

Genetic Identification of Predominant Bacteria Isolated from Student Kitchen

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The domestic kitchen represents one of the most significant areas associated with the prevalence of foodborne disease. Literature related to exploring and discussing bacterial contamination and sanitation practices in shared kitchens is scarce. The aim of this study is to carry out genetic identification of 2 of the predominant bacterial isolates previously obtained from a shared student kitchen. Partial 16S rDNA gene sequencing was used to further identify two selected isolates. Gene sequencing revealed the 2 selected isolates to be closely related to *Enterobacter cloacae* (99% sequence similarity) and *Pseudomonas* sp. (100% sequence similarity); this is in agreement with the previous presumptive identifications.

Keywords: Food borne diseases, domestic kitchens, shared student kitchen, Partial 16S rDNA gene sequencing, *Enterobacter cloacae*, and *Pseudomonas* sp.

Foodborne disease leads to substantial morbidity and mortality all over the world¹, and is thus considered a severe public health problem². In 2010, there were 56,991 recorded cases of food poisoning illness in England and Wales³. However, it is generally accepted that actual figures may be orders of magnitude higher than those recorded due to significant under-reporting amongst other factors^{2,4}.

Campylobacter jejuni and *Salmonella* spp. are the main culprits responsible for a significant proportion of reported cases of foodborne disease in the UK^{2,5-7}. This is explained by the close association of these species with chicken, a common component of the UK diet. Numerous surveys have been conducted to assess the extent of contamination of raw poultry sold in the UK, and they have revealed that up to 30% is

contaminated with salmonellae and up to 90% with campylobacters⁷.

Salmonella spp. and *Campylobacter* spp. are widely recognized as the most common pathogens isolated in human cases of bacterial gastroenteritis^{2,8} although it has been reported that *Campylobacter* is more often implicated in gastrointestinal infection than *Salmonella*⁴. *Campylobacter* is also considered to be the leading bacterial cause of diarrheal disease/infectious intestinal disease (IID) in the developed world⁸. In addition to *Salmonella* and *Campylobacter*, it has been reported that *Shigella* and Shiga toxin and *Escherichia coli* (STEC) O157 significantly contribute to the incidence of foodborne disease in the United States⁹. Other foodborne pathogens include *Staphylococcus aureus*, rotavirus and hepatitis A virus¹.

Foodborne outbreaks of *Listeria monocytogenes*, responsible for septicemia and meningoenzephalitis in the immunocompromised and the elderly, have been reported¹⁰. L.

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monocytogenes is common in the environment and has caused several cases of hospital-acquired/nosocomial listeriosis, including in neonates, in the UK. Nosocomial outbreaks of Salmonella infection, particularly Salmonella enteritidis, have also been recorded¹¹.

Bacterial contamination in the domestic environment was first comprehensively investigated by Finch *et al.* in (1978)¹². In this study, samples were isolated from various sites in the kitchen, bathroom, living room, toilet and hall of 21 homes and the bacterial flora therein examined. Several studies followed assessing the levels of microbial contamination in the domestic kitchen and the home environment^{6, 13-16} highlighting the growing significance of the home, and the kitchen in particular, as locations for harbouring pathogens responsible for foodborne disease. Scott *et al.*'s study in 1982¹⁵ was similar to that of Finch *et al.*¹² but on a much larger scale, looking at microbial contamination in 251 domestic houses by sampling 60 sites in the bathroom, toilet and kitchen. Speirs *et al.*¹⁶ presented findings from the investigation of a wider range of sites (76 in total) in 13 domestic kitchens together with focusing on specific sites with potential for cross-contamination in a further 33 kitchens. Josephson *et al.*⁶ looked at the effectiveness of different cleaning regimes through a two year study that involved the quantification of specific bacterial pathogens and indicator organisms in 10 household kitchens in the United States in the presence, both 'casual' and 'targeted', and absence of disinfectant cleaner use. Rusin *et al.*¹⁴ examined fourteen sites in the kitchen and bathroom of 15 households over an extended period of time to determine which of these had the highest levels of contamination with faecal coliform, coliform and heterotrophic plate count (HPC) bacteria. Their study also included an investigation of the effectiveness of various cleaning and disinfection regimes using hypochlorite cleaners. Finally, Haysom¹³ recognised that previous kitchen studies only looked at samples taken from one point in time and decided to investigate the change of microbial load in domestic kitchens over a 24-hour period.

The above studies provide substantial evidence confirming the presence of viable microorganisms across various sites in domestic kitchens. However, the type of organism together

with its likelihood of contaminating food very much determine how significant this microbial contamination is in relation to human health¹⁷.

The present study was carried out to provide genetic identification of 2 of the predominant bacterial isolates previously obtained from a shared student kitchen.

MATERIALS AND METHODS

Selected Isolates

Isolate D5, previously identified as a member of the Enterobacteriaceae family.

Isolate S2, previously identified as *Pseudomonas* spp.

Bacterial DNA Extraction

Two of the bacterial isolates obtained from the student kitchen were chosen for sequencing and were sub-cultured on nutrient agar. A few bacterial colonies were aseptically removed from each plate using a sterile wire loop and homogenised in 100µl of nanopure water in a reaction tube. The reaction tubes containing the bacterial suspensions were then heated in a boiling water bath to 100°C for 10 min and then centrifuged for 10 min (10,000 x g). The resulting supernatants were used as templates for PCR (Polymerase Chain Reaction).

DNA amplification using PCR

The primers 8FPL1 (52 -GAG TTT GAT CCT GGC TCA G-32) and 806R (52 -GGA CTA CCA GGG TAT CTA AT-32) at a concentration of 5µM each were used to amplify the partial 16S rRNA gene sequences. Each PCR consisted of the following:

- 25µl Red *Taq* DNA polymerase ready mix.
- Forward and reverse primers (1µl each; 5µM).
- 18µl nanopure water.
- 5µl template DNA.

A TGradient PCR machine (Biometra, Goettingen, Germany) was used to run 35 of the following thermal cycles: 94°C (1 min), 53°C (1 min), and 72°C (1 min). A 15 minute chain elongation step (72°C) was included in the final cycle.

Agarose gel electrophoresis

The quality of the extracted DNA was assessed using 0.8% agarose gel electrophoresis. 0.4g of agarose powder was dissolved in 50ml

Table 1. Sequencing of PCR amplicons derived from two predominant isolates^a

| Isolate | Sequence length (bp) | Ambiguity | | Most closely related species (% sequence similarity) |
|---------|----------------------|-----------|-----|--|
| | | bp | % | |
| D5 | 761 | 8 | 1.1 | <i>Enterobacter cloacae</i> FR821644.1 (99) |
| S2 | 750 | 3 | 0.4 | <i>Pseudomonas</i> sp. EU573784.1 (100) |

^a Compared to published sequences, according to BLAST searches

of 1 x TAE buffer solution (Sigma-Aldrich, St. Louis, USA) by heating in a microwave. Nucleic acid stain (NBS Biologicals Ltd., Cambridgeshire, UK; 5µl) was added to the molten agarose solution and transferred into the electrophoresis tank. Each DNA sample (5µl) was loaded into the wells supplemented with a loading buffer (1 x TAE buffer solution). An additional well was filled with 5µl of a DNA ladder (HyperLadder IV; Bioline Reagents Ltd., London, UK). The gel was subjected to electrophoresis for 30 minutes and then visualised under UV light.

Sequencing and identification of isolates

The method used for partial 16S ribosomal DNA gene sequencing of isolates was adapted from McBain *et al.*¹⁸. Following purification, the amplification products were sequenced using the primers described above. FinchTV (available from <http://www.geospiza.com/Products/finchtv.shtml>) was used to read the partial 16S rDNA gene sequences, and both compiled sequences were subjected to a BLAST search against those in the EMBL nucleotide database.

RESULTS

Partial 16S ribosomal DNA gene sequencing results, based on PCR of the selected isolates D5 and S2 (previously identified), are presented in Table 1.

Gene sequencing revealed isolates D5 and S2 to be closely related to *Enterobacter cloacae* (99% sequence similarity) and *Pseudomonas* sp. (100% sequence similarity) respectively; this is in agreement with the previous identifications.

DISCUSSION

Partial 16S ribosomal DNA gene sequencing of two selected predominant isolates

was conducted in order to confirm their identities (results shown in Table. 1). The first selected isolate (D5) that was obtained from the sink drain sample, and previously identified as a species of Enterobacteriaceae by means of morphological and biochemical testing, was further identified as *Enterobacter cloacae*. This finding concurs with several other bacteriological studies of the domestic kitchen which also reported the isolation of *Enterobacter cloacae* from sink sites^{12,15,16}. The second isolate (S2) that was an oxidase positive, gram-negative rod, obtained from the sponge sample, and previously identified as *Pseudomonas* sp., was confirmed as *Pseudomonas* sp.

These gene sequencing results are therefore in agreement with the previous presumptive identifications made by means of phenotypic methods for the samples tested, and may imply that the remaining presumptive identifications were fairly accurate. This is supported by a study in 1991 by Garaizar *et al.*¹⁹ who found that there was a good agreement between genotypic and the more conventional phenotypic methods when discriminating among *Enterobacter cloacae* isolates. Despite this, the identifications made by phenotypic methods should still be interpreted with caution due to the reliance of these methods on more subjective determination in relation to cellular morphology, Gram-reaction and biochemical tests, which poses problems in terms of consistent identification²⁰.

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