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The use of whole-genome sequencing to investigate a foodborne-associated outbreak in a mental healthcare institution

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Summary

Salmonella enterica serotype Enteritidis (*Salmonella* Enteritidis) is a cause of foodborne disease outbreaks globally. Outbreaks have been reported in South Africa. Whole-genome sequencing (WGS) has revolutionised the investigation of foodborne-associated outbreaks by identifying the principal sources of the outbreaks. We describe the investigation of a *Salmonella* Enteritidis outbreak that was reported in July 2024 from a mental health institution in Gauteng, South Africa. The Centre for Enteric Diseases of the National Institute for Communicable Diseases, a division of the National Health Laboratory Service, received 18 bacterial isolates that included those from food retention samples, a food handler, and symptomatic patients. These isolates were confirmed as *Salmonella* Enteritidis by standard phenotypic serotyping methods. WGS was performed using Illumina NextSeq technology. Core-genome multilocus sequence typing (cgMLST) of 3 002 genes was used to identify genetic relatedness of the isolates. Serotyping identified all 18 isolates as *Salmonella* Enteritidis, and WGS confirmed this finding. Analysis of the WGS data using cgMLST showed that all isolates associated with the outbreak had ≤2 allele differences, indicating highly genetically related *Salmonella* Enteritidis strains. WGS did not identify any genetic determinants associated with antimicrobial resistance. We concluded that the pathogen associated with the foodborne disease outbreak was *Salmonella* Enteritidis. Epidemiological investigation suggests that the likely source was contamination of the food by the food handler. Our recommendation was that the mental healthcare institution enhance its food hygiene practices, including general cleanliness, storing foods appropriately, and stricter hand hygiene.

Introduction

Non-typhoidal *Salmonella* (NTS) is a well-known cause of foodborne diseases globally. According to the World Health Organization (WHO), unsafe food causes 600 million cases of foodborne diseases worldwide annually. Furthermore, the WHO reports that the largest burden of NTS-associated foodborne disease is in the African region; however, this is under-reported in literature.[1] *Salmonella enterica* serovar Enteritidis (*Salmonella* Enteritidis) remains the most common NTS serotype associated with foodborne disease outbreaks in the European Union and the USA.[2]

In 2011, South Africa reported that *Salmonella* Enteritidis replaced *Salmonella* Typhimurium as the predominant cause of Salmonellosis in the country.[3] Currently, two serovars account for ~71% of all clinical *Salmonella* isolates in South Africa (*Salmonella* Enteritidis; ~53% and *Salmonella* Typhimurium; ~18%).[4]

Although symptoms of *Salmonella* Enteritidis infection are relatively mild, in children and elderly patients, the infection is associated with severe dehydration and can become life-threatening. *Salmonella* Enteritidis causes invasive and non-invasive disease and significantly impacts morbidity and mortality in low- and middle-income countries (LMICs).[5]

Whole-genome sequencing (WGS) is increasingly used globally as a tool to investigate foodborne disease outbreaks.[6] In 2017, the Centre for Enteric Diseases (CED) of the National Institute for Communicable Diseases (NICD), a division of the National Health Laboratory Service, implemented the use of WGS analysis of diarrhoeal disease-associated bacterial pathogens to assist in food- and waterborne disease outbreak investigations. Since then, three outbreaks caused by *Salmonella* Enteritidis at a variety of institutions (day-care centre, hospital, and restaurant) in South Africa have been reported. In these reports, WGS was used as a diagnostic tool.[6,7]

In South Africa, a foodborne illness outbreak is a category 1 notifiable medical condition and must be notified to public health authorities (i.e., the National Department of Health) within 24 hours. The case definition of a foodborne illness outbreak is "any food poisoning incident involving two or more individuals that are epidemiologically linked to a common food or beverage source". [8]

Here we describe a foodborne disease outbreak of *Salmonella* Enteritidis in a mental healthcare institution in the Gauteng province, South Africa, in July 2024, where WGS analysis was pivotal in the investigation. To the best of our knowledge, this is the first report on the African continent where *Salmonella* Enteritidis was investigated and confirmed, using WGS, for isolates obtained from food retention samples, a food handler, and symptomatic patients from an institution. This publication aims to increase the number of reports of foodborne disease outbreaks on the African continent to emphasise the importance of food safety practices and to highlight the usefulness of WGS in investigating outbreaks and identifying the source of outbreaks.

Methods

Study setting

On 30 July 2024, the CED was notified by the respective provincial surveillance team of a potential foodborne disease outbreak at a mental healthcare institution in Gauteng. Patients from the institution presented to a private hospital with similar gastrointestinal symptoms that occurred over a similar date of onset.

Study design

This is a descriptive analysis of the outbreak investigation.

Epidemiological investigations

An epidemiological investigation was undertaken by the respective district surveillance team. The team visited both the mental health institution and the private hospital to gather further epidemiological information. This included a thorough history from patients, staff, and food handlers with respect to food consumption, time of symptom onset, symptoms experienced, and travel history. Standardised foodborne disease outbreak case investigation forms (CIFs) were completed and submitted to the CED, together with a line list of 27 symptomatic patients, which included staff and patients. Clinical (stool specimens or rectal swabs), environmental, and food retention samples were collected and submitted to both public and private

laboratories. Available food retention samples from before the onset of symptoms were collected and sent to a private laboratory for testing. At the time, the CED advised that collection of clinical samples should include those from food handlers and symptomatic patients only.

Statistical analysis

The line list and available CIFs were combined and captured on Microsoft Excel. Descriptive analyses were conducted using R version 4.4.1 (R Foundation for Statistical Computing, Vienna, Austria). The epidemic curve and bar graphs were constructed using Microsoft Excel.

Laboratory investigations

Stool specimens or rectal swabs were initially processed at peripheral laboratories using standard microbiological methods. Cultured *Salmonella* isolates were then referred to the CED, where confirmation of all isolates was performed by standard serotyping techniques using the White-Kauffmann-Le Minor scheme.[9] Genomic DNA was extracted from cultures using an Invitrogen PureLink Microbiome DNA Purification Kit (Invitrogen, Waltham, Massachusetts, USA). WGS was performed using the Illumina NextSeq 2000, with DNA libraries prepared using the Illumina DNA Prep Kit with an insert size of 300-400 bp, followed by 2 x 150 paired-end sequencing runs with ~80x coverage. Raw reads were analysed using the JEKESA bioinformatics pipeline version 1.0 (https://github.com/stanikae/jekesa) which incorporates multiple bioinformatics analysis tools. Raw reads were also uploaded and further investigated at the EnteroBase web-based platform (http://enterobase.warwick.ac.uk/species/index/senterica). EnteroBase analysis included serotype confirmation using various genomic serotyping tools and a genomic comparison of isolates based on core-genome multilocus sequence typing (cgMLST) data, using the 'cgMLST V2 + HierCC V1' scheme. Phylogenetic cluster analysis of cgMLST data was depicted using a GrapeTree-generated minimum spanning tree using the EnteroBase 'MSTree V2' algorithm. cgMLST was used to investigate the phylogeny of the *Salmonella* Enteritidis isolates so clusters could be identified.

Availability of sequencing data

All WGS data were uploaded to the public EnteroBase platform (http://enterobase.warwick.ac.uk/species/index/senterica) and are freely available. In addition, sequencing data were deposited into the European Nucleotide Archive under the project accession number PRJEB39546.

Results

Epidemiology

A line list of 27 symptomatic individuals was provided to the CED. Twenty-one of the 27 symptomatic individuals were patients residing at the mental healthcare institution, and six were staff members. The staff members consisted of one food handler, three nursing staff, one cleaner, and one security guard. The reported dates of illness onset were from 28–31 July 2024. Two patients presented with onset of symptoms on 28 July 2024, with the majority presenting thereafter (29–31 July 2024) (Figure 1). Importantly, the food handler reported a history of gastrointestinal symptoms prior to 28 July 2024 but continued to prepare meals at the institution despite being symptomatic. Eight of 27 (30%) patients gave a clear history of food consumption the day before symptom onset. The types of food consumed varied, but the majority ate a combination of eggs, chicken, roast beef, roast vegetables, pork, and rice.

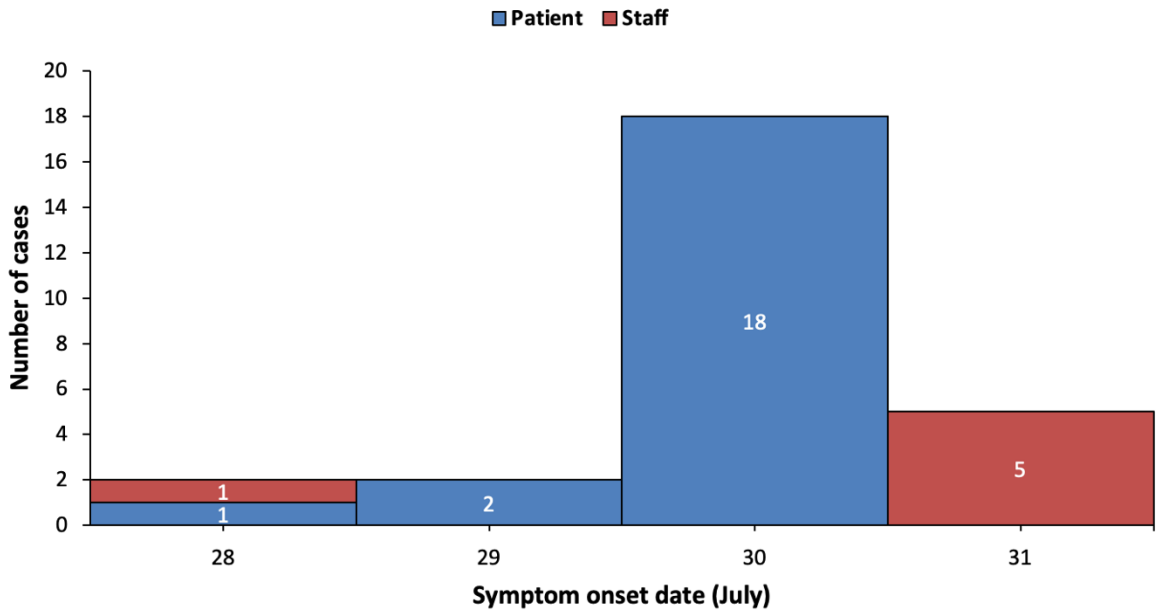

Figure 1. Gastroenteritis cases by date of symptom onset at a mental healthcare institution in the Gauteng province, South Africa, July 2024 (N=27).

Patients' ages ranged from 18–63 years with a median age of 46 years (Figure 2). The majority of cases were female (20/27; 74%). Twenty-three patients were taken to private hospitals and general practitioners and presented with one or more of the following symptoms: diarrhoea, vomiting, abdominal cramps, joint pain, headache, syncope, and generalised body aches. Diarrhoea (22/26; 85%), abdominal cramps (8/26; 31%), and vomiting (6/26; 23%) were the most common symptoms. A total of 19 clinical specimens was collected from 27 symptomatic patients and processed at peripheral laboratories. Ten patients were admitted, treated with intravenous antibiotics, and subsequently discharged. No deaths were reported.

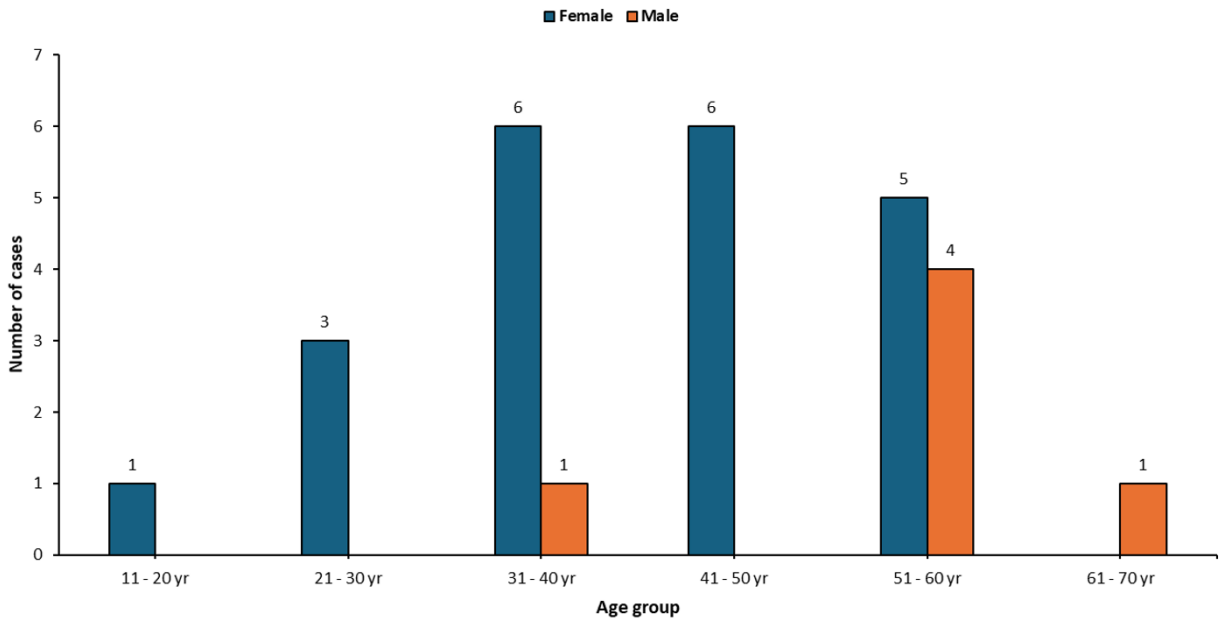

Figure 2. Age and sex distribution of gastroenteritis cases at a mental healthcare institution in the Gauteng province, South Africa (N=27).

Laboratory tests

Sixteen (16/19; 84%) tested positive for *Salmonella* spp. on culture, while three were negative for *Salmonella* spp. (Figure 3). Of the food retention samples tested at the private laboratory, two were positive for *Salmonella* spp.; one from a roast beef dish and one from a roast vegetable dish. Subsequently, the CED received 18 *Salmonella spp*. isolates from referring laboratories. These included two isolates from food retention samples, one isolate from a food handler, and 15 isolates from patients at the mental healthcare institution.

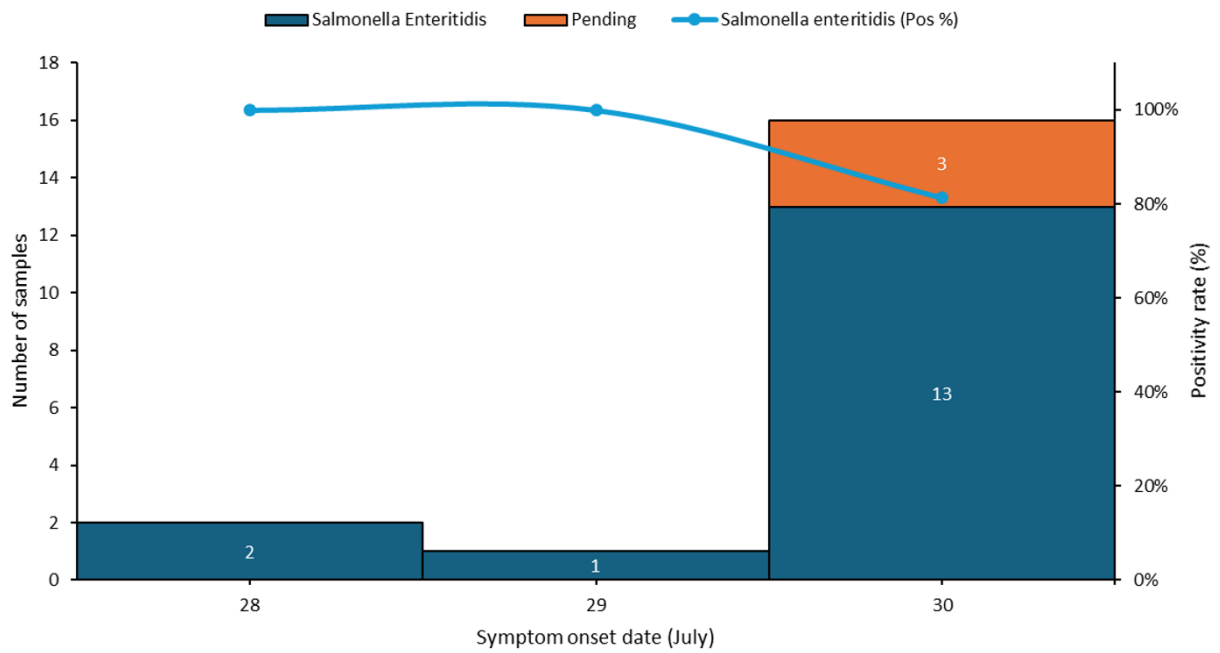

Figure 3. *Salmonella* Enteritidis positivity rate of specimens tested from a gastroenteritis outbreak at a mental healthcare institution in the Gauteng province, South Africa (n=19). Food isolates are not included because here we show patient symptom onset in relation to the number of positive samples.

The CED confirmed all isolates as *Salmonella* Enteritidis with standard serotyping techniques. Core genome multilocus sequence typing showed that all isolates associated with the outbreak showed ≤2 allele differences, indicating highly related *Salmonella* Enteritidis strains (Figure 4). No genes known to be associated with antimicrobial resistance were identified on WGS, and therefore all isolates were considered to be susceptible to all antibiotics.

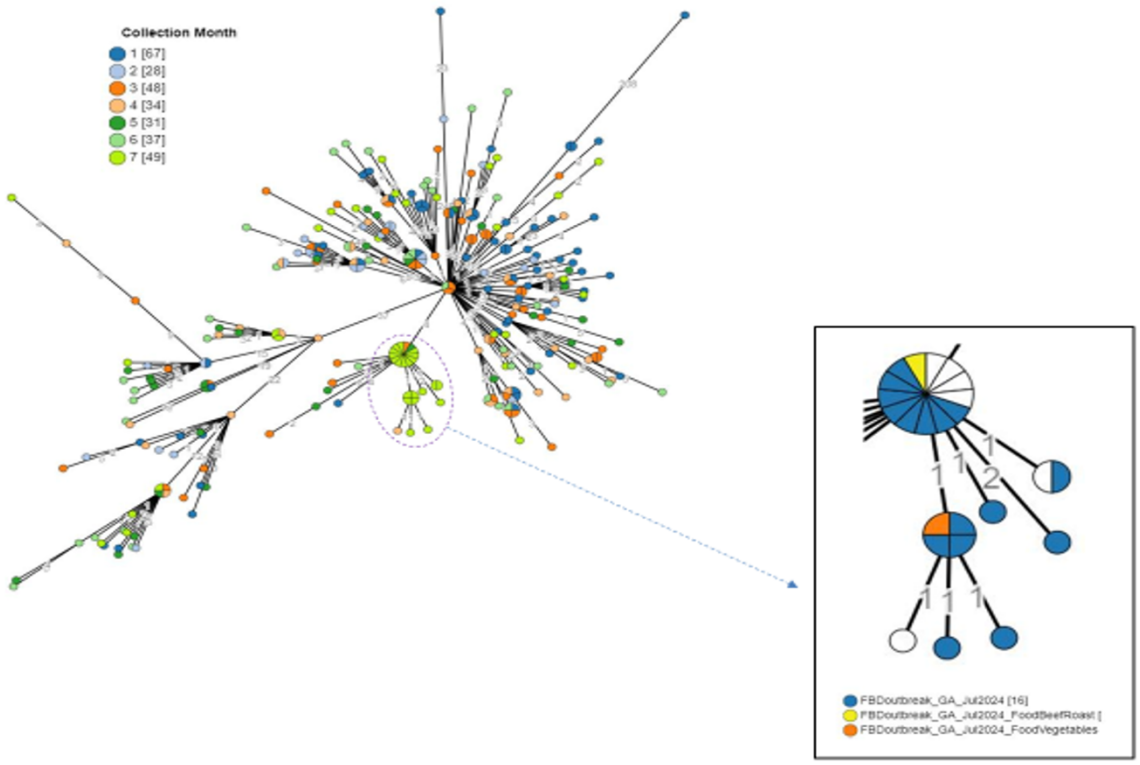

Figure 4. Minimum spanning tree drawn using cgMLST data from *Salmonella* Enteritidis isolates were sourced from the Gauteng province, South Africa, January–July 2024, as part of routine national non-typhoidal *Salmonella* (NTS*)* surveillance. The isolates highlighted in the box were sourced from the gastroenteritis outbreak at a mental healthcare institution in Gauteng and were compared to other *Salmonella* Enteritidis isolates collected as part of routine NTS surveillance from Gauteng between January–July 2024. The circular nodes represent isolate(s). The larger circular node indicates that a greater number of isolates are genetically related. The segments within a circular node are indicative of the number of isolates. Isolates positioned within the same circular node showed no allelic differences when comparing one isolate against another. The number values between adjacent nodes indicate the number of allele differences between connecting nodes (isolates). The highly related *Salmonella* Enteritidis strains on all outbreak-associated isolates are indicated.

Discussion

Salmonella Enteritidis is a well-described source of foodborne disease outbreaks associated with eggs and poultry worldwide.[10–12] The use of WGS has revolutionised the investigation of foodborne disease outbreaks, as well as strengthened the capacity of surveillance systems to rapidly detect epidemiologically linked cases that are difficult to identify, e.g., in international outbreaks that have been linked to a common food source.[10] In 2014, the largest European multi-county outbreak of *Salmonella* Enteritidis was linked to egg producers in Bavaria, Germany. Soon after, in 2015, a salmonellosis outbreak was reported in the United Kingdom, and through the use of WGS, these *Salmonella* Enteritidis isolates were phylogenetically linked to isolates found in Spain, and subsequently, all the cases were linked to chicken eggs.[10,11] In South Africa, following the introduction of WGS as a surveillance tool, three foodborne disease outbreaks caused by *Salmonella* Enteritidis at various institutions (a day-care centre, a hospital, and a restaurant) have been investigated using WGS and reported in the literature.[6,7]

It is becoming increasingly important to identify the principal sources of foodborne disease outbreaks to interrupt the transmission and reduce the burden of disease globally.[13] Food handlers play a crucial role in the spread of *Salmonella,* as they are responsible for food preparation and serving. A food handler is defined as "any person who directly handles packaged or unpackaged food, food equipment and utensils, or food contact surfaces, and is therefore expected to comply with food hygiene requirements".[14] Studies have shown that food handlers can harbour intestinal parasites, *Salmonella spp.,* and *Shigella spp*., and can therefore contaminate food by physical contact where hand hygiene is not strictly adhered to.[13] Food handlers can also cross-contaminate foods by allowing contact of raw foods with cooked foods. It is therefore imperative that food handlers have mandatory food safety training and protocols as a way to prevent foodborne diseases.[14]

The foodborne disease outbreak that occurred in the mental healthcare institution over the period 28–31 July 2024 was reported to the relevant authorities timeously. *Salmonella* Enteritidis was identified as the cause of the diarrhoeal disease outbreak, as it was isolated from patients, food samples, and a food handler. Genetic relatedness of the isolates analysed by cgMLST confirmed that all isolates from the outbreak showed ≤2 allele differences, indicating that all 18 isolates were genetically closely related. Both staff members and patients at the institution were symptomatic, indicating that common contaminated food sources were circulating within the institution. Interestingly, the food handler reported symptoms of diarrhoeal disease before 28 July 2024 but continued to prepare food at the institution despite being symptomatic. We hypothesised that the food handler was the probable source of the outbreak. The isolation of *Salmonella* Enteritidis from both the roast beef and roast vegetable dishes supports this hypothesis because this organism is mostly associated with outbreaks related to eggs, egg products, and poultry, and is rarely reported as a cause of foodborne illness associated with beef or vegetable dishes.[9] Therefore, initial contamination by the food handler probably resulted in the roast beef and roast vegetables being contaminated. Furthermore, both dishes had been served the evening before the onset of symptoms of the first patient, which is in keeping with an incubation period of six to 72 hours for *Salmonella* Enteritidis. The CED therefore recommended that

the institution enhance its food hygiene practices, including general cleanliness, storing foods appropriately, and hand washing.

There are some limitations in our study. Institutional specimens were not processed for all patients; therefore, fewer isolates were available for WGS. There was limited epidemiological information available for staff members of the institution. Additionally, no information on environmental assessment was available to us; however, recommendations can still be made regarding general food safety practices.

Conclusion

Using WGS, our investigation showed that the foodborne disease outbreak at the mental healthcare institution was caused by a highly related strain of *Salmonella* Enteritidis. The food handler was strongly implicated as a potential source of the outbreak.

Recommendations

- The management of care institutions should provide proper training to professional food handlers and encourage them to be vigilant while preparing food and enforce that they must observe hygienic rules of food preparation.
- Any food handler who suffers from fever, diarrhoea, vomiting, or any visible infected skin lesions must report this to their employer immediately. Employers must protect employee confidentiality and prevent discrimination or loss of income related to illness reporting.
- Institutions and other healthcare personnel must report foodborne illness outbreaks to increase the accuracy of the burden of disease, thereby raising awareness on foodborne disease outbreaks and food safety.
- Institutions must emphasise that the WHO's *five keys to safer food* serve as the basis for food handlers:
    - Keep clean (this includes hands, surfaces, and utensils);
    - Separate raw and cooked (keep raw meat and poultry separate to avoid cross-contamination);
    - Cook thoroughly (cooking food at the right temperature kills harmful bacteria);
    - Keep food at safe temperatures (refrigerate perishable foods); and
    - Use safe water and raw materials.

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Ethical considerations

Ethics Clearance certificate no. M230985; Human Research Ethics Committee; University of the Witwatersrand, Johannesburg.

Conflicts of interest

The authors declare no competing interests.

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