Inhibition of bacteria on agar surfaces by vapour phase triclosan


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Sir,

Triclosan is the most widely used member of the bisphenol family of disinfectants, which are characterized by their high intrinsic activities and proportional low solubility in water. Unfortunately, they also share ineffectiveness against pseudomonads and an ability to select for resistance in Staphylococcus aureus. In recent years, an increasing number of domestic plastic and cleaning products incorporating triclosan have been marketed under the Microban trade mark.

In their paper in this journal on triclosan-impregnated food-storage boxes, Braid & Wale described, but were "unable to account for the complete suppression of growth of S. aureus on one side of open agar plates that were directly adjacent to the triclosan-impregnated wall of the 9 L boxes used". This effect is reproducible and we have also demonstrated it, under experimental conditions, by exposure of strains of both S. aureus and Escherichia coli to a proprietary Microban household cleaning fluid and a 5% solution of triclosan. The degree of inhibition appears to be proportional to both the concentration of the agent and the respective susceptibility of the test strains.

The authors also found it difficult to equate the vapour phase of triclosan with the pattern of inhibition found, asking why all of their plates had not been affected equally and evenly; however, we believe that the pattern of suppression seen can be entirely explained in terms of classical zone formation theory. In our experiments, we inoculated nutrient agar plates with our test organisms, as if for a conventional test. Triclosan molecules reaching the bacterial cells would be expected to preferentially solubilize in lipids present in the membranes and ultimately enter and inhibit the cell. This will occur until the point is reached where the concentration of inhibitory molecules is only just sufficient to inhibit all of the cells (critical population) and a zone edge will form. From then on, since the number of target sites is multiplying as a result of bacterial growth, the eventual vapour pressure obtained will never be sufficient to prevent the visible zone from forming. The flattened zones seen in the figures presented by Braid & Wale, and also seen in our work, can be explained by the geometry of the plates and are paralleled by those seen when a disc is placed too near the edge of a Petri dish.

Controversially, Braid & Wale concluded their article by suggesting that triclosan impregnated storage boxes are "potentially useful for storage of foods for short periods (e.g. lunch boxes or overnight) where refrigeration is not possible". In the light of their own findings, we question this proposition. Only when bacteria were suspended in broth directly in contact with the triclosan-impregnated plastic was there inhibition of growth for some species. When the bacteria were spiral-plated onto solid media the authors observed no significant reduction in colony counts compared to controls, other
than where total inhibition zones had formed. The important observation to make is that, outside of the inhibition zone, the inoculated cells multiplied to form visible colonies despite being in the triclosan vapour for up to 72 h at 22°C. This is entirely in accord with zone theory and, of course, everyday experience with diffusion susceptibility tests. It is unlikely therefore that at ambient temperatures perishable foods, in a triclosan-impregnated lunch box, would keep Pseudomonas putrefaciens in conditions simulating domestic use. Journal of Antimicrobial Chemotherapy 49, 87–94.


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First description of CTX-M-15-producing Klebsiella pneumoniae in Turkey

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Sir,

At the beginning of the 1990s, extended-spectrum β-lactamase (ESBL)-producing Gram-negative bacteria, exhibiting a higher level of resistance to cefotaxime than to cefazidime, were described in Germany (1990), France and Argentina (1992). These ESBLs were named CTX-M type β-lactamases, owing to their high level of activity against cefotaxime. Such Ambler class A β-lactamases have <40% identity with the β-lactamases of the TEM and SHV series. Twenty-nine different genes encoding CTX-Ms have been characterized so far. They have been identified mostly from Enterobacteriaceae isolated from various geographical areas, mainly from South America, Europe and East Asia. Unlike most CTX-Ms, CTX-M-15, CTX-M-16 and CTX-M-19 hydrolyse cefazidime at a higher rate than cefotaxime.

We report here the first detection of a clinical isolate from Turkey that expresses CTX-M-15.

In September 2001, a multiresistant isolate of Klebsiella pneumoniae (KP7881) was isolated from a urine sample of a patient hospitalized in the surgical unit of the Istanbul Faculty Hospital. This isolate was resistant to all β-lactams except imipenem. MICs of β-lactams were determined by an agar dilution method (Table 1). A cefotaxime/co-amoxiclav synergy test was slightly positive, whereas a cefotaxime/co-amoxiclav synergy test remained negative. The isoelectric points (pI) of culture extracts of this isolate were 5.6, 6.1, 7.4, 8.2 and 8.6. Plasmid DNA was isolated by the Kieser procedure and transformed into Escherichia coli DH10B by electroporation as described. Four antibiotic resistance phenotypes of transformants were obtained: three gave different ESBL phenotypes and another exhibited resistance to penicillins and co-amoxiclav. The MICs of β-lactams for the four transformants and the parental isolate are shown in Table 1. Transformant 1 was resistant to cefotaxime and of intermediate susceptibility to cefazidime. It was also resistant to kanamycin, tobramycin, amikacin, netilmicin, spectinomycin, tetracycline, sulphonamides, trimethoprim and chloramphenicol. PCR amplification, performed with DNA of transformant 1 as template using bla_CTX-M-specific primers, was positive, and sequencing of the PCR product identified the bla_CTX-M-15 gene. Using PCR experiments, an insertion sequence ISεcp1 was identified upstream of the 5′ end of this gene, with primers hybridizing to the ends of this insertion sequence. The pI value of this ESBL was 8.6, and the bla_CTX-M-15 gene was located on a 60 kb plasmid. Two other transformants (transformants 2 and 3) exhibited an ESBL phenotype with a higher resistance to cefotaxime than to cefotaxime. Using primers specific for the SHV gene, bla_SHV-12 (pI 8.2) was identified in transformant 2 located on a 70 kb plasmid, and in transformant 3 on a 190 kb plasmid. In addition, in transformant 3, three other β-lactamase genes were identified [bla_TEM-2 (pI 5.4), bla_OXA-1 (pI 7.4) and bla_OXA-10 (pI 6.1)] also using primers specific for the TEM gene, the OXA-1 gene and the OXA-10 gene. Transformant 4, exhibiting a restricted spectrum β-lactamase phenotype, encoded two β-lactamases that corresponded to TEM-2 and OXA-10, and was located on a 140 kb plasmid.

K. pneumoniae KP7881 was resistant to cefoxitin and moxalactam, which was not explained by any of the ESBLs identified. However, culture extracts of that isolate did not hydrolyse these β-lactams (data not shown). In a disc diffusion test on Mueller–Hinton agar containing cloxacillin 250 mg/L, the presence of cloxacillin did not inhibit the cephalosporinase activity, which confirmed the absence of a plasmid-mediated cephalosporinase. Thus, the outer membrane protein profile was studied by sodium dodecyl sulphate-polyacrylamide gel electrophoresis analysis. A major ~35 kDa outer membrane protein of K. pneumoniae was lacking (data not shown), suggesting that cefoxitin and moxalactam resistance could have been related to a permeability defect.

β-Lactamase CTX-M-15, which differs from CTX-M-3 by a single amino acid substitution Asp-240 to Gly, was identified first in India and in Japan (GenBank accession no. AY013478), and was subsequently detected in Canada, Russia, Bulgaria, Poland and France. Its report now in Turkey suggests that the bla_CTX-M-15 gene may be highly prevalent worldwide. Moreover, as found previously in India and Poland, K. pneumoniae KP7881 also possesses ISεcp1 located upstream of the bla_CTX-M-15 gene. The ISεcp1 element was located at...