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Rapid molecular methods for epidemiological investigation of food-borne outbreaks

Key Messages

1. Pulsed-field gel electrophoresis (PFGE) was used to type more than 1000 strains of salmonellae belonging to the major serotypes isolated in Hong Kong. Some strains of *S enteritidis* probably belonged to a few clusters, whereas almost all strains of the other serotypes tested were different and due to sporadic spread. ERIC and BOX typing may be used as quick alternatives to PFGE in discriminating strains.
2. A total of 128 strains of *Shigella flexneri* and *S sonnei* were typed by PFGE, ERIC and BOX typing. All except 22 strains of *S sonnei* belonged to a heterogeneous population.

Introduction

Salmonellae and shigellae are important causes of diarrhoeal disease in Hong Kong, the former being the most common bacterial pathogens isolated from stool.[1-4] They have the potential to cause outbreaks, as they are readily transmissible via contaminated food or drinks as well as the faecal-oral route. Patients who have recovered may carry the organism for extended periods during which they act as a source of infection, especially if they are food handlers. This can lead to grave consequences in terms of public health and health care burden.

To prevent such outbreaks, prompt identification of sources of infection, in addition to strict adherence to infection control practices is required. Microbiological investigation of diarrhoeal cases is routinely performed by culture, identification and typing of the causative organisms. Various methods involving characterising bacterial DNA or protein have been developed, but no single technique is considered definitive. Thus, at least two methods should be used and results collated. Bacterial evolution leads to minor divergence among strains of the same outbreak, rather than novel genetic changes. Thus, typing results of strains isolated within a short period should not be affected by bacterial evolution.

We aimed to develop discriminatory, simple, quick and economical methods for genotyping of *Salmonella* and *Shigella* species for routine infection control. We also aimed to optimise and evaluate these methods by investigating both genuses isolated during 1990-2004.

Methods

This study was conducted from September 2005 to August 2007. All surviving *Salmonella* serotypes with >50 strains isolated during 1990-2004 from hospitals in the New Territories East Cluster, Hong Kong SAR were tested. Some serotypes that were isolated in smaller numbers were randomly selected for testing. All surviving *Shigella* stains of *S flexneri* and *S sonnei* isolated during 1996-2004 were also tested. Strains of *S typhimurium*, *S enteritidis*, *S derby*, *S typhi*, *S flexneri* and *S sonnei* that were tested in previous studies were not tested. The tested strains were subjected to pulsed-field gel electrophoresis (PFGE),[5] and then randomised for variable number tandem repeats (VNTR) typing[6] (using EXCEL gels to separate amplification products), multilocus sequence typing (MLST),[7] rDNA spacer region typing,[8] ERIC-PCR typing,[9] BOX typing,[9] IS200 typing (for salmonellae only),[10] and amplified fragment length polymorphism (AFLP)[11] analysis.

Results

Salmonella sp

Out of the 2234 strains of salmonellae isolated during 1990-2004, 1155 surviving strains belonging to 24 serotypes were tested, giving a survival rate of 52%. All strains were subjected to PFGE, a method regarded as the gold standard to which results of other typing methods are compared. The 1155 strains (representing 55% of the total number of strains isolated) were pulsotyped using *Xba*I-digested

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DNA. More than 50% of the strains of each serotype were tested except for *S anatum*, *S derby*, *S newport* and *S stanley*. A total of 49 strains of *S flexneri* and 97 of *S sonnei* were tested.

S enteritidis, *S typhimurium* and *S derby* were the three most common *Salmonella* serotypes isolated. We only studied strains of *S enteritidis* and *S typhimurium* isolated during 1997-2004, strains of *S derby* isolated during 1995-2004, and strains of *S typhi* isolated during 1998-2004. Those isolated outside these periods had been tested in previous studies. For the remaining 21 serotypes, strains isolated during 1990-2004 were tested.

The isolates were from 565 male and 599 female patients (male:female=1:1.06); 41% of the patients were aged ≤1 year, 13% were aged 2-9 years, 25% were aged 10-39 years, and 21% were aged 40-99 years. During the 5 months from June to October, ≥10% of isolates were obtained each month, constituting 57% of isolates. Isolates obtained during the other 7 months ranged from 3% (February) to 9% (May).

Each strain with a unique PFGE banding pattern was assigned an individual pattern. The patterns were analysed, using the BioNumerics software (Applied Maths, Saint-Martens-Latem, Belgium), by cluster analysis using the Dice coefficient for band matching with 1.0% position tolerance and an unweighted pair group method with an averaging algorithm. Patterns with ≥90% similarity were placed in the same group. *S typhimurium*, *S enteritidis*, *S derby* and *S london* were then tested by VNTR typing. The bands differed by approximately 6 bp in size and could only be discerned by separating on EXCEL gels. However, no difference in banding patterns was observed in all the tested strains. For MLST typing, the same banding patterns were obtained using different primers[7] for all *S typhimurium*, *S enteritidis*, *S derby* and *S london* strains tested. For rDNA spacer region typing, the same strains of *S typhimurium*, *S enteritidis*, *S derby* and *S london* were typed. Similarly, all banding patterns were the same. ERIC and BOX typing were applied in the typing of 8-90 each of the 12 serotypes tested. Patterns obtained using both methods were combined and analysed. Almost all banding patterns were distinctly different and were comparable to those of PFGE. However, patterns with ≥90% similarity should not be grouped since the differences were too small. Grouping these patterns together would give a false impression of relatedness of the strains. Both IS200 typing and AFLP analysis failed to give discriminatory banding patterns on the tested strains.

Shigella sp

Of the 241 strains of *S flexneri* and *S sonnei* obtained during 1996-2004, 88 strains survived and were tested, giving a survival rate of 37%. Both *Shigella* sp were isolated throughout the year with no preponderance during any particular season. All except two of the 40 *S flexneri* strains tested belonged to an individual PFGE pattern. Eight of these patterns could be grouped into four groups each with two patterns that were ≥90% similar, whereas there were nine patterns that could be grouped into two groups each with four and five similar patterns in one group. Similarly, all except two strains of *S sonnei* belonged to a distinct PFGE pattern. While 42 strains could be grouped into 11 groups, there was one group that comprised 22 strains. ERIC and BOX typing also revealed that strains of both *Shigella* sp were heterogeneous.

Discussion

Only about 50% of *Salmonella* and *Shigella* isolates survived for testing. The survival rate was low, as isolated strains were stored on agar slants in bijoux bottles and could dry up if the screw caps were not tightly replaced or the bottles misplaced.

S enteritidis, *S typhimurium* and *S derby* remained the most common *Salmonella* serotypes isolated, with approximately 300-1000 single patient strains isolated during 1990-2004. These were followed by *S london*, *S anatum* and *S blockley* with >100 strains isolated during the same period. The other serotypes had <100 strains isolated.

Using PFGE, we were able to reveal the diverse heterogeneity of strains of the more common *Salmonella* serotypes as well as *S flexneri* and *S sonnei*. Almost all the strains of each *Salmonella* serotypes and the two *Shigella* sp were quite distinct, as inferred from their different PFGE patterns. Thus, we most likely experienced sporadic salmonella and shigella infections in the community. Probably, there were a few clusters of *S enteritidis*, *S flexneri* and *S sonnei* circulating, eg, one cluster comprising 15 *S enteritidis* strains that belonged to 10 individual patterns that were ≥90% related, and one cluster comprising 22 *S sonnei* strains that belonged to 20 patterns with ≥90% similarity.

It was disappointing to find that typing methods such as VNTR typing, MLST typing, rDNA spacer region typing, IS200 typing, and AFLP were not useful in distinguishing our strains, as almost all showed the same banding patterns. ERIC and BOX typing were probably satisfactory for discriminating individual strains, but their use in determining strain similarities was questionable. ERIC and BOX typing might be a rapid alternative to PFGE in typing salmonellae or shigellae, as they could provide results within the same day and were more economical and much less technically demanding than PFGE.

However, no two typing methods could provide the same results. Although PFGE was regarded as the gold standard, it could only detect differences within the fragments containing the restriction sites of the digesting enzymes, whereas ERIC and BOX typing could only detect differences in regions between the primers. Thus, at least two methods should be used and the results combined in

order to obtain more reliable interpretation.

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