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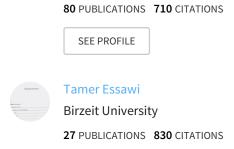
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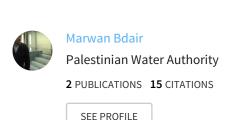
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**EPIDEMIOLOGY** 

## Isolation and characterisation of Shiga toxigenic Escherichia coli strains from northern Palestine

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Shiga toxigenic *Escherichia coli* (STEC) isolates from symptomatic and asymptomatic patients in northern Palestine in 1999 were screened for serotype O157 and characterised for virulence genes by multiplex PCR assay. Of the 176 STEC isolates, 124 (70.5%) were of serotype O157. All these isolates carried the gene for Shiga toxin type 1 ( $stx_1$ ) and 112 (90.3%) carried  $stx_2$ . The intimin encoding gene locus *eae* was detected in 16 isolates (12.9%) and the enterohaemolysin encoding gene, hlyA, in 18 (14.5%). Statistical analysis showed a significant association between the presence of *eaeA* and hlyA, either alone or combined with  $stx_1$  and  $stx_2$  genes in O157 isolates from symptomatic infection. ERIC-PCR analysis of DNA from 80 serotype O157 isolates revealed three major clonal populations.

#### Introduction

Shiga toxigenic Escherichia coli (STEC) are an important cause of gastrointestinal disease in man. Infection with these organisms may result in lifethreatening complications such as haemolytic-uraemic syndrome (HUS) and thrombotic thrombocytopenic purpura [1, 2]. Within the STEC family, certain strains, such as those of serotype O157 or those that have particular combinations of putative virulence factors, appear to be more virulent for man [1-3]. Numerous outbreaks of STEC-related disease have been attributed to serotype O157, although the latter accounts for only c. 60% of isolates in outbreaks [4]. Indeed, several strains of other serotypes have been implicated in both sporadic infections and outbreaks caused by STEC. In some studies, non-O157 strains have represented 20% [5] and 30% [6] of STEC isolated.

Several virulence factors have been described in STEC, including Shiga toxin type 1 (Stx1, encoded by  $stx_1$ ), Shiga toxin type 2 (Stx2,  $stx_2$ ), intimin (eaeA) and the plasmid-borne enterohaemolysin encoded by enterohaemorrhagic  $E.\ coli\ (EHEC)\ hlyA\ [2,7-9]$ . The present study was initiated to assess the importance of STEC as an aetiological agent of acute diarrhoea among Palestinians. The presence of  $stx_1$ ,  $stx_2$ , eaeA, hlyA and

Received 16 May 2001; revised version received 12 Nov. 2001; accepted 21 Nov. 2001.

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O157 *rfbE* genes was tested for simultaneously by a multiplex PCR [2] and the clonal structure of the population of isolates was examined by genomic DNA fingerprinting by enterobacterial repetitive intergenic concensus-PCR (ERIC-PCR) [10].

#### Materials and methods

STEC isolates

A total of 250 stool samples was collected during an outbreak of diarrhoea between February and June 1999 in the north of Palestine. Samples were obtained from symptomatic patients, i.e., those with diarrhoea with or without blood or with clinical signs of HUS, and asymptomatic patients. Samples were plated on Mac-Conkey agar, incubated overnight at 37°C, and a loopful of growth from the first inoculation streak was suspended in 0.5 ml of distilled water and boiled for 10 min. After centrifugation of the lysate, the supernate was used in PCR.

### Multiplex PCR

The  $stx_1$ ,  $stx_2$ , eaeA, hlyA and O157 rfbE gene sequences were detected with the primer pairs described previously [2]. Details of the nucleotide sequence, the specific gene region amplified and the size of the PCR product for each primer pair are given in Table 1. Samples  $(5 \mu l)$  of each extract were amplified in 50- $\mu l$  reaction mixtures with 35 PCR cycles each consisting of 1 min of denaturation at