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A rapid, reliable method for the extraction from avian faeces of total bacterial DNA to be used as a template for the detection of antibiotic resistance genes

J Antimicrob Chemother 2001; **47**: 241–243

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Sir,
Recent reports, for example that published by the House of Lords,¹ have highlighted concern over the role played by

antibiotics added to animal feedstuffs in antimicrobial resistance of human pathogens. It has been established for some time that the use of antibiotics in animal feedstuffs leads to the selection of antibiotic-resistant bacteria. It has been found that resistance persists after the antibiotic has been withdrawn.^{2,3} Furthermore, the mobility of antibiotic resistance determinants has been highlighted by the discovery of resistance in animal rearing facilities that did not use antibiotics in feedstuffs.^{4,5} Concern over the scale of this problem has resulted in Sweden banning the use of all antibiotics in feedstuffs and to the European Union banning the use of avoparcin, bacitracin zinc, spiramycin, tylosin phosphate and virginiamycin in animal feedstuffs.

Most concern about antimicrobial resistance has focused on the enteric bacteria, mainly *Escherichia coli* and salmonellae, since they are recognized human pathogens. The bowel microflora is diverse, however, and, in addition to pathogens, contains species that may harbour transferable antibiotic resistance determinants, some of which may be non-cultivable. These bacteria thus act as a silent gene pool for the potential dissemination of antibiotic resistance determinants. The most appropriate way to study the occur-

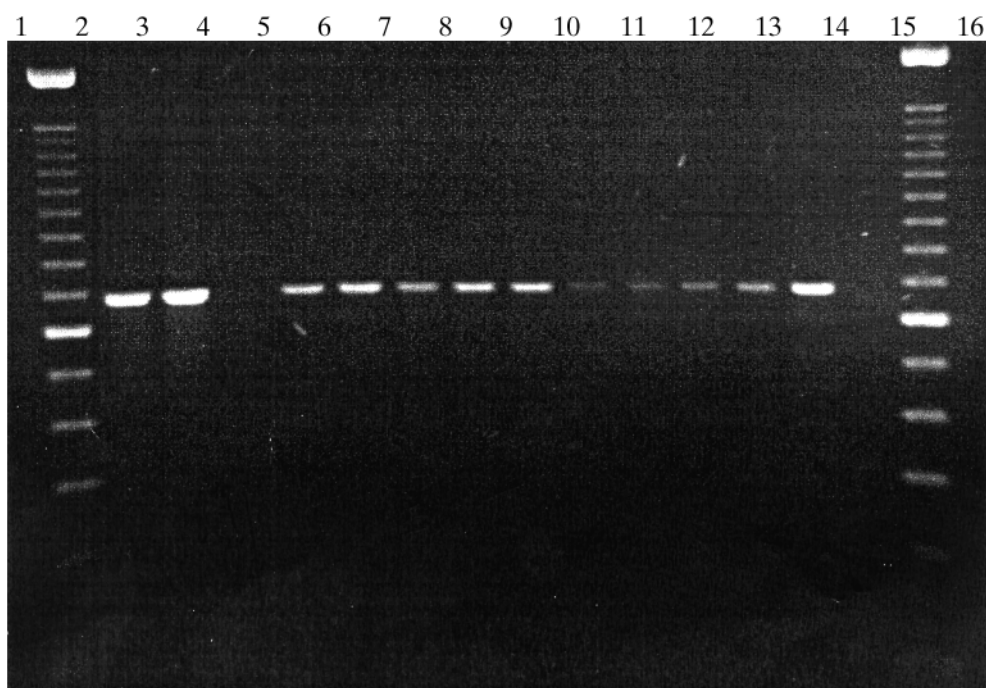


Figure. PCR amplification of the *bla*_{TEM} gene from chicken faecal material. Lanes 1 and 16, 100 bp ladder; lane 2, UB1780 carrying a chromosomal copy of *bla*_{TEM}; lane 3, plasmid pUC18; lane 4, UB5201 lacking *bla*_{TEM}; lanes 5–9, faecal material from five birds fed conventional maize; lanes 10–14, faecal material from five birds fed transgenic maize; lane 15, water blank.

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rence of antibiotic resistance determinants in the total bowel population is by examination of faeces using PCR. This well-established technique has the required sensitivity and specificity, can be used to study non-cultivable organisms and bypasses the need for bacterial culture, which the examination of a complex mixed population would require. The extraction from faeces of sufficient suitably pure bacterial DNA for PCR amplification has proved problematic because of the large amount of PCR inhibitors present. This is a particular problem for bird faecal material, which is mixed with urine. Most of the published methods are either labour intensive or require a cultural enrichment step, thereby rendering them unsuitable for examination of the total faecal population.⁶

As part of an ongoing research project we have used the QIAamp DNA Stool Mini Kit (Qiagen Ltd, Crawley, UK) to extract sufficient, suitably pure bacterial DNA from chicken faeces to enable the detection of TEM-type β -lactamase genes using PCR. Typical results are shown in the Figure. The greater response of the five samples collected from birds fed conventional maize compared with transgenic maize reflects a shorter time from collection of faecal material to processing. Although the kit has been optimized for use with human faeces, we have achieved consistent success with chicken faeces using the manufacturer's protocol, which has been optimized to increase the ratio of microbial DNA extracted to host animal cellular DNA extracted. At present we have used only stored frozen faeces; we have no reason to doubt that similar, if not better, results would be achieved using fresh faeces. The kit has been shown to be suitable for extraction of DNA from both Gram-negative and Gram-positive organisms (results not shown). Briefly, the bacterial cells are lysed in a lysis buffer at 70 °C, PCR inhibitors present in the sample are adsorbed on 'InhibitEX', proteins are digested using proteinase K and the DNA is purified on QIAamp spin columns. The DNA is eluted in a final volume of 200 μ L, 5 μ L of which is used in a PCR with a total volume of 50 μ L. The inclusion of bovine serum albumin in the PCR at a final concentra-

tion of 0.1 μ g/ μ L is recommended by the manufacturer and does indeed seem to increase the robustness of the procedure. The isolation procedure takes approximately 1 h. It is not only more rapid than other methods we have used, but also provides greater reproducibility and reliability. About 25–30 specimens can be screened per day. This method extends study of the ecology of genes encoding antibiotic resistance by facilitating their detection in poultry faeces.

Acknowledgements

This work was supported financially by a grant from the Food Standards Agency.

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