INTRODUCTION

The detection of pathogens in ready-to-eat food samples requires urgent and timely confirmation for effective public health action. The current protocols used by the HPA FW&E Microbiology Network laboratories require additional biochemical & phenotypic tests which can take up to a further three days once the organism has been isolated.

There is a need for a rapid culture confirmation technique for confirmation /identification of food-borne pathogens, particularly in outbreaks.

Real Time PCR assays have been developed for the detection & identification of food-borne pathogens, providing rapid & accurate results with high sensitivity & specificity.

The FW&E Microbiology Network laboratories at Leeds and Preston investigated the suitability a real-time PCR-based method for the rapid detection and identification of Campylobacter coli, C. jejuni, Listeria spp., L. monocytogenes, Salmonella spp., Vero-cytotoxin producing Escherichia coli and E. coli O157.

MATERIALS & METHODS

• Bacterial colonies from a total of 77 naturally contaminated food samples were tested in this study, including presumptive isolates of: Campylobacter spp. (15), E. coli O157 (6), Listeria spp. (29) & Salmonella spp. (27; Table 1).

• Up to five colony forming units were tested from each sample by molecular methods and by standard routine culture.

• Colonies were picked from selective agar, inoculated onto standard confirmation media and the bacterial growth was then emulsified in 0.5ml of PCR grade water. The same water aliquot was used for all isolates selected from each sample, providing a pooled result.

• Bacterial suspensions were heated at 95°C for 15 mins to kill viable organisms.

• 30µl of bacterial suspension was amplified using lyophilised assays designed specifically for detection of food pathogens: Salmonella spp.; Listeria spp.; L. monocytogenes; C. jejuni; C. coli; vero-cytotoxin producing E. coli and E. coli O157 (Applied Biosystems) on the TaqMan 7500 Fast Real Time PCR machine (Applied Biosystems).

• An internal positive control (IPC) was incorporated for each extract, positive & negative control were included for each PCR run.

• Data was analysed with the 7500 Fast System SDS software (version 1.4; Applied Biosystems) using the auto baseline setting and a manual threshold of 0.2 was used for all assays, except for the Listeria spp. assay where a manual threshold of 0.55 was used. A Ct value cut-off point of >35 was applied to determine negative samples.

RESULTS

• Initial testing of 77 naturally contaminated samples has shown 100% concordance between culture- & molecular-based confirmation/identification of presumptive pathogens. No false negative & false positive results were obtained (Table 1).

Table 1. Culture Confirmation Results

<table>
<thead>
<tr>
<th>Target Assay</th>
<th>No. of samples</th>
<th>PCR results</th>
<th>Culture results</th>
<th>PCR Assays</th>
</tr>
</thead>
<tbody>
<tr>
<td>Campylobacter coli</td>
<td>15</td>
<td>2</td>
<td>13</td>
<td>Positive</td>
</tr>
<tr>
<td>Campylobacter jejuni</td>
<td>15</td>
<td>12</td>
<td>3</td>
<td>Negative</td>
</tr>
<tr>
<td>Escherichia coli O157:H1</td>
<td>6</td>
<td>0</td>
<td>6</td>
<td>Positive</td>
</tr>
<tr>
<td>Listeria species</td>
<td>29</td>
<td>15</td>
<td>14</td>
<td>Positive</td>
</tr>
<tr>
<td>Listeria monocytogenes</td>
<td>29</td>
<td>7</td>
<td>22</td>
<td>Negative</td>
</tr>
<tr>
<td>Salmonella species</td>
<td>27</td>
<td>3</td>
<td>24</td>
<td>Positive</td>
</tr>
</tbody>
</table>

DISCUSSION

• Real time PCR assays were as sensitive as traditional culture–based methods for confirming the identity of presumptive pathogens from bacterial cultures.

• The molecular-based method employs two simple steps for rapid identification of food-borne pathogens directly from culture plates (Figure 1).

• The direct addition of killed suspensions into the lyophilised PCR assays significantly reduces the identification time for food-borne pathogens (Figure 2) and provides accurate results with high sensitivity and specificity (Table 1).

• The inclusion of an IPC assay allows the identification of false negative results that may occur due to the presence of PCR inhibitors in the isolate suspension.

• Testing is continuing alongside routine culture confirmation to provide robust validation data for roll-out to all HPA FW&E Microbiology Network laboratories.

• The introduction of molecular confirmation/identification of presumptive pathogens from ready-to-eat foods represents a major service development for improved public health management of food-borne infections.

ACKNOWLEDGEMENTS

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