Short reports

Rapid screening for toxigenic Corynebacterium diphtheriae by the polymerase chain reaction

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Abstract

The polymerase chain reaction (PCR) was used to discriminate between toxigenic and non-toxigenic isolates of *Corynebacterium diphtheriae*. Primers specific to the diphtheria toxin gene were used to amplify a toxin gene fragment from simple boiled-cell preparations. Eight recent clinical isolates and four reference strains were tested. The result of the PCR agreed with the traditional toxigenicity assays (the Elek test and guinea pig inoculation) in all cases. PCR has several advantages over the Elek test: it gives a same-day result, it works on colonies taken from selective media, and it detects the toxin gene in mixed cultures. One potential drawback is that the PCR might give a false positive result with the occasional isolate carrying an inactive toxin gene. The good predictive value of a negative PCR result, however, should make it a valuable screening test.

Diphtheria is now rare in the United Kingdom but this is no cause for complacency. Several outbreaks of diphtheria have occurred in developed countries (including the United Kingdom) in recent decades,[1-3] and the disease is still endemic in the Third World. A serological survey in Britain showed that 35% of the population were susceptible to infection, despite high vaccine uptake.[4] There is thus a constant risk that diphtheria may be imported into the United Kingdom and, once here, spread widely.[3]

It is precisely because diphtheria is now such a rare disease in the United Kingdom that great emphasis must be placed on rapid and accurate bacteriology.[5] Brooks and Joynson point out in a recent Association of Clinical Pathologists' broadsheet that the most important test on any suspicious isolate is for toxigenicity, and that this must be done without delay.[5] They claim, however, that "the in vitro modified Elek's immunodiffusion test is ideal for the clinical laboratory."[5] This point is debatable—many view the Elek test as technically demanding, unreliable in inexperienced hands, and slow. In a national quality assessment trial nearly a third of British laboratories reported incorrect results[6] and many, if not most, British laboratories have now abandoned the in-house Elek test and prefer to send isolates as promptly as possible to a reference laboratory.

The aim of this study was to investigate an alternative technique for toxigenicity testing of *C diphtheriae*. The polymerase chain reaction (PCR) has already found many applications in diagnostic bacteriology.[7]

Methods

Eight recent clinical isolates of *C diphtheriae* (four toxigenic and four non-toxigenic) and four NCTC strains were tested. The toxigenic isolates came from pharyngeal swabs taken in England and Wales in 1989 and 1990, and were positive in both the guinea pig and Elek tests (performed by the Diphtheria Reference Laboratory of the PHLS). The non-toxigenic isolates were also all from England and Wales, and were negative in the Elek test. The four NCTC strains included the three Elek controls, and one additional toxigenic strain.

Two primers based on published diphtheria toxin gene sequences (5'-CACTTTTAGTGCTGCGAGAACCTTCGTCA-3' and 5'-GAAAACTTTTCTTCGTACCACGGGACTAA-3')[8-10] were used to amplify a 246 base pair fragment of the toxin gene from boiled-cell preparations. Samples were prepared as follows: After overnight growth on Hoyle's tellurite blood agar a loopful of bacteria was inoculated into 1 ml of distilled water in a 1·5 ml polypropylene tube. Each sample was boiled for 15 minutes, then centrifuged for one minute. Supernatant (1 μl) was added to each 50 μl reaction. PCRs were performed using AmpliTaq polymerase (Perkin Elmer Cetus) in accordance with the manufacturer's instructions. After an initial denaturation step (96°C for two minutes), 40 amplification cycles (94°C for 30 seconds, 37°C for 30 seconds, 72°C for two minutes) were performed under thermocouple control on a Hybaid Thermal Reactor, with a final 10 minute extension step at 72°C. Samples were subjected to rapid electrophoresis on a 1·8% agarose gel containing ethidium bromide, and visualised on an ultraviolet transilluminator.

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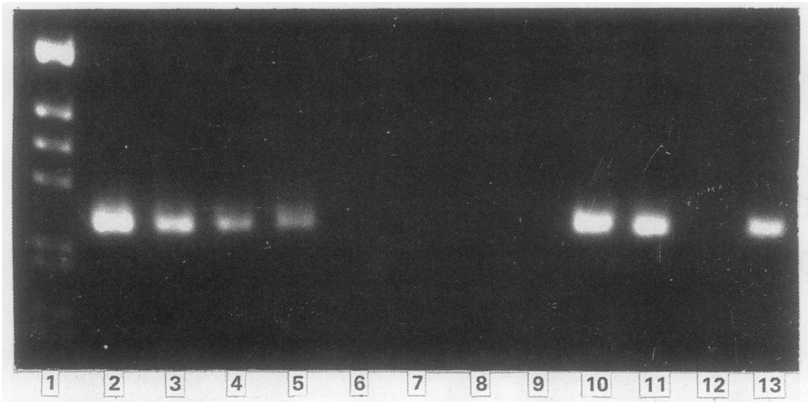

Lane 1: markers—134 base pairs, 154 base pairs, 201 base pairs, 220 base pairs, 298 base pairs, 344 base pairs, 394 base pairs, 506 base pairs
Lanes 2–5: Elek positive and guinea pig positive clinical isolates of C diphtheriae
Lanes 6–9: Elek negative clinical isolates of C diphtheriae
Lane 10: NCTC 3984 weakly toxigenic Elek control
Lane 11: NCTC 10648 strongly toxigenic Elek control
Lane 12: NCTC 10356 non-toxigenic Elek control
Lane 13: NCTC 5014 a toxigenic strain

Results

All of the samples from the seven toxigenic strains could be clearly distinguished from those from the five non-toxigenic strains (Figure). A single band of the predicted size was seen in all the toxigenic samples and in none of the non-toxigenic samples. Identical results were obtained in three separate assays, all done on fresh subcultures.

Discussion

The PCR toxigenicity assay gave accurate and reproducible results on all of the strains and isolates tested. The PCR has several advantages over the Elek test and other immunoassays: it is a same-day test, taking only five to six hours from the selection of colonies to the final result; it is easy to perform and gives clear cut results; its constituents have a long shelf-life (over a year for AmpliTaq polymerase); it can be applied to colonies taken directly from selective media (the Elek test requires an additional incubation step on a non-inhibitory medium); it works on mixed cultures, including mixtures of toxigenic and non-toxigenic strains (troublesome mixtures are sometimes isolated from clinical specimens;[2] and, finally, it requires none of the highly variable biological reagents, such as antisera, used in immunoassays. *Taq* polymerase is now widely available commercially, and, given appropriate equipment, olignucleotides can be synthesised in any laboratory in under 24 hours. This last fact means that the assay could be set up very rapidly anywhere in the world, which could be life saving during an outbreak.

One potential problem with the PCR is that it might give false positive results with isolates of *C diphtheriae*, which carry the *tox* gene, but are unable to produce the intact toxin. Such isolates are probably rare, except in certain defined localities,[11] and would be picked up on further tests, such as guinea pig inoculation.

To see whether isolates carrying inactive toxin genes do indeed present a problem for the PCR assay, further work on a range of non-toxigenic isolates is envisaged (additional isolates would be welcome). Even if some occasional isolates do give a false positive result in the PCR, the good predictive value of a negative assay should make it an valuable screening test.

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