Resistance to antibiotics threatens our ability to control bacterial pathogens. It is clear that the persistence of cells containing resistance determinants is promoted by the strong selective pressure imposed by antibiotic use. This problem has been exacerbated by inappropriate and excessive use of antibiotics in both medicine and animal production. Concern has also been raised that inappropriate use of biocides contributes to the selection of resistant bacterial strains. This may occur because detoxification mechanisms for biocides and antibiotics are shared, or via selection for biocide resistance genes that are physically linked to antibiotic resistance genes and their mobile DNA vectors. Mobile DNA elements carry integrons and their mobile DNA vectors. In this review I will illustrate the latter phenomenon using the evolutionary history of the class 1 integron as an example, and then examine whether the increasing trend towards indiscriminate use of biocides in homes and consumer products might result in the selection of novel genetic elements that will have negative and unpredictable consequences for human health.

Mobile DNA elements that carry integrons, and their associated antibiotic resistance genes, can now be found in the majority of Gram-negative pathogens in hospitals and animal production facilities. The diversity of genes involved and the complexity of the DNA elements that carry them appears to be increasing. Studying this pool of genes and vectors in pathogens and in the general environment could help control the recruitment of further resistance determinants. We can also learn about the process of recruitment by reconstructing the evolutionary history of existing resistance elements.

Class 1 integrons have played a significant role in the spread of antibiotic resistance, and are a good example of a genetic element whose evolutionary history can inform our understanding of resistance phenomena. Integrons generally are a diverse family of elements that are capable of acquiring and expressing exogenous genes. Their basic structure is similar, consisting of a gene for a site-specific tyrosine recombinase (intI), an associated recombination site (attI), and an array of gene cassettes inserted sequentially into the attI site by the recombinase (Figure 1A). Hundreds of classes of integrons have now been described, grouped by the homology of their respective tyrosine recombinases. However, only classes 1, 2 and 3 are of clinical relevance. Of these, class 1 integrons were the first to be described, and are still of the most importance.

The appearance of class 1 integrons in clinical contexts coincided with the widespread use of antibiotics. Initial clues to their origin lay in the conservation of key sequence features in all class 1 integrons of clinical relevance, strongly suggesting that they were all derived from a very recent common ancestor. Clinical class 1 integrons mainly carry antibiotic resistance genes and are normally found on plasmids. However, it has recently been shown that class 1 integrons are also common on the chromosomes of environmental Betaproteobacteria, where they are not associated with known resistance genes. Examination of the structure of clinical class 1 integrons shows that their recent common ancestor arose when one of these chromosomal class 1 integrons was captured by a transposon of the Tn402 family (Figure 1B). This event generated a hybrid DNA element with two key characteristics: the ability of the integron to sample diverse gene cassettes from the environmental pool of these elements, and the ability of the Tn402 transposon to target res sites, notably those of the Tn21 subfamily of transposons. These transposons are, in turn, carried on conjugative plasmids, thus giving the integron and associated gene cassettes the ability to mobilise between both cells and species.

Class 1 integrons attached to complete Tn402 transposition machinery can be readily recovered from environmental samples. Recently, mapping of one of these integrons showed that the integron cassette array did not contain any antimicrobial resistance genes, but rather carried gene cassettes of unknown function (Figure 1C) (Sajjad, A. unpublished observations). Consequently, descendants of the original structure resulting from capture of a class 1 integron by Tn402 are still circulating in the general environment. This raises the question as to what selective forces fixed the Tn402-class 1 integrons in human clinical or commensal flora. There is strong circumstantial evidence to suggest that it may have been the use of disinfectants, and in particular, the quaternary ammonium compounds.

When class 1 integrons from freshwater sediment and biofilms are examined, more than half of them carry qac cassettes that confer resistance to quaternary ammonium compounds, but virtually none carry known antibiotic resistance genes. Thus there was little chance of Tn402 capturing an integron that carried...
an antibiotic resistance gene, but a greater than 50% chance of capturing an integron with a qac cassette. This early involvement of qac is strongly supported by the observation that a modified qacE cassette is an almost universal feature of extant clinical class 1 integrons. Also, quaternary ammonium compounds have been used as disinfectants since the 1930s, pre-dating antibiotic class 1 integrons is often terminated by the gene cassette showing that the class 1 integrons from Tn402 carry a complete cassette array. This early involvement of qacE cassette in clinical antibiotic resistance more generally. There are two accepted mechanisms by which this might occur. The first is cross-resistance, where a biocide resistance mechanism is also able to mediate resistance to antibiotics. There are, for instance, a number of examples of efflux pumps that export both quaternary ammonium compounds and other antimicrobial agents. The second mechanism is that of co-resistance, where selection on biocide resistance determinants results in co-selection for antibiotic resistance genes physically linked on the same genetic element. Selection for integrons carrying both qac and antibiotic resistance genes has been demonstrated in environments polluted with quaternary ammonium compounds. A similar phenomenon, the co-selection of antibiotic and metal resistance, has been recently reviewed. Although long-term exposure to quaternary ammonium compounds did not alter the pattern of antimicrobial resistance in biofilm communities, a randomised trial involving exposure to quaternary ammonium compounds in households resulted in significant co-selection of biocide and antibiotic resistance.

There is a third, and possibly more sinister, relationship between biocide use and the generation of antibiotic resistance. It may be that the very use of antimicrobial agents, including biocides, actually increases the basal rate of bacterial evolution, enhances lateral gene transfer, and promotes the generation of novel DNA elements. Antimicrobial compounds induce environmental stress on bacterial cells, stimulating mechanisms that increase evolvability and activating transcription cascades. Cells within stressed populations can become hypermutators through defects in DNA repair mechanisms, thereby increasing the likelihood of resistance mutations appearing. It has also been demonstrated that multidrug environments can accelerate the evolution of...
resistance in *E. coli*. Lysis of dead cells during antimicrobial treatment releases DNA into the environment, where it is available for transformation and, in at least some cases, antibiotic exposure can increase the transformability of pathogens. It is true that these cases do not directly deal with biocides, but it is reasonable to suggest that biocides generate the same general effects, these being increases in both mutation and lateral transfer rates.

Finally, it is also likely that the ecology of human-dominated ecosystems helps to stimulate the generation of novel genetic elements. Exposure of cells that contain integrons to various antibiotics induces an SOS response that, in turn, controls recombination events. Consequently, the release of antibiotics in human waste streams is likely to have an impact on the activity and complexity of large gene cassette arrays found in environmental samples. Human waste streams simultaneously release resistance determinants, their DNA vectors and the very antimicrobial agents that maintain them by selection. Wastewater, therefore, brings together diverse plasmids and integrons in an environment that also contains sub-inhibitory concentrations of the agents to which they exhibit resistance. This creates a ‘genetic reactor’ where recombination and lateral transfer allow DNA elements borne by pathogens and commensals to interact with the vast diversity of mobile elements found in environmental bacteria.

The use of biocides is an essential element of good practice for preventing the transmission of infectious diseases, and should not be discouraged in this context. However, the indiscriminate and unnecessary use of biocides in an expanding range of household products has the potential to worsen the antibiotic crisis, through mechanisms of cross-resistance and co-selection, and, importantly, by potentially altering the mode and tempo of bacterial evolution. The application of any strategy to control bacterial growth will ultimately alter the evolutionary dynamics between humans, their commensals and their pathogens. None of this should be news to those who accept the inevitability of bacterial evolution. It was succinctly put in 1998: “Resistance stems from misguided efforts to try to sterilize our environment.”

**Acknowledgements**

Thanks to Hatch Stokes, Ian Paulsen and Andy Beattie for discussions and comments on drafts of the manuscript. This work is supported by the ARC and NH&MRC.

**References**


**Biography**

Michael Gillings is Professor of Molecular Evolution in the Department of Biological Sciences at Macquarie University. His research interests lie in the exploration of genetic diversity in a range of organisms. Current projects investigate lateral gene transfer in bacteria, population structure in freshwater fish and sharks, and molecular prospecting as a means of recovering novel genes and gene products.