Retrospective use of whole genome sequencing to better understand an outbreak of \textit{Salmonella enterica} serovar Mbandaka in New South Wales, Australia

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**Introduction:** \textit{Salmonella enterica} serovar Mbandaka is an infrequent cause of salmonellosis in New South Wales (NSW) with an average of 17 cases reported annually. This study examined the added value of whole genome sequencing (WGS) for investigating a non-point source outbreak of \textit{Salmonella} ser. Mbandaka with limited geographical spread.

**Methods:** In February 2016, an increase in \textit{Salmonella} ser. Mbandaka was noted in New South Wales, and an investigation was initiated. A WGS study was conducted three months after the initial investigation, analysing the outbreak \textit{Salmonella} ser. Mbandaka isolates along with 17 human and non-human reference strains from 2010 to 2015.

**Results:** WGS analysis distinguished the original outbreak cases ($n = 29$) into two main clusters: Cluster A ($n = 11$) and Cluster B ($n = 6$); there were also 12 sporadic cases. Reanalysis of food consumption histories of cases by WGS cluster provided additional specificity when assessing associations.

**Discussion:** WGS has been widely acknowledged as a promising high-resolution typing tool for enteric pathogens. This study was one of the first to apply WGS to a geographically limited cluster of salmonellosis in Australia. WGS clearly distinguished the outbreak cases into distinct clusters, demonstrating its potential value for use in real time to support non-point source foodborne disease outbreaks of limited geographical spread.

\textit{Salmonella enterica} serovar Mbandaka is a relatively uncommon \textit{Salmonella} serovar in New South Wales (NSW) with an average of 17 cases notified per year over the past 10 years.\textsuperscript{1} \textit{Salmonella} ser. Mbandaka cases reported in Australia have been acquired locally and overseas in India, Africa, Indonesia, Mexico and China.\textsuperscript{2} In Australia, \textit{Salmonella} ser. Mbandaka has been isolated from foods such as chicken, peanut butter, turkey meat and curry powder.\textsuperscript{2} Whole genome sequencing (WGS) is a high-resolution typing method that can help foodborne disease investigators distinguish outbreak cases from non-outbreak cases.\textsuperscript{3} WGS has been used for public health surveillance in the United States of America, United Kingdom of Great Britain and Northern Ireland, and the European Union.\textsuperscript{4-6} In Australia, several jurisdictional reference laboratories are developing WGS capacity and evaluating its utility for routine surveillance of enteric pathogens.\textsuperscript{7} This study examined the potential added value of WGS in assisting investigators identify the source of a community outbreak of \textit{Salmonella} ser. Mbandaka with limited geographical spread.

**METHODS**

In February 2016, an increase in \textit{Salmonella} ser. Mbandaka notifications was noted in the Hunter New England and Central Coast local health districts of NSW. A confirmed case was defined as any resident or visitor to NSW with laboratory-confirmed \textit{Salmonella} ser. Mbandaka infection and symptom onset from 1 January 2016 to 30 April 2016. Individuals meeting the case definition were interviewed by phone, beginning 22 February 2016, using a standard \textit{Salmonella} hypothesis-generating questionnaire to collect demographic, clinical and risk factor information, including travel and food consumption histories during the seven days before illness onset. For

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The food consumption histories were reanalysed based on the clusters identified by WGS and compared to the data from the 2016 Victorian Food Consumption study. Data were entered and analysed in EpiInfo (Version 7) and Microsoft Excel.

Ethics statement

This work was part of an outbreak investigation and did not require ethical review and oversight by a Human Research Ethics Committee.

RESULTS

From 1 January 2016 to 30 April 2016, 29 cases of salmonellosis caused by Salmonella ser. Mbandaka were notified. The epidemic curve of cases investigated as part of this outbreak is shown in Fig. 1. Illness onset dates ranged from 15 January to 14 April 2016. Seven case patients were hospitalized and no patients died. Patients were aged from 1 to 89 years with a median age of 48 years, 14 (48%) lived in the Hunter New England Local Health District, 16 (55%) were male and three (10%) were of Aboriginal origin. Commonly reported symptoms included diarrhoea (n = 21, 95%), lethargy (n = 17, 85%), abdominal pain (n = 14, 64%), fever (n = 13, 62%) and vomiting (n = 12, 55%). Symptoms continued for 1–10 days (median five days).

The initial (pre-WGS) investigation did not identify any common eating establishments or shopping venues among cases. Processed cheese was identified to have a higher-than-expected consumption frequency among cases; it was consumed by 64% of cases when the expected consumption frequencies in a healthy population was 22% (binomial probability P = 0.0008). However, on closer analysis, several different brands of processed cheese and places of purchase were indicated, and in the absence of additional cases, no food safety investigation was initiated. Other foods with a higher-than-expected consumption frequency included watermelon (63%, P = 0.0341, P = 0.04), onion (69%, P = 0.0574, P = 0.07) and green capsicum (53%, P = 0.0599, P = 0.07).

WGS analysis distinguished the original outbreak cases into two main clusters: Cluster A, which included 11 cases with an SNP distance between 12 and 82, and Cluster B, which included six cases with an SNP distance...
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**DISCUSSION**

Internationally, WGS is increasingly being used for enhanced foodborne disease surveillance and response due to its discrimination power for typing cases and tracing infection sources and its similar turnaround times to other laboratory techniques.\(^5\)\(^6\) WGS is also being used to understand disease transmission pathways and determinates of transmission, monitor pathogen evolution and adaptation, identify infections with epidemic potential and refine control strategies.\(^9\)

In the United States, WGS is replacing pulsed-field gel electrophoresis for subtyping foodborne pathogens for outbreak surveillance.\(^10\) WGS of foodborne pathogens is used for regulatory purposes by the US Food and Drug Administration\(^5\) and has proven valuable in outbreak investigations for differentiating sources of contamination.\(^11\)\(^12\) In European Union countries and in the United Kingdom, WGS is increasingly being used for foodborne disease outbreak investigations and national surveillance of infectious diseases.\(^4\)\(^6\) In Australia, WGS is acknowledged as a promising typing alternative; however, it is not yet in widespread use due to limitations in standardized quality control and data interpretation, cost and infrastructure.\(^13\)\(^14\) WGS is being piloted by OzFoodNet, an Australian Department of Health foodborne disease surveillance and response network, and has been successfully applied in multijurisdictional foodborne disease outbreaks and for routine surveillance of *Listeria monocytogenes*.\(^3\)\(^15\)

This *Salmonella* ser. Mbandaka study was one of the first in Australia to apply WGS to a geographically limited cluster of *Salmonella*. Although the WGS was not conducted in real time, its potential to support an outbreak investigation was demonstrated. WGS was able to differentiate the outbreak cases of *Salmonella* ser. Mbandaka into distinct clusters and sporadic cases. Analysis of food consumption histories based on
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Fig. 2. Phylogenetic tree generated from whole-genome SNPs for outbreak and non-outbreak cases of Salmonella ser. Mbandaka in NSW

phylogenetic cluster suggests two concurrent outbreaks of Salmonella ser. Mbandaka may have occurred in NSW. If WGS had been conducted in real time, affected individuals would have been reinterviewed to collect additional details on food items of interest and further analysis conducted. Our findings support an earlier study in NSW that applied WGS retrospectively to five epidemiologically confirmed community outbreaks of Salmonella enterica serovar Typhimurium and found that WGS significantly increased the resolution of investigations. Their study also found that for one of the outbreaks, the food source was contaminated with more than one strain of Salmonella ser. Typhimurium, highlighting the need to assess both laboratory and epidemiological information during an investigation.

Data from the Victorian Food Consumption study allowed investigators to estimate expected food consumption frequencies in a healthy population and, using binomial probabilities, compare them to the food consumption frequencies among the outbreak cases. This method allows for rapid hypothesis generation to guide further environmental and epidemiological investigations. The absence of an equivalent NSW food consumption data set was a limitation of this study. It was assumed that food consumption habits and available foods in Victoria and NSW were similar enough to permit hypothesis generation. Given the potential for differences in food habits or food availability between the two populations, the associations derived need to be interpreted with caution and used for hypothesis generating rather than testing. The rapid development in advanced laboratory tools also presents challenges for public health practitioners. As public health reference laboratories have been adopting WGS, clinical laboratories are increasingly relying on culture-independent multiplexed molecular panels to test
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In conclusion, this study highlighted the potential value of WGS in supporting epidemiologists to investigate a relatively small, non-point source foodborne disease outbreak in a community. If conducted in real time, WGS could have assisted with potential source detection to guide further investigations and to aid control efforts. The continued application of WGS to support foodborne disease outbreak investigations in Australia will contribute to a global understanding of its potential to control outbreaks in a more timely and efficient manner.

**Conflicts of interest**

None.

**Funding information**

None.

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Table 1. Food consumption frequencies among all cases and by key clusters identified by WGS

| Food Item     | All cases | WGS – Cluster A | WGS – Cluster B | Reference* |
|---------------|-----------|-----------------|-----------------|------------|
|               | Ate food  | Total %         | Ate food Total %| Ate food Total %| Reference* |
| Tomato        | 11 13     | 85%             | 9 9 100%        | 2 3 67%    | 497 665 75% |
| Carrot        | 12 16     | 75%             | 9 10 90%        | 3 4 75%    | 534 662 81% |
| Potato        | 10 14     | 71%             | 9 9 100%        | 1 3 33%**  | 546 667 82% |
| Onion         | 11 16     | 69%             | 8 10 80%**      | 3 4 75%    | 307 666 46% |
| Chicken pieces| 11 16     | 69%             | 6 10 60%        | 3 4 75%    | 406 666 61% |
| Black pepper  | 11 16     | 69%             | 8 10 80%        | 3 4 75%    | 427 666 64% |
| Processed cheese | 9 14     | 64%**           | 8 9 89%**       | 1 3 33%    | 149 665 22% |
| Free range eggs| 7 11      | 64%             | 4 6 67%         | 2 3 67%    | 291 446 65% |
| Eggs (any)    | 7 11      | 64%             | 7 9 78%         | 3 4 75%    | 446 665 67% |
| Watermelon    | 10 16     | 63%**           | 8 10 80%**      | 2 4 50%    | 245 667 37% |
| Apple         | 10 16     | 63%             | 6 10 60%        | 3 4 75%    | 446 667 67% |
| Banana        | 10 16     | 63%             | 6 10 60%        | 3 4 75%    | 464 667 70% |
| Beef mince    | 8 14      | 57%             | 7 8 88%**       | 1 4 25%    | 332 663 50% |
| Green capsicum| 8 15      | 53%             | 6 10 66%**      | 2 4 50%    | 209 667 31% |
| Grapes        | 8 15      | 53%             | 4 9 44%         | 3 4 75%    | 375 666 56% |
| Red capsicum  | 8 15      | 53%             | 6 9 67%         | 2 4 50%    | 304 667 46% |
| Broccoli      | 8 15      | 53%             | 6 9 67%         | 2 4 50%    | 348 665 52% |
| Cucumber      | 8 15      | 53%             | 5 9 56%         | 3 4 75%    | 383 665 58% |

* Reference = data from 2016 Victorian Food Consumption study

** Difference from reference statistically significant (< 0.05)

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stool specimens for enteric pathogens. The move away from culturing enteric pathogens will reduce the number of isolates available for typing by WGS or other culture-dependent typing methods. In response, scientists are working to develop metagenomic sequencing-based tools to characterize stool specimens without the need for culture. As these developments continue to evolve, health practitioners will need to understand how they will impact surveillance systems, outbreak detection and response activities.

In conclusion, this study highlighted the potential value of WGS in supporting epidemiologists to investigate a relatively small, non-point source foodborne disease outbreak in a community. If conducted in real time, WGS could have assisted with potential source detection to guide further investigations and to aid control efforts. The continued application of WGS to support foodborne disease outbreak investigations in Australia will contribute to a global understanding of its potential to control outbreaks in a more timely and efficient manner.
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