Ac, Th | V, Cr, Mn, Fe(II), Co, Ni, Cu(II), Zn, Ga, As, Rh, Pb(IV), Sn, Sb, Cu(I), Pd, Ag, Cd, Ir, Pt, Au, Hg, Ti, Pb(II) |

Modified from Nieboer & Richardson (1980) and Duffus (2002).
and protein misfolding repair transcriptional responses, and hydrogen peroxide scavenging defence (Baker et al., 2010). According to the Irving–Williams series, copper has a higher affinity than other first-row transition metals for ligands, and displacement of iron from iron–sulphur clusters by copper in liquid culture experiments has been reported to be an important mechanism of copper toxicity (Macomber & Imlay, 2009). There is also a role for copper and ROS in phagosome killing of bacteria (reviewed by German et al., 2013).

The rapid killing of bacteria on solid copper surfaces is thought to be due to cellular damage caused by very high local concentrations of copper dissolving from the surface, which causes membrane rupture, coupled with ROS generation leading to further cellular destruction, including degradation of plasmid and chromosomal DNA (Grass et al., 2011).

**Silver**

Silver (as well as gold) is the second most toxic metal to *E. coli* (Nies, 1999). Silver ions cause the inhibition of respiration, membrane damage and destruction of the proton motive force. The interaction of Ag⁺ with thiol groups in membrane proteins/enzymes is thought to be a major mechanism of toxicity, with data suggesting that the key toxicity event is interactions between Ag⁺ and respiratory chain enzymes (Holt & Bard, 2005). Proteomic studies have shown that ionic and nanoparticle silver cause destabilization of the outer membrane, collapse of the cytoplasmic membrane potential and depletion of intracellular ATP levels in *E. coli*, consistent with interference with the respiratory chain (Lok et al., 2006, 2007; Du et al., 2012). Other evidence suggests that although still toxic to bacteria under anaerobic conditions, under aerobic conditions intracellular Ag⁺ ions also cause ROS generation and interference with DNA replication (Park et al., 2009), increased membrane permeability and increased sensitivity to antibiotics (Morones-Ramirez et al., 2013). There is some disagreement about which ROS are important in this mechanism of Ag⁺-mediated damage. Park et al. (2009) suggest Ag⁺-mediated superoxide radical generation in *E. coli* and *S. aureus*, whilst in *Staphylococcus epidermidis* Gordon et al. (2010) suggest generation of hydroxyl radical ions through release of iron from proteins by Ag⁺ binding thiol groups, leading indirectly to hydroxyl radical formation. Other work in *Vibrio cholera* showed that low levels of Ag⁺-cause collapse of the proton motive force and proton leakage, and the cytoplasmic membrane is the major target for low levels of silver ions (Dibrov et al., 2002). Silver cations also cause rapid and extensive loss of membrane integrity in *S. aureus* (Randall et al., 2013).

**Arsenic and antimony**

Arsenic toxicity depends on the nature of the arsenic compound. Inorganic arsenic toxicity occurs through allosteric inhibition of essential metabolic enzymes, with arsenite being more toxic than arsenate (Cooksey, 2012). Arsenate is an analogue of phosphate, and can enter cells via phosphate uptake systems and inhibit oxidative phosphorylation. Arsenite can enter cells via aquaglyceroporins and bind to thiol groups in proteins, and has been reported to bind to the vicinal thiols in pyruvate dehydrogenase and 2-oxo-glutarate dehydrogenase, affecting cellular respiration (Oremland & Stolz, 2003). There is evidence that the presence of arsenic in cells leads to the generation of ROS and reactive nitrogen species. One known mechanism for this is that arsine (ASH₃) and methylated derivatives can generate methylarsinyl peroxy radicals, which damage DNA (Cooksey, 2012), but inorganic arsenic has also been implicated in ROS generation and disruption of signal transduction pathways (Kumagai & Sumi, 2007). Arsenic and antimony share some chemical and toxicological properties, and therefore may share modes of toxicity.

**Bacterial metal ion homeostasis and resistance to toxic metals**

The natural exposure of bacteria to bioavailable metals (both essential and toxic) has occurred over billions of years since the expansion of oxic environments that accompanied the great oxidation event (Barkay et al., 2010), and this exposure has likely been the driver for the evolution of the ability of micro-organisms to control cellular levels of these bioavailable oxidized metal ions. These metals are sometimes found in high concentrations due to volcanic activity or other natural geological events. Bacteria have also been exposed to lethal concentrations of these metals through anthropogenic releases of toxic metals into the environment, such as mining, smelting, manufacturing, fossil fuel burning and numerous other industrial applications, often at high localized concentrations, as well as the deliberate use of metals as antimicrobials and pesticides. Thus, bacteria have evolved mechanisms to acquire essential metals, control the intracellular levels of these metals and eliminate those that in excess are deleterious. Similarly, systems for removing from the cell, or modifying, purely toxic metals have also evolved and have been selected.

Antimicrobial metals have multiple and different cellular targets, and there are limited options available for bacteria to mitigate or nullify the effects of metal toxicity. Therefore, the potential resistance strategies that they can employ are limited to extracellular or intracellular sequestration of the metal, reduction in permeability, alteration of target sites, enzymic detoxification or efflux of the metal ions (Hobman & Brown, 1997). These resistance mechanisms are conceptually similar to the potential mechanisms of antibiotic resistance (Courvalin, 2008). Most of the mechanisms of resistance to metals that have been well characterized at the genetic level in bacteria are linked to enzymic detoxification or efflux of metals from the cell. This is because unlike organic antimicrobial compounds, which can be broken
down or inactivated by enzymic cleavage, metals are immutable and bacterial metal import systems or porins are not sufficiently discriminatory to allow entry to the cell only metal ions that are required. Metal ion chaperones may also be subverted to bind to the ‘wrong’ metal.

Mechanisms of antimicrobial metal resistance

Although mechanisms such as methylation or demethylation of metals (which are often by-products of normal cellular metabolism), generalized antimicrobial efflux through multidrug efflux systems and stress response mechanisms may contribute to fortuitous metal ion tolerance/resistance or damage repair, specific metal ion resistance mechanisms are usually characterized by a metal ion-specific response regulator, which controls the expression of structural resistance genes. The products of these genes produce a metal ion-specific efflux protein or protein complex and/or enzyme(s) that alter the metal ion into a form less toxic to the bacterial cell (Silver & Phung, 1996). There may be other proteins encoded by the resistance mechanism, their functions ranging from metal ion chaperone to metal ion transporter or metal ion reductase. The simplest general mechanism of resistance is therefore a metal-specific efflux system.

The specific resistance mechanisms for mercury, copper, silver and arsenic and antimony are discussed in detail below.

Mercuric ion resistance

Resistance to mercuric ions is believed to be an ancient resistance mechanism, evolving after the biosphere became widely oxygenated, and has been found widely in bacteria and Archaea (Barkay et al., 2010). The mechanism of mercuric ion resistance to inorganic mercuric ions (narrow-spectrum resistance) is unusual for a metal ion resistance mechanism and counter intuitive (Fig. 1). Rather than direct efflux of the metal, the simplest inorganic mercuric ion resistance operon in Gram-negative bacteria, from Tn501, encodes proteins that chaperone divalent mercuric ions (Hg^{2+}) in the periplasm using MerP. Hg^{2+} ions are imported across the cytoplasmic membrane via MerT into the cytoplasm, where they are reduced to essentially non-toxic metallic mercury (Hg^{0}) by mercuric reductase (MerA). Metallic mercury is volatile at room temperature and pressure, and leaves the bacterial cell by passive diffusion. Mercuric ion resistance is a very good example of how a resistance mechanism is determined by the chemistry of the metal, as MerA requires reducing equivalents to reduce Hg^{2+} to Hg^{0} and has to import Hg^{2+} to the cytoplasm in order to do this. MerP and MerT appear to prevent Hg^{2+} from damaging the cell during this process (Morby et al., 1995). In Gram-negative bacteria, regulation of the mer mercury resistance operon is through the activator MerR, with secondary regulation of the operon via MerD (reviewed by Brown et al., 2003). Resistance to organomercurials (or broad-spectrum mercuric ion resistance) is conferred via organomercurial lyase (MerB). MerB cleaves the carbon-mercury bond in organomercurial compounds, working with a narrow-spectrum mer operon and is regulated by an organomercurial-responsive MerR. MerE is an additional inorganic and organic mercury importer (Kiyono et al., 2009). Other Gram-negative mercuric ion resistance operons encode additional mercuric ion import proteins [e.g. MerC in the Tn21 mer operon (Sahlman et al., 1997) and MerF in pMER327/419 (Hobman et al., 1994; Wilson et al., 2000)]. Most of the work on our understanding of mercury resistance has come from studies on the classic mercury resistances from Tn501 and Tn21 in Gram-negative bacteria.

The mechanism of mercuric ion resistance in Gram-positive bacteria is broadly the same as that in Gram-negative bacteria, but details of the regulation and mercuric ion import systems differ slightly. The mer operons in Gram-positive bacteria have been best characterized in the plasmid pLZ58 mer from S. aureus and in different Bacillus strains. The S. aureus mer resistance contains merR, merA, merB and merT homologues, and some additional ORFs, as do the Bacillus mer resistance operons, which confer broad-spectrum mercury resistance (Chu et al., 1992; Gupta et al., 1999b). There is now evidence that mercury resistance in some S. aureus strains is carried on the SCCmercury element (Staphylococcus cassette chromosome; reviewed by Malachowa & DeLeo, 2010). Excellent and comprehensive reviews of mercuric ion resistance in bacteria are available (Barkay et al., 2003; Barkay & Wagner-Döbler, 2005).

Copper homeostasis and resistance

Copper homeostasis and copper resistance mechanisms have evolved because copper is an essential metal that can be toxic at higher intracellular concentrations, and copper is involved in host defence against pathogens.

Bacterial cells have systems that control ‘normal’ levels of copper and other systems that confer resistance to very high levels of copper. In E. coli, there are two chromosomally encoded copper homeostasis mechanisms, the cue and cus systems, both of which have components that modify the charge in ionic copper and efflux it (the model for the mechanism is shown in Fig. 2). In the cue system, a MerR family copper-responsive transcriptional activator, CueR, regulates expression of a copper efflux P1-type ATPase, CopA and of CueO, a multi-copper oxidase (Outten et al., 2000; Petersen & Møller, 2000; Stoyanov et al., 2001). In the cus system, a two-component regulator CusRS activates expression of cusCFBA; CusCBA is a tripartite RND (resistance–nodulation–cell division) family silver/copper effluxer and CusF is a periplasmic metallo-chaperone (Munson et al., 2000). Whilst the cue system is induced under very low external copper concentrations, the cus system has been reported to be induced under higher external levels of copper and may be important.
under anaerobic conditions (Munson et al., 2000). The AcrD and MdtABC multidrug efflux pumps in Enterobacter cloacae have also been reported to efflux copper and other antimicrobials when NlpE, an outer membrane lipoprotein that functions during envelope stress responses, is overexpressed (Nishino et al., 2010). In Salmonella, enterobactin and TolC are involved in copper detoxification (Pontel et al., 2014).

In addition to the cue and cus systems, some E. coli strains isolated from pigs fed on copper-supplemented feed carry a plasmid-borne copper resistance system, pco, which confers additional copper resistance (Tetaz & Luke, 1983; Williams et al., 1993; Brown et al., 1995). The pco copper resistance from E. coli plasmid pRJ1004 contains seven ORFs, designated pcoABCDRSE (Rouch & Brown, 1997). The current model for the mechanism of resistance to copper salts conferred by pco is shown in Fig. 3. Gene expression from the pco operon is regulated by PcoRS, a two-component regulator system homologous to the CopRS, CusRS and SitRS regulators (from the pMG101 silver resistance plasmid) (Munson et al., 2000). PcoR regulates expression of the pcoABCD genes from one promoter and pcoE from a separate promoter (Rouch & Brown, 1997). PcoA, C and E are periplasmic proteins, PcoB is an outer membrane protein and PcoD is an inner membrane protein. PcoA is a multi-copper oxidase and may have a similar function to CuoO, oxidizing Cu(I) to less toxic Cu(II). PcoC is a copper chaperone (Djoko et al., 2008) and PcoE may act as a periplasmic first line of defence copper ‘sponge’ protein, binding copper whilst the remainder of the Pco proteins are expressed (Zimmermann et al., 2012). PcoB is a predicted outer membrane protein that may interact with PcoA, which could either oxidize Cu(I) to the less toxic Cu(II) or act to sequester oxidized catechol siderophores, which per se can reduce cupric ions to the more toxic cuprous ions. CueO may also act to prevent this by directly oxidizing catechols (Grass et al., 2004). PcoC and PcoD are required for full copper resistance (reviewed by Rensing & Grass, 2003). Homologues of pco (albeit lacking some of the genes seen in pRJ1004 pco resistance) called cop have been identified in plant-saprophytic and -pathogenic bacteria from crops treated with copper fungicides (Bender & Cooksey, 1987; Cooksey et al., 1990).

Gram-positive bacteria have a different copper homeostasis mechanism, which is probably best understood in Enterococcus hirae (reviewed by Solioz et al., 2010). This mechanism involves import of copper into the cytoplasm via (a different) CopA, an ATPase and binding of excess cytoplasmic copper by a copper chaperone (CopZ), which donates it to either a copper export ATPase (CopB) or CopY, which is a copper-responsive repressor of gene expression for the Enterococcus hirae cop operon. The mechanism of copper homeostasis in Lactococcus lactis appears to be different as both CopA and CopB act as efflux ATPases. Recently, a plasmid-encoded copper efflux ATPase and a multicropper oxidase have been found in Listeria monocytogenes (Kuenne et al., 2010). A Cpx-type ATPase copper resistance efflux pump encoded by the tcrB gene has also been found on a conjugative plasmid carried by an Enterobacteriaceae.
by *Enterococcus faecium* from pigs and is related to the *copYZAB* operon in *Ent. hirae*. This has also found in farmed chickens and calves, and is linked to macrolide and glycopeptide resistance (Hasman & Aarestrup, 2002). CsoR, a copper-sensing repressor, regulates expression of the *copZA* promoter in response to intracellular copper in *Bacillus subtilis* and *Staphylococcus* (Liu et al., 2007; Smaldone & Helmann, 2007; Baker et al., 2011).

There are several excellent and comprehensive review articles on copper resistance, which describe the genetics and biochemistry of resistance and the role of copper resistance in pathogenicity in great detail (e.g. Rensing & Grass, 2003; Osman & Cavet, 2008; Solioz et al., 2010; Dupont et al., 2011; German et al., 2013; Chaturvedi & Henderson, 2014).

**Silver tolerance and resistance**

Although bacterial silver resistance has been reported sporadically since the 1960s (for reviews, see Clement & Jarrett, 1994; Silver et al., 2006; Chopra, 2007; Mijnendonk et al., 2013), the pMG101 *sil* system remains the only system characterized in any detail at the genetic level. The 182 kb transferrable IncHI-2 group plasmid pMG101 from *Salmonella enterica* serovar Typhimurium was isolated in 1973 from fatal infections in a burns unit in Massachusetts General Hospital, Boston, USA. Plasmid pMG101 confers resistance to chloramphenicol, ampicillin, tetracycline, streptomycin, sulphamamide, mercury, tellurium and silver (McHugh et al., 1975; Gupta et al., 1999b).

The proposed silver resistance mechanism has been predicted via DNA sequencing and comparison to the *E. coli* *cop* and *cus* copper resistance. Several small clones of the *sil* operon confer partial silver resistance (Gupta et al., 1999a). The *cus* system is known to confer resistance to low levels of silver [and was called *agr* by Franke et al. (2001, 2003) and *cus* by Munson et al. (2000)], and some of the *sil* genes from pMG101 are closely related to the *cus* genes. There is 71 % identity between *SilIC* and *CusC*, 67 % identity between *SilIB* and *CusB*, and 87 % identity between *SilIA* and *CusA*, which form the efflux protein complexes *SilCBA* and *CusCBA*, respectively (Gupta et al., 1999a).

The proposed mechanism of silver resistance is shown in Fig. 4, in which a two-component silver-responsive transcriptional regulation system *SilRS* (homologous to *CusRS* and *PcoRS*) controls expression of a silver efflux ATPase, *SilP*, the tripartite *SilCBA* silver effluxer and *SilF*, which is believed to be a periplasmic silver chaperone. Several other genes or ORFs are present in the *sil* system. *SilE* has a role in periplasmic silver binding (S. Silver, personal communication), and there is a small ORF between *silA* and *silP* named *orf105* that could encode a hypothetical protein of 105 aa, but which is of unknown function. There is another silver resistance system in the environmental bacterium *Cupriavidus metallidurans* CH34, which is composed of *silCBA* and located on one of two large plasmids, pMOL28 (Murgeay et al., 2003, Monchy et al., 2007).

Homologues of the *sil* system have been detected using *sil*-specific primers in IncH1-2 group plasmids from Gram-negative bacteria (Gupta et al., 2001), in oral bacteria (Davis et al., 2005), in nosocomial isolates of *Enterobacter cloacae* (Kremer & Hoffmann, 2012), a silver-resistant *Enterobacter cloacae* leg ulcer isolate (Su¨tterlin et al., 2012), some Gram-negative bacteria isolated from wounds (Woods et al., 2009) and, surprisingly, in *S. aureus* (Loh et al., 2009). There are also some reports of silver-resistant pathogens which carry the *sil* genes that were isolated from burns units and even from the silver-containing burns creams (Pirnay et al., 2003).
Arsenic and antimony resistance

Arsenic resistance is very widespread amongst both Gram-negative and -positive bacteria, probably reflecting the wide distribution of arsenic in the environment and its use as an antimicrobial (Silver & Phung, 2005). Arsenic resistance was first identified in Gram-positive bacteria by Novick & Roth (1968) and in Gram-negative bacteria shortly afterwards. Arsenic resistance operons in bacteria confer resistance to arsenite (As(III)), arsenate (As(V)) and antimonite (Sb(III)). The minimum arsenic resistance operon consists of *arsR, arsB* and *arsC*, which encode, respectively, an arsenite-responsive trans-acting transcriptional repressor protein, an arsenite antiporter and an arsenate reductase. Some Gram-negative arsenic resistance operons (such as the *E. coli* plasmid-borne arsenic resistance carried on R773) also carry two additional genes: *arsD* and *arsA*. ArsA has an ATPase function and binds as a dimer to ArsB forming an ATP-energized effluxer, which is more efficient at arsenite efflux than ArsB alone. ArsD has a minor role in transcription, but has recently been found to act as a metallochaperone for arsenite efflux via ArsAB (Lin et al., 2006). Previous work has shown that in the absence of ArsA, ArsB confers lower levels of arsenite resistance by translocating these ions into the periplasm using energy derived either from the proton-pumping respiratory chain or from F_0F_1-ATPase (Dey & Rosen, 1995). The Gram-positive arsenic resistance found on *S. aureus* plasmid pI258 comprises the simpler *arsRBC* system. The current model for arsenic and antimony resistance conferred by *ars* operons is shown in Fig. 5.

There is an additional chromosomal arsenic resistance mechanism that has recently been found in some bacteria (e.g. *Alcaligenes faecalis, Thiomonas sp.*). This mechanism involves the use of arsenate as a terminal electron acceptor in the absence of oxygen, with a respiratory arsenite oxidase from the periplasm and a respiratory arsenate reductase converting arsenite to the less toxic arsenate (as part of a chemolitho-autotrophic lifestyle), and acting as a terminal electron acceptor during anaerobic heterotrophic growth. Some of these arsenate-respiring bacteria also carry the classic arsenite resistance genes and can tolerate very high levels of arsenate (Silver & Phung, 2005).

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**Fig. 3.** Model for plasmid-borne (*pco*) copper resistance from *E. coli* plasmid pRJ1004. Modified from Rensing & Grass (2003), Djoko *et al.* (2008) and Zimmermann *et al.* (2012). The *pco* system operates in addition to the chromosomal *cue* and *cus* systems. The *pco* copper resistance from *E. coli* plasmid pRJ1004 contains seven ORFs: *pcoABCDRS* (Rouch & Brown, 1997). Copper enters the periplasm, possibly through porins, and gene expression from the *pco* operon is regulated by the two-component sensor kinase regulator system PcoRS, which responds to the presence of copper. Phosphorylated PcoR regulates expression of the *pcoABCDRS* genes from one promoter and *pcoE* from a separate promoter (Rouch & Brown, 1997). There is a likely role for pigments/catechol siderophores in *pco* copper resistance, although this is not well understood. PcoE acts as a first line of defence, copper ‘sponge’ protein, binding copper in the periplasm, whilst the other Pco proteins are expressed. PcoA is a multi-copper oxidase which oxidizes Cu(I) to less toxic Cu(II) bound to PcoC in the periplasm (Djoko *et al.*, 2008). PcoB is a predicted outer membrane protein that may interact with PcoA/C to export copper (Djoko *et al.*, 2008) or may act to sequestrate oxidized catechol siderophores (Rensing & Grass, 2003). PcoD is an inner membrane-spanning protein that may import copper into the cytoplasm or PcoC may provide copper to PcoD, for loading onto PcoA, which is exported to the periplasm via the twin-arginine translocation pathway (Rensing & Grass, 2003; Djoko *et al.*, 2008).
Antibiotic and antimicrobial metal ion resistances are often carried on mobile genetic elements in bacteria from the ‘antibiotic era’

Bacterial resistance to antimicrobial metals in clinically important bacteria was first reported in the early 1960s in *S. aureus* isolated from surgical wounds. This was attributed by Moore (1960) to the use of mercuric ions used to disinfect catgut used in sutures, and by other workers to the use of mercury-containing diuretics (Hall, 1970) or disinfectants (Porter et al., 1982). Mercuric ion resistance (Hg\(^{R}\)) was then found to be genetically linked to *S. aureus* penicillinase plasmids (Richmond & John, 1964) and arsenic resistance was first identified in Gram-positive bacteria by Novick & Roth (1968), who found that *S. aureus* penicillinase plasmids carried resistance to arsenate, arsenite/antimony, lead, cadmium/zinc, mercury and bismuth. Meynell & Datta (1966) isolated R (resistance) plasmids from clinical *E. coli* strains such as R46, which conferred tetracycline, ampicillin, streptomycin, sulphonamide and arsenic resistance, whilst Smith (1967) also found resistance to mercuric ions, nickel and cobalt in clinical isolates of *E. coli* and *Salmonella* sp. These resistances were later found to be located on plasmids or mobile genetic elements such as transposons. Elek & Higney (1970) also identified arsenic, mercury and copper resistance in *E. coli* causing urinary tract infections using resistogram typing. One of these strains contained the classic R plasmid R773, which also conferred resistance to tetracycline and streptomycin. The Hammersmith Hospital Collection of resistance plasmids collected from the early 1960s onwards contained 25 % Hg\(^{R}\) plasmids (Schottel et al., 1974), whilst other studies showed up to 60 % of hospital isolate strains at that time were Hg\(^{R}\). Since then, metal ion resistance genes have been regularly detected in bacteria isolated from clinical, environmental, agricultural, domestic and wild animal, and human sources.

The first descriptions of the mechanisms of antimicrobial metal resistance started in the late 1960s, and the detailed mechanisms of resistance and the genes encoding the resistance mechanisms have been studied since then.

More recently, clinical interest in antimicrobial metal resistances has decreased, but there is increasing evidence that antibiotic and metal ion resistances are linked, as they are carried on the same mobile genetic elements, e.g. transposons and plasmids (Frost et al., 2005; Baker-Austin et al., 2006; Summers, 2006; Mindlin & Petrova, 2013).

Antimicrobial metal ion resistances were carried on mobile genetic elements in bacteria from the ‘pre-antibiotic era’

Our first understanding of bacterial antimicrobial metal ion resistance came from clinical bacteria from the ‘antibiotic era’, which were originally isolated because they were resistant to antibiotics. However, subsequent investigations showed that as well as resistance to antimicrobial metals in contemporaneous strains of clinically important bacteria, antimicrobial metal resistance was also present in ‘pre-antibiotic era’ clinical isolates stored by E. D. G. Murray at Hammersmith Hospital between 1917 and 1954 (although very low numbers of these strains were antibiotic resistant). Within the collection there were significant

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**Fig. 4.** Hypothetical model for silver resistance encoded on *Salmonella* Typhimurium plasmid pMG101. Modified from Silver et al. (2006). Silver ions (Ag\(^{+}\)) enter the cell and are detected in the periplasm by SilS, the sensor component of a two-component silver-responsive transcriptional regulation system, SilRS. The SilRS sensor–regulator regulates its own expression via phosphorylated SilR and that of silE, which is believed to have a role in periplasmic silver binding. SilRS also regulates the expression of silICBA, silF, silP and a small ORF ort105, the product of which is of unknown function. SilCBA is a tripartite RND family silver effluxer, SilF is believed to encode a small periplasmic silver chaperone and SilP is an efflux ATPase. The model predicts that SilCBA effluxes Ag\(^{+}\) directly to the outside of the cell and Ag\(^{+}\) (by homology to the *cus* system) can directly enter the SilCBA complex from either the cytoplasm, the periplasm or SilF. SilE may work like its homologue PcoE, as a periplasmic metal-binding ‘sponge’ giving initial protection against Ag\(^{+}\) damage.
numbers of strains carrying plasmid-borne resistance to K₂TeO₄ (11/433), CuSO₄ (68/433) and NaAsO₂ (61/433), but fewer to HgCl₂ (3/433). Resistance to silver was not tested (Hughes & Datta, 1983). The incompatibility groups of these plasmids were the same as those found today (Datta & Hughes, 1983).

**Tn21 subgroup transposons drug (resistance) ‘mules’**

One of the best known examples of how metal ion resistance and antibiotic resistance genes are genetically linked is the understanding that Tn21 family mercuric ion resistance transposons carry class 1 integrons. These integrons are not mobile themselves, but are responsible for the acquisition and expression of antibiotic resistance cassettes (Liebert et al., 1999).

Sulphonamides were introduced into Japan during World War II, and streptomycin, chloramphenicol and tetracycline were introduced in 1950 to tackle serious shigellosis problems. *Shigella dysenteriae* strains were isolated in 1952 that were resistant to sulphonamides, and isolation of strains resistant to sulphonamides, streptomycin, chloramphenicol and tetracycline first occurred in 1955 (reviewed by Watanabe, 1963). Experimental findings that these antibiotic resistances could be transferred from *Shigella* sp. to *E. coli* K-12 led to the realization that these resistances were associated with ‘resistance transfer factors’ or plasmids. Plasmid R100 (also independently isolated as R222 or NR1) is a classic example of a multi-resistance plasmid that was first isolated in Japan some time during the early to mid-1950s (Nakaya et al., 1960; Davies, 1995). R100 carries resistances to tetracycline, chloramphenicol, sulphonamides and aminoglycosides (Liebert et al., 1999). The mercury resistance transposon Tn21 carried on R100 can justly be regarded as the paradigm for a particular class of mercuric ion resistance found in Gram-negative bacteria and for how a metal ion resistance transposable element performs another role acting as a drug (resistance) ‘mule’ carrying integron elements that acquire, reassort and express antimicrobial resistance genes. In the case of Tn21, In2, the integron carried by it, contains the *sulI* (sulphonamide resistance), *qacE* (partially deleted quaternary ammonium compound resistance) and *aadA1* (aminoglycoside adenylyltransferase) resistance genes (Liebert et al., 1999). Thus, although mercury compounds are now rarely (if at all) used as antimicrobials in agriculture and medicine, class I integrons are being carried by mercuric ion resistance transposons in Gram-negative pathogens that are of current concern.

Examination of the Hg^R_ plasmids from the Murray collection showed that the Hg^R_ determinant carried on one of them was very similar to Tn21 (but was flanked at each end by copies of IS3075, lacked the integron and had a small deletion at the site where In2 was inserted into the transposon) (Hobman et al., 2002; Essa et al., 2003). In a separate study, a 10,000-year-old Siberian permafrost bacterial isolate was found to contain a transposon that was virtually identical to Tn21, but lacked the integron (Kholodii et al., 2003). These pre-antibiotic-era Tn21-like mercury resistances lacking In2 are consistent with a model for the stepwise evolution of Tn21 ancestor mercury resistance transposons into multi-resistance transposons.

**Fig. 5. Arsenic and antimony resistance operon from *E. coli* plasmid R773. Modified from Silver (1998), Nies (1999) and Kruger et al. (2013). Arsenate (AsO₄³⁻), and arsenite (AsO₂⁻) or antimonite (SbO₂⁻) can enter the *E. coli* cell via specific or non-specific transport systems (arsenate can enter cells via the phosphate import system). ArsR is an arsenite-responsive trans-acting transcriptional repressor protein that senses As³⁺ in the cytoplasm and regulates expression of the structural arsenic resistance genes. ArsC is an arsenate reductase that is required to reduce arsenate to arsenic so that it can be effluxed by ArsB, an arsenite antiporter. ArsA has an ATPase function and binds as a dimer to ArsB, forming an ATP-energized effluxer, which is more efficient for arsenite efflux than ArsB alone. ArsD has a minor role in transcription, but has recently been found to act as a metallochaperone for arsenite efflux via ArsAB (Lin et al., 2006).**
Table 2. Tn21-like mercury resistance transposons and associated integrons, and other mercury resistances, in sequenced plasmids or bacterial chromosomes from pathogens

<table>
<thead>
<tr>
<th>Strain</th>
<th>Genetic element</th>
<th>Additional information</th>
<th>Reference</th>
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<tr>
<td><strong>Tn21-related mercury resistances</strong></td>
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<tr>
<td><em>Aeromonas salmonicida</em> subsp. <em>salmonecida</em> A449</td>
<td>Tn21 subfamily composite transposon containing an In2 integron encoding resistance to streptomycin/spectinomycin, quaternary ammonia compounds, sulphonamides and chloramphenicol</td>
<td>Causative agent of furunculosis in fish</td>
<td>Reith <em>et al.</em> (2008)</td>
</tr>
<tr>
<td><em>Acinetobacter baumannii</em> AYE</td>
<td>Tn21-like element contained within an 86 kb chromosomal ‘resistance island’ (AbaR1) containing 45 antimicrobial resistance genes</td>
<td>Epidemic strain in French hospitals; multiple antibiotic resistant</td>
<td>Fournier <em>et al.</em> (2006)</td>
</tr>
<tr>
<td><em>Acinetobacter baumannii</em> 3208</td>
<td>Chromosomal resistance island AbaR5, carrying residual sequences of Tn1696 (Tn21-related transposable element carrying In4)</td>
<td>Australia 1997 blood infection isolate</td>
<td>Post &amp; Hall (2009)</td>
</tr>
<tr>
<td><em>Acinetobacter baumannii</em> AB0057</td>
<td>Tn21-like element contained within an 86 kb chromosomal ‘resistance island’ AbaR3</td>
<td>USA 2005 MDR bloodstream isolate</td>
<td>Adams <em>et al.</em> (2008)</td>
</tr>
<tr>
<td><em>E. coli</em> O42</td>
<td>Tn21 subfamily-related Tn2411 carrying mercuric chloride resistance, and resistances to sulphonamide, streptomycin and ethidium bromide on an In2-like integron; Tn2411 is flanked by tetracycline and chloramphenicol resistances</td>
<td>Enteraggregative <em>E. coli</em>-type strain isolated in Bangladesh in 1970</td>
<td>Chaudhuri <em>et al.</em> (2010)</td>
</tr>
<tr>
<td><em>E. coli</em> EAHEC O26:H- strain O6877</td>
<td>Plasmid pO26-CRL contains a Tn21 subfamily composite transposon containing an In2 integron encoding resistance to mercuric chloride, trimethoprim, β-lactams, sulphathiozole, streptomycin and kanamycin</td>
<td>Australian isolate of enterohaemorrhagic <em>E. coli</em> isolated in the 1990s</td>
<td>Venturini <em>et al.</em> (2010)</td>
</tr>
<tr>
<td><em>E. coli</em> EAHEC O104:H4 TY2482</td>
<td>Genomic island 3 (GI-3)</td>
<td>German epidemic enteraggregative haemorrhagic <em>E. coli</em> outbreak 2011; MDR; related to <em>E. coli</em> 55989 Outbreak in a neonatal Intensive Care Unit; IncL/M plasmid carrying a Tn3-like transposon carrying blaTEM-1a and Tn6234 carrying blaFOX-7 [an AmpC-type extended spectrum β-lactamase (ESBL)] and other resistance determinants</td>
<td>Crossman (2011), Grad <em>et al.</em> (2013) Di Pilato <em>et al.</em> (2014)</td>
</tr>
<tr>
<td><em>Klebsiella pneumoniae</em></td>
<td>Plasmid pFOX-7a carrying a Tn1696 derivative Tn6234</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em> PA7</td>
<td>Tn21-like element contained within a chromosomal ‘transposon dump’</td>
<td>Non-respiratory clinical isolate from Argentina; unusually antibiotic resistant, and contains Tn21, Tn1721 and Tn5393</td>
<td>Roy <em>et al.</em> (2010)</td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em></td>
<td>Plasmid R10003</td>
<td>Tn1696 carrying In4; originally isolated in the 1970s; resistance to gentamicin, streptomycin, spectinomycin and chloramphenicol; independent evolution by insertion of In4 into a Tn5036-like transposon</td>
<td>Partridge <em>et al.</em> (2011)</td>
</tr>
<tr>
<td><em>Salmonella Typhi</em> CT18</td>
<td>Plasmid pHCM1</td>
<td>Is5075-flanked mer resistance related to Tn21 Isolated in 2002 in Karachi, Pakistan</td>
<td>Parkhill <em>et al.</em> (2001)</td>
</tr>
<tr>
<td><em>Salmonella Paratyphi</em> A</td>
<td>Plasmid pAKU_1 IncHI-1 group, similar backbone to pHCM1 and pRK27; 24 kb composite multi-drug resistance transposon</td>
<td></td>
<td>Holt <em>et al.</em> (2007)</td>
</tr>
<tr>
<td>Strain Genetic element Additional information Reference</td>
<td></td>
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<td>-------------------------------------------------------</td>
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<td></td>
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</tr>
<tr>
<td>Salmonella Typhimurium</td>
<td>Isolated from a human gastroenteritis sufferer in 2000; et al. (2011)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plasmid R100 containing Tn21</td>
<td>The index isolate of Tn21; isolated in Japan in the mid-1950s; Tn21 contains In2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mycobacterium abscessus</td>
<td>Strain originally described in 1953 from a human knee carriage of the merA gene has been noted in</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Staphylococcus aureus Tn6009: a combination of Tn916 and merR carried on a 23 kb plasmid similar to pMM23 from</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E. coli</td>
<td>Whole-genome and whole-plasmid sequencing of medically important bacteria is now showing the presence of Tn21-related multi-resistance transposons in multiple strains – this will be discussed further below (also see Table 2).</td>
<td></td>
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</tr>
</tbody>
</table>

**Microbial genomes: snapshots of the evolution of pathogen resistance repertoires?**

The dramatic increase in the number of draft or complete microbial genome sequences produced over the last 10 years or so has provided us with information on the content of bacterial genomes and, particularly with high-throughput sequencing technologies, on the evolution of bacterial pathogens. These genome sequences also allow us to examine the prevalence of antimicrobial metal resistance in recent isolates of medically or agriculturally important bacteria, including the genomes of existing, ‘new’, emerging and re-emerging pathogens, opportunistic pathogens, and ‘pathogenic commensals’ (Alekshun & Levy, 2006). Many of these pathogens are niche pathogens, or opportunistic healthcare-associated infections in critically ill or immune compromised patients. They also represent acute clinical problems because they are MDR pathogens presenting challenges to treatment.

[Table 2](#)
What is the evidence from these genome sequences that antimicrobial metal resistances are contributing to the broader MDR pathogen problem? We have examined evidence for carriage of mercury, copper/silver and arsenic/antimony resistance in microbial genome sequences, and will discuss them next.

**Mercury resistance: still here, but why?**

Mercury resistance transposons related to Tn21 and the similar Tn1696 can be found on pathogen plasmids or chromosomes, associated with antibiotic resistance cassettes carried on integrons. Table 2 shows examples of these resistances from recently sequenced pathogens, some of which were originally isolated when mercury was still used as an antimicrobial and others that were isolated more recently. Examples of more recently isolated mer transposons include those carrying the TEM-24 ESBL resistance in the integron (Novais et al., 2010), examples of MDR *Acinetobacter baumannii*, *Yersinia pestis* and *Salmonella Typhimurium*, and the recent *E. coli* O104:H4 mass food-poisoning outbreak isolate from 2010 (Fig. 6). The widespread persistence of mercury resistance transposons in pathogens is at first sight surprising given that mercury compounds are apparently rarely used as antimicrobials.

**Copper and silver resistance genes: a previously under-remarked genetic linkage?**

The pMG101 *sil* plasmid-borne silver resistance (Gupta et al., 1999a) and the independently isolated *pco* plasmid copper resistance (Tetaz & Luke, 1983) are the most well characterized silver and copper resistances. During the annotation of the genome sequence of the enterohaemorrhagic *E. coli* H10407 (Crossman et al., 2010), we noted a chromosomal genetic arrangement where the *pco* and *sil* operons were adjacent to each other. Subsequent searches of other plasmid and genome sequences (Table 3) have identified this arrangement (or similar) in a range of different Gram-negative bacteria, on both plasmids and chromosomes (Fig. 7), including in the German *E. coli* O104:H4 isolate from the 2010 mass outbreak, avian pathogenic *E. coli* (Johnson et al., 2006) and livestock isolates (unpublished). This raises a number of unresolved questions regarding the contribution of *sil* and *pco* to silver and copper resistance, whether these contribute to in vivo survival of pathogens in macrophages, cross-regulation and co-selection of these genes, as well as their mobility, and the consequences of this on MDR resistance, particularly in agricultural environments where high levels of copper are used in feed and as antimicrobials, but also in environments where silver is used as an antimicrobial.
Table 3. Examples of pco/sil gene clusters in sequenced bacterial type strains and pathogens

<table>
<thead>
<tr>
<th>Strain</th>
<th>Genetic element</th>
<th>Additional information</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Citrobacter sp. 30_2</td>
<td>pco/sil</td>
<td>Reference genome for the Human Microbiome Project sequenced by the Broad Institute</td>
<td>GenBank assembly GCA_00158355.2</td>
</tr>
<tr>
<td>Cronobacter sakazakii BAA-894</td>
<td>Chromosomal location of pco/sil</td>
<td>Type strain for bacterial meningitis associated with infant formula milk</td>
<td>Kucerova et al. (2010)</td>
</tr>
<tr>
<td>Enterobacter cloacae subsp.</td>
<td>Reported to encode 37 multi-drug efflux proteins, 7 antimicrobial peptide resistance proteins, 11 β-lactamases; multiple metal ion resistances: chromosome: 2 × sil, 3 × ars, 1 × mer and 1 × cop operon; plasmid PECL_A: 1 × sil, 1 × ars, 2 × mer, 1 × cop and 1 × ter</td>
<td>Type strain isolated in 1890 from human cerebrospinal fluid by Edwin Oakes Jordan</td>
<td>Ren et al. (2010)</td>
</tr>
<tr>
<td>E. coli ATCC 13047</td>
<td></td>
<td>Nationally nosocomial outbreak in the Netherlands; plasmid related to R478</td>
<td></td>
</tr>
<tr>
<td>Enterobacter hormaechei</td>
<td>Plasmid pQC carried in Enterobacter hormaechei hospital outbreak strain; resistance to aminoglycosides and third-generation cephalosporins; reduced sensitivity to fluoroquinolones</td>
<td>Isolated from the gut of <em>Anopheles gambiae</em> mosquito</td>
<td></td>
</tr>
<tr>
<td>Enterobacter hormaechei</td>
<td></td>
<td>Test strain for testing antimicrobial handwashes and assaying antimicrobial preservatives</td>
<td></td>
</tr>
<tr>
<td>Enterobacter sp. Ag1</td>
<td>Chromosomal location of pco/sil</td>
<td>National epidemic enterohaemorrhagic <em>E. coli</em> outbreak 2011</td>
<td>Grad et al. (2013) and this article</td>
</tr>
<tr>
<td>E. coli H10407</td>
<td></td>
<td>Multiresistance plasmid pUHU293.2 carrying sil, pco and ars resistance with multiple antibiotic resistances; resembles <em>E. coli</em> ST131 plasmids</td>
<td>Chen et al. (2004)</td>
</tr>
<tr>
<td>Enterobacter hormaechei</td>
<td></td>
<td>Enterotoxigenic <em>E. coli</em>-type strain from Bangladesh</td>
<td>Crossman et al. (2010)</td>
</tr>
<tr>
<td>E. coli APEC O1</td>
<td></td>
<td>Plasmid found in avian pathogenic <em>E. coli</em> isolates in USA</td>
<td>Johnson et al. (2006)</td>
</tr>
<tr>
<td>E. coli EAHEC O104 : H4</td>
<td></td>
<td>German epidemic enteroaggregative haemorrhagic <em>E. coli</em> outbreak 2011</td>
<td></td>
</tr>
<tr>
<td>Klebsiella pneumoniae CG43</td>
<td></td>
<td>Influenza-2 plasmid from Taiwan, hospital isolate 2005 nosocomial outbreak isolate from Sweden; CTX-M-15 ESBL</td>
<td></td>
</tr>
<tr>
<td>Klebsiella pneumoniae</td>
<td></td>
<td>Resembles <em>E. coli</em> ST131 plasmids</td>
<td></td>
</tr>
<tr>
<td>Serratia marcescens</td>
<td></td>
<td>IncHI-2 group plasmid isolated in USA in 1969</td>
<td>Gilmour et al. (2004)</td>
</tr>
<tr>
<td>Salmonella Typhimurium</td>
<td></td>
<td>Isolated from a burns unit at Boston General Hospital, Boston, USA 1973; IncHI-2 group plasmid containing prototypical sil operon; plasmid is partially sequenced</td>
<td>McHugh et al. (1975)</td>
</tr>
<tr>
<td>sil only</td>
<td></td>
<td>Avian pathogenic <em>E. coli</em> isolated from a chicken with colibacillosis</td>
<td>Gupta et al. (1999a)</td>
</tr>
<tr>
<td>APEC E. coli</td>
<td></td>
<td>Avian pathogenic <em>E. coli</em> isolated from a chicken with colibacillosis</td>
<td>Johnson et al. (2005)</td>
</tr>
</tbody>
</table>
Arsenic/antimony resistance: not gone away, nor likely to?

Bacterial arsenic and antimony resistance is currently of marginal interest to human medicine, but resistance is still found widely in bacteria of medical importance (Table 4). Environmental exposure to arsenic or antimony, the continued use of antimonite in treating leishmaniasis, exposure of human populations to arsenic-contaminated drinking water (Perry et al., 2011, 2013), the use of arsenic compounds as rodenticides, and the current and historic use of arsenic compounds in animal husbandry could all have provided direct selection for carriage of arsenic/antimony resistance in commensal and pathogenic microbial flora, and may still be doing so (Eppinger et al., 2012). Co-selection of arsenic resistance alongside other antimicrobial resistances in IncH1-2 plasmids has also been proposed as an explanation for the continued retention of arsenic resistance (Ryan & Colleran, 2002), but environmental arsenical selection may also be contributing to MDR pathogen selection.

The state we are in and how we got here

Amidst the current worldwide concerns about antibiotic resistance, it could be argued that antimicrobial metal ion resistance is of marginal importance to medical microbiology because antimicrobial metals are currently of limited clinical significance, although their use is growing again. Despite limited or discontinued use of these metals, mercury, copper, silver, arsenic and antimony resistances are still in evidence (Reva & Bezuidt, 2012). These resistance genes are often found associated with antibiotic resistance gene cassettes on the same mobile genetic elements, or these antimicrobial metal resistances are carried on MDR elements, where presumably the fitness loss of carrying them is either unimportant or outweighed by the advantages there are to carrying them, because resistance is still needed. It is interesting that the transposons carrying mercury resistance genes found in clinically important pathogens are often carrying a far heavier ‘payload’ of antibiotic resistance genes.

The original ‘R’ (resistance) plasmids isolated in the 1960s and 1970s conferred multiple antibiotic and metal ion resistances on their hosts, and high levels of Hg\(^\text{R}\) and As\(^\text{R}\) bacteria were found in healthcare environments. It was reasonably assumed that this was at least in part due to mercury (Porter et al., 1982) and arsenic compounds being widely used in medicine. There is current and clear evidence of the linkage of metal ion and antibiotic resistance gene carriage in bacteria in sewage treatment plants (Davies &
## Table 4. Arsenic resistance in recently sequenced strains

<table>
<thead>
<tr>
<th>Strain</th>
<th>Genetic element</th>
<th>Additional information</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Acinetobacter baumannii</em></td>
<td>Arsenic resistance contained within an 86 kb chromosomal resistance island (AbaR1), which is a composite transposon containing 45 antimicrobial resistance genes</td>
<td>Epidemic strain in French hospitals; multiply antibiotic and antimicrobial resistant</td>
<td>Fournier et al. (2006)</td>
</tr>
<tr>
<td><em>Acinetobacter baumannii</em></td>
<td>Multiple antimicrobial resistance: arsenate, mercury and multiple antibiotic resistance carried on AbaR3, which shares resistance island homology with AYE strain</td>
<td>Isolated in 2004 from a patient at Walter Reed Army Medical Center, USA</td>
<td>Adams et al. (2008)</td>
</tr>
<tr>
<td><em>Acinetobacter baumannii</em></td>
<td>Arsenic and multiple antimicrobial resistance carried on the AbaR5, similar to AbaR3</td>
<td>Isolated in 1997 in a hospital in Sydney, Australia, from a blood sample</td>
<td>Post &amp; Hall (2009)</td>
</tr>
<tr>
<td><em>Burkholderia cenocepacia</em></td>
<td>Resistant to aminoglycosides, macrolides, (\beta)-lactams, imipenem and piperacillin, cotrimoxazole (trimethoprim/sulphamethoxazole) and also exhibits intermediate resistance to fluoroquinolones; carries arsenic resistance</td>
<td>Epidemic pathogen of cystic fibrosis patients; isolated from the sputum of a cystic fibrosis patient in 1989 in Edinburgh; UK index case of the ET12 lineage</td>
<td>Holden et al. (2009)</td>
</tr>
<tr>
<td><em>Campylobacter jejuni</em></td>
<td>Carries a four-gene arsenic resistance cluster; resistant to cephalosporins, (\beta)-lactams and sulphonamides</td>
<td>Originally isolated from a chicken carcass</td>
<td>Fouts et al. (2005), Wang et al. (2009)</td>
</tr>
<tr>
<td><em>Klebsiella pneumoniae</em></td>
<td>Plasmid pUHU239.2: a CTX-M-15-encoding multiresistance plasmid; confers resistance to (\beta)-lactams, aminoglycosides, tetracyclines, trimethoprim, sulphonamides, quaternary ammonium ions, macrolides, silver, copper and arsenic</td>
<td><em>Klebsiella pneumoniae</em> strain involved in a large nosocomial outbreak in Uppsala University Hospital between 2005 and 2011</td>
<td>Sandegren et al. (2012)</td>
</tr>
<tr>
<td><em>Listeria monocytogenes</em></td>
<td>Serotype 4b strains contain a 35 kb genomic island containing arsenic and cadmium resistance</td>
<td>Isolated in 1983 from a food-borne listeriosis outbreak; the Scott A strain is widely used as a reference strain</td>
<td>Briers et al. (2011), Lee et al. (2013, 2014), Sampei et al. (2010) Gilmour et al. (2004)</td>
</tr>
<tr>
<td><em>Salmonella Typhimurium</em></td>
<td>Plasmid R64; resistance to streptomycin, tetracycline and arsenic</td>
<td>Isolated in 1983 from a food-borne listeriosis outbreak; the Scott A strain is widely used as a reference strain</td>
<td>Briers et al. (2011), Lee et al. (2013, 2014), Sampei et al. (2010) Gilmour et al. (2004)</td>
</tr>
<tr>
<td><em>Serratia marcescens</em></td>
<td>Plasmid R478; also confers resistance to tetracycline, chloramphenicol, kanamycin, mercury, copper and tellurite</td>
<td>Isolated in 1997 in a hospital in Sydney, Australia, from a blood sample</td>
<td>Post &amp; Hall (2009)</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>30 kb SCC(_{\text{mcx}}) element carrying mecA, blaZ and arsenic resistance</td>
<td>Isolated in 2010 in Ireland</td>
<td>Shore et al. (2011)</td>
</tr>
<tr>
<td><em>Staphylococcus capitis</em></td>
<td>Novel 60.9 kb composite SCC(<em>{\text{mcx}}) metilcillin resistance, and an SCC(</em>{\text{cadari/cop}}) carrying arsenic copper and cadmium resistance</td>
<td>Isolated in 2007 in France from an infant with late-onset sepsis in a neonatal intensive care unit</td>
<td>Martins-Simões et al. (2013)</td>
</tr>
<tr>
<td><em>Staphylococcus haemolyticus</em></td>
<td>28 kb SCC(_{\text{mcx}}) (SH32) carrying the metilcillin-resistance mec gene complex, arsenic and copper resistance</td>
<td>Isolated in 2003 in China from the blood of inpatient in a hospital</td>
<td>Yu et al. (2014)</td>
</tr>
<tr>
<td><em>Staphylococcus</em></td>
<td>Carries a novel SCC(_{\text{mcx57995}}) element; resistance to oxacillin, penicillin, chloramphenicol, tetracycline, kanamycin, gentamicin, streptomycin, erythromycin, clindamycin and ciprofloxacin; arsenic, cadmium and copper resistance</td>
<td>Meticillin-resistant <em>Staphylococcus pseudointermedius</em> is an emerging problem in animal healthcare and can cause severe infections in humans</td>
<td>Perreten et al. (2013)</td>
</tr>
<tr>
<td><em>Staphylococcus</em></td>
<td>Carries a novel SCC(_{\text{mcx57995}}) element; resistance to oxacillin, penicillin, chloramphenicol, tetracycline, kanamycin, gentamicin, streptomycin, erythromycin, clindamycin and ciprofloxacin; arsenic, cadmium and copper resistance</td>
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<td>Perreten et al. (2013)</td>
<td></td>
</tr>
<tr>
<td><em>Stenotrophomonas maltophilia</em></td>
<td>Carries resistance to cadmium, lead, cobalt, zinc, mercury, silver, selenite, tellurite and uranium</td>
<td>Increasingly important as a nosocomial pathogen of cystic fibrosis patients and the immunocompromised Tn2502 confers resistance to arsenite and arsenate</td>
<td>Crossman et al. (2008), Pages et al. (2008), Neyt et al. (1997) Eppinger et al. (2012)</td>
</tr>
<tr>
<td><em>Yersinia enterocolitica</em></td>
<td>Arsenic resistance carried on the 70 kb pYV virulence plasmid</td>
<td>Increasingly important as a nosocomial pathogen of cystic fibrosis patients and the immunocompromised Tn2502 confers resistance to arsenite and arsenate</td>
<td>Crossman et al. (2008), Pages et al. (2008), Neyt et al. (1997) Eppinger et al. (2012)</td>
</tr>
<tr>
<td><em>Yersinia pestis</em></td>
<td>Carries four plasmids, each of which carries Tn2503 encoding arsenic resistance related to Tn2502 carried on <em>Yersinia pestis</em> JAVA 9 pYV virulence plasmid</td>
<td>Increasingly important as a nosocomial pathogen of cystic fibrosis patients and the immunocompromised Tn2502 confers resistance to arsenite and arsenate</td>
<td>Crossman et al. (2008), Pages et al. (2008), Neyt et al. (1997) Eppinger et al. (2012)</td>
</tr>
</tbody>
</table>
Davies, 2010 and references therein), as well as in terrestrial and aquatic environments (Berg et al., 2005; Stepanauskas et al., 2006; Wright et al., 2006, 2008; Skurnik et al., 2010). Moreover, there is a considerable literature on the problem of antibiotic resistance/metal resistance co-selection (Baker-Austin et al., 2006; Singer et al., 2006; Stepanauskas et al., 2006; Alekshun & Levy, 2007; Aminov & Mackie, 2007; Allen et al., 2010). Thus, whilst the use of mercury and arsenic in medicine has declined, and copper and silver have limited uses, antimicrobial metal resistance genes to these (and other) metals are persisting, and are co-selected with other antimicrobial resistance genes.

Herein lies the problem. Summers (2002, 2006) has already elegantly argued that although antimicrobial resistance has traditionally been viewed as a treatment (failure) problem, the propagation of resistance to antimicrobials is actually an ecological problem, and that both human and agricultural uses of antimicrobials have contributed to this situation. Summers (2006) has also argued that understanding the role of the agricultural and commensal microbiota, and the mobile genetic elements involved in resistance gene movement, is also very important in understanding these multidrug resistance and transmission phenomena. We can find nothing to disagree with there: the role of commensal bacteria as a reservoir for antimicrobial resistance genes is now gaining more recognition (e.g. Fricke et al., 2009; Marshall et al., 2009) and it has been long recognized that antibiotic resistance in agricultural bacteria is a significant problem (reviewed by Khachatourians, 1998; Wise et al., 1998; Levy & Marshall, 2004; Silbergeld et al., 2008). The phenomenon of antimicrobial metal resistance gene emergence and spread is, in our opinion, conceptually identical to the problem of the evolution and dissemination of antibiotic resistance as outlined by Courvalin (2005, 2008). One strategy proposed to reduce antibiotic resistance is to attempt to delay the emergence and dissemination of resistance to new antibiotics (Courvalin, 2008). Unfortunately, we cannot delay the emergence of antimicrobial metal resistance. It has already happened, and those resistances are still apparently highly successful, widespread and mobile in Gram-negative bacteria, and may also be important in Gram-positive bacteria such as Staphylococcus aureus. Is it now time to have a serious debate about the non-medical uses of antimicrobial metals in relation to the dissemination of multidrug resistance? Or are we too late? Will new formulations and uses of antimicrobial metals overcome existing resistance mechanisms? Or will lethal selection drive evolution of resistance?

Conclusions

Bacterial antimicrobial metal ion resistances, which have been found in pathogens and non-pathogens, were present long before microbiologists realized that these resistances existed. Even now, the genetic elements encoding metal ion resistance appear to be playing a powerful role in facilitating multi-drug resistance and horizontal gene transfer, through co-carriage and/or co-selection of antibiotic resistance with the metal resistances. The presence of antimicrobial metal resistance genes in bacteria not only reflects the anthropocentric view of microbiology (Aziz, 2009), which is the history of human antimicrobial use in infectious disease (Toleman & Walsh, 2011), but also microbial exposure to these metals from industry and agriculture; and preating all human uses: the exposure of micro-organisms over millennia to localized high levels of bioavailable toxic metals from natural environmental releases and interactions with organisms that predate humans. The continuing widespread presence of antimicrobial metal resistance genes often intimately associated with other antimicrobial resistance genes suggests that it is unlikely that they are going to go away soon, and we must take resistance gene co-carriage and co-selection into account when we think about strategies to combat antimicrobial and antibiotic resistance. Persistence of these metal resistance genes points to what the future for antibiotic resistance gene persistence could be.

Acknowledgements

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Bacterial antimicrobial metal ion resistance


