Biocide use, integrons and novel genetic elements

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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. In this brief 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[1,2]. 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[3-5]. 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[6-8]. 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[6,9]. However, only classes 1, 2 and 3 are of clinical relevance. Of these, class 1 integrons were the first to be described[10], and are still of the most importance.

The appearance of class 1 integrons in clinical contexts coincided with the widespread use of antibiotics[11]. 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[7]. 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 Tn*402* family (Figure 1B)[7,8]. 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[12], and the ability of the Tn*402* transposon to target *res* sites, notably those of the Tn*21*-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[8].

Class 1 integrons attached to complete Tn*402* 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 Tn*402* are still circulating in the general environment. This raises the question as to what selective forces fixed the Tn*402*-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[13].

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[12,13]. Thus there was little chance of Tn*402* capturing an integron that carried

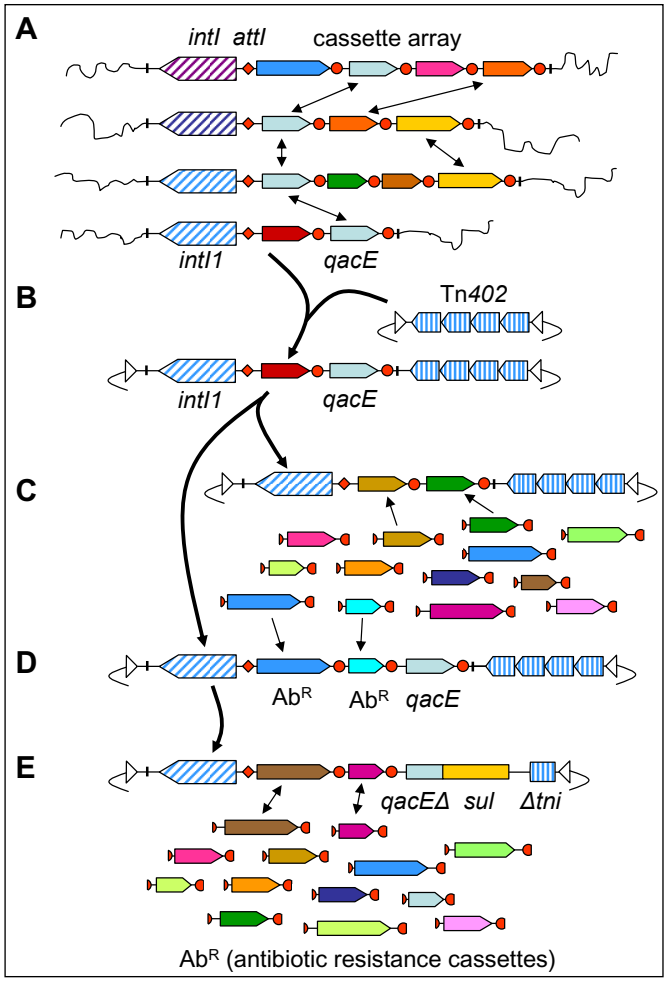

Figure 1. A model for the origin of clinical Class 1 integrons
(A) Generalised structure of chromosomal integrons: Integrons contain a gene for a site-specific tyrosine recombinase, called an integron-integrase (*intI*). The integrase protein catalyses the recombination of gene cassettes into the *attI* site (red diamond). Gene cassettes are composed of an open reading frame (solid colour arrows) and a secondary recombination site (*attC,* red circles). This activity results in a tandem array of gene cassettes, that can in some circumstances contain hundreds of different genes, represented here by different colours. Gene cassettes can be exchanged between integrons resident on the chromosomes of different species. In this schematic, the sequence boundaries of the integrons are represented by the small vertical bars, and the wavy lines are the chromosomal DNAs into which the integron is inserted. Integron-integrase genes (diagonal stripes) are shown in different colours to represent different homology groups.
(B) Capture of a class 1 integron by a Tn*402* transposon: The chromosomal class 1 integron from a member of the *Betaproteobacteria* is inserted into the Tn*402* tranposon. It is likely that the original capture event included a *qac* gene cassette (solid light blue arrow) that conferred resistance to quaternary ammonium compounds (see text for explanation). The transposon gave the integron the ability to easily move between different genetic elements and thus onto conjugative plasmids that could readily move between cells.
(C) Acquisition of gene cassettes (shown linearised here): The activity of the Tn*402* Class 1 integron allowed the rearrangement and acquisition of diverse cassettes from the environmental pool of these elements. Class 1 integrons that carry neither *qac* nor antibiotic resistance can be recovered from environmental samples.
(D) Acquisition of antibiotic resistance cassettes: Descendants of the original structure resulting from capture of a class 1 integron by Tn*402* were able to capture gene cassettes conferring antibiotic resistance from the environmental gene cassette pool. Their movement and spread into human pathogens and commensals may have been driven by antibiotic selection, or by selection on the *qac* cassette.
(E) Modification of the Tn*402* integron: The class 1 integrons circulating in human-dominated ecosystems have undergone additional modifications, including insertion of a gene for sulphonamide resistance (*sul1*) into *qac*, thereby deleting the terminal portion of the cassette. Various deletions have also inactivated the transposition module (*Δtni*). Variants of the original class 1 element have acquired and mobilised well over 100 gene cassettes conferring antibiotic resistance.

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[14]. Also, quaternary ammonium compounds have been used as disinfectants since the 1930s[15], pre-dating antibiotic use and thus providing an earlier opportunity for selection to act. It should be mentioned that the extant *qacE* cassette in clinical class 1 integrons is often terminated by the *sul1* gene (Figure 1E)[14], which confers resistance to sulfonamides, and that these compounds were also in use in the 1930s–1940s[15]. However, the class 1 integrons from Tn*402* and Tn*6007* carry a complete *qacE* gene cassette[16,17] showing that *qacE* predates the insertion of *sul1*.

The likely evolutionary history of the clinical class 1 integron is a clear indication that biocide use has the potential to select for novel genetic elements with impacts on human health. There has been considerable speculation that ongoing and increased biocide use will enhance the appearance and spread of antibiotic resistance more generally[18-22]. 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[18]. 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[23]. A similar phenomenon, the co-selection of antibiotic and metal resistance, has been recently reviewed[24]. Although long-term exposure to quaternary ammonium compounds did not alter the pattern of antimicrobial resistance in biofilm communities[20], a randomised trial involving exposure to quaternary ammonium compounds in households resulted in significant co-selection of biocide and antibiotic resistance[22].

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[25] and activating transcription cascades[26]. Cells within stressed populations can become hypermutators through defects in DNA repair mechanisms[27], 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*[28]. 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[29]. 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 *intI*-mediated recombination events[30]. Consequently, the release of antibiotics in human waste streams[31,32] is likely to have an impact on the activity and complexity of large gene cassette arrays carried by the diverse chromosomal integrons found in environmental samples. Human waste streams simultaneously release resistance determinants, their DNA vectors and the very antimicrobial agents that maintain them by selection[32]. Wastewater, therefore, brings together these diverse plasmids and integrons in an environment that also contains sub-inhibitory concentrations of the agents to which they exhibit resistance[33]. 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[31].

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[19]. 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[18,22]. 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"[34].

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Biography

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