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Original Research Article

Prevalence of *Salmonella typhimurium* infection related to street food consumption

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A B S T R A C T

A panel of Microbiologists investigated an outbreak of gastroenteritis with a local street food in April-May 2012. The infecting agent was *Salmonella typhimurium*. A case control study was conducted to identify the source of infection. The food materials served also took into version for the presence of *S. typhimurium*. A total of 24 cases and 6 controls were recruited to take part in this study. The major constraint of this study was the small sample size and small number of controls and selective food samples. Despite this, a strong association with illness and consumption of those street side foods was detected and this was further supported by environmental indication. The investigation concluded that the cause of the infection was putatively foods available in street side including coconut chutney and Kurma either on their own or as an ingredient used for preparation. The workers those are working the street hotels are also accountable for this infection spread. The nail cuts and stool samples from the workers also supported the presence of *S. typhimurium* by culturing, biochemical confirmation and antibiotic resistance. The investigation and control measures led to an improvement in hygiene practices at the restaurants and contributed to the voluntary recall of the contaminated foods. The results of the study also build upon other evidence that street foods related salmonellosis is now common in Thanjavur, India and attention to commercial practices at production and processing is overdue. As control measures, the research team provided the protective materials like hand gloves, spoon covers to avoid the food contamination.

Keywords

Salmonella typhimurium; Street foods; case control study.

Introduction

In India, more number of food borne illness are estimated annually costing of estimated numerous crores per year and *Salmonella typhimurium* is the atypical type of samonellosis causing gastrointestinal diseases. Many of these

infections are preventable by appropriate interventions and surveillance help to recognize the control actions (Hall *et al*., 2005; CDC, 2009). The much involvement of Health departments under ministry of health need more interventional research and surveillance for food borne diseases and disease potentially transmitted by food to monitor trends in illness, detect outbreaks, inform preventive measures and evaluation of the efficacy of interventional efforts. These observations are not satisfactory in India compared to other countries.

More food borne diseases manifest as mild self limiting gastroenteritis, with only 25% of affected people seeking medical attention (Majowicz *et al.,* 2005; Hall *et al.,* 2006). Consequently surveillance data collected by health departments underestimate the true burden of the diseases. *Salmonella typhimurium* infection commonly results in symptoms such as abdominal pain, diarrhoea, fever, nausea and vomiting. The bacteria transmitted via ingestion, usually of food contaminated by the feces of an infected person or animal. The incubation period of *Salmonella* can range between 6 to 72 hours, but is more commonly between 12 to 36 hours. There have also been instances of longer incubation period of up to 16 days (Heymann, 2008; Amalie *et al.*, 2009).

Most of studies depicted that the isolates of *S. typhimurium* harboring various resistance genes with broad spectrum drug resistance including resistance of fluoroquinolones through chromosomal mutation as a major cause of concern (Threlfall, 2005; Pillai and Prakash, 1993). Further rapid differentiation of salmonella isolates is often required in outbreak situations to identify additional cases and for source tracking. A variety of genotyping methods have been used for this purpose including plasmid profiling, ribotyping, amplified fragment length polymorphism (AFLP) and pulse field gel electrophoresis (Best *et al*., 2007; Vicki *et al.,* 2009). A recent developed DNA fingerprinting technique, multiple locus variable number tandem repeat analysis (MLVA) has become very useful for this purpose. The polymerase chain reaction (PCR) based technique have good discriminatory power between the strains of *S. typhimurium*. This strain variations and characterization is based on the differences in amplified DNA fragments at various loci in the salmonella genome, due to varying numbers of short sequenced DNA tandem repeats (VNTR) at these sites (Vicki *et al.,* 2009).

Salmonella transmission to food processing plants and food production equipment is a serious public health issue. *Salmonella* can enter the food chain at any point; crop, farm, livestock feed, food manufacturing, processing and retailing (Wong *et al*., 2002). A number of workers handle animals during slaughter and processing and contamination is possible when Salmonella or any other pathogen is present on the equipment or the workers hands or clothings (Steffi *et al.,* 2012). Contamination most often occurs during explicit slaughter changes including bleeding, skinning, evisceration and pre-processing carcass handling. These factors contribute toward outbreaks of salmonella infections. Raw fruits and vegetables contaminated during slicing have been implicated in several food borne outbreaks, as have foods contaminated by food handlers who did not adhere to proper hygienic standards and practice proper hand washing techniques (Albreiki *et al*., 2004; Jegadeeshkumar *et al*., 2010; Steffi *et al*., 2012).

In this study area there is no previous out-break observed related to *Salmonella* infection among the hotel workers. In India, the working time for hotel workers is not determined properly due to the less salary packages, the hotel workers may continue the work even they have symptoms of *Salmonella* infection. According to the *Salmonella* carriers status this is the first work in this area on to check the prone infections status (carter) among the hotel workers. The prone persons could be responsible for infection spreading.

Materials and Methods

Epidemiological examination

Initial interviews with cases and control of the subjects included in this study were conducted to allow the generation of hypothesis and to guide further investigations. All the subjects included were interviewed using the standard questionnaire investigating exposures in the 7 days prior to onset of illness (Amalie *et al*., 2009). This led to the hypothesis that the outbreak was linked to a local restaurant, affecting those who attended for breakfast and dinner. To test this hypothesis, a case control study was developed based upon the restaurant's breakfast and dinner menu.

Before the study started, case was defined as "any person who ate at the restaurants during April 2012 and developed symptoms of gastroenteritis defines as two or more gastrointestinal symptoms", later it was revised as "any person who ate breakfast and dinner during the period of 27th April and 17 may 2012 and who developed symptoms of gastroenteritis after exposure". Probable cases were those met this definition and confirmed cases were those who met the definition and had a fecal culture positive to Salmonella.

Controls were selected via convenience sampling resulted in the recruitment of 6 controls. Data were analyzed using odds rations and stratification to remove the effects of confounding between some menu items. Case control analysis and stratification were performed using Stata 9.

Environmental examination

General hygiene practices, food storage, preparation procedures and refrigerator condition were examined. Samples of sambar, coconut chutney, onion chutney, kurma, coconut milk and idly were taken from the restaurant and street side hotels for microbiological testing. The samples were transferred to Microbiology laboratory under aseptic procedures and it was processed immediately without storage.

Laboratory examination

Sample Collection

The study setting was various hotels and road side restaurants in Thanjavur district in Tamilnadu, India. Samples were collected from total of 2 hotels and 8 road side open restaurants in Thanjavur in small wide mouthed sterile containers from the hotel workers and the possibility was 16 only. Before collection of the sample the surface was sterilized by distilled water for preventing the entry of contaminants. The collected samples are processed as soon as possible. Labeling plays a vital role, so the sterile containers should have appropriate label with name, date of collection and age (Steffi *et al.,* 2012).

Processing of finger swab and nail cuts

A total number of 22 finger swabs and nail cuts were collected and assessed bacteriologically. Sterile cotton swabs was

prepared and dipped in saline and dispensed into screw cap tubes. Using the swab finger scrapings and nail cuts were collected from each hotel workers and it was aseptically swabbed on appropriate media like DCA, Hektoen enteric agar and MacConkey agar and kept it for incubation and colonies were observed after 2 to 3 days. From the colonies gram staining and motility test was prepared and observed and further confirmation of *Salmonella* biochemical tests was performed.

Results and Discussion

Total of 24 cases were indentified after consuming food at the restaurants. Among them, 22 subsequently agreed to participate in the case control study. The age of cases ranged from 7 to 55 years, with that 9 (41%) of those affected being female. Cases ate breakfast and dinner at the street side hotels and restaurants between 27th April and 17 may 2012. The common symptoms found are diarrhea (100%), fever (72.7%), nausea (72.7%) and vomiting (59.1%) – the frequent reported signs and symptoms. Of those ill, 18 (81.8%) people consulted physician and 4 (18.2%) were hospitalized. The odds ration with confidence intervals and P values were calculated for each item on the breakfast and dinner included. These results suggest that the most likely source of infections due to *Salmonella* was coconut chutney, coconut milk and kurma with odds ratio of 42, 19 and 19 respectively.

Despite of large confidence intervals both these results were statistically significant. The Odds ratios for onion chutney (OR 8.00), idly (OR 6.12), water (OR 4.00) and sambar (0.66) was elevated but not statistically significant. Stratification of foods with elevated odds ratios was analyzed to adjust for possible confounding and was included in Table 2.

After stratification, the coconut chutney and kurma could not be separated. This was because almost all those who ate coconut chutney also had kurma. The odds ratio for coconut chutney remained elevated and statistically noteworthy with onion chutney (OR 30.00) and sambar (OR 42.00). Similarly, the odds ratio for kurma remained high when stratified with onion chutney (OR 13.50) and sambar (OR 18.00), where the odds ratios had confidence intervals higher than one and were statistically significant.

Onion chutney (OR 2.22) had elevated odds ratio when stratified against kurma but this was no longer statistically significant. The odds ratio of onion chutney, when stratified with coconut chutney remained elevated at 2.20 but this also not statistically significant. The other combinations were not adjusted.

The possibility that the coconut chutney and kurma may have been the vehicle of infection was raised during the initial examination of the street side hotels and restaurants. The proper supervision and advices gave the hotel owners regarding the dangers of serving coconut chutney that prepared long back and request them to serve freshly prepared to reduce the risk. The process involving the preparation of storage of kurma (rich in coconut) was identified as a possible problem. The storage in the low temperature is also advisable; however this varies depending on customer predilection.

Information from the cook advised that they had investigated microbiologically, may have been responsible for the contamination.

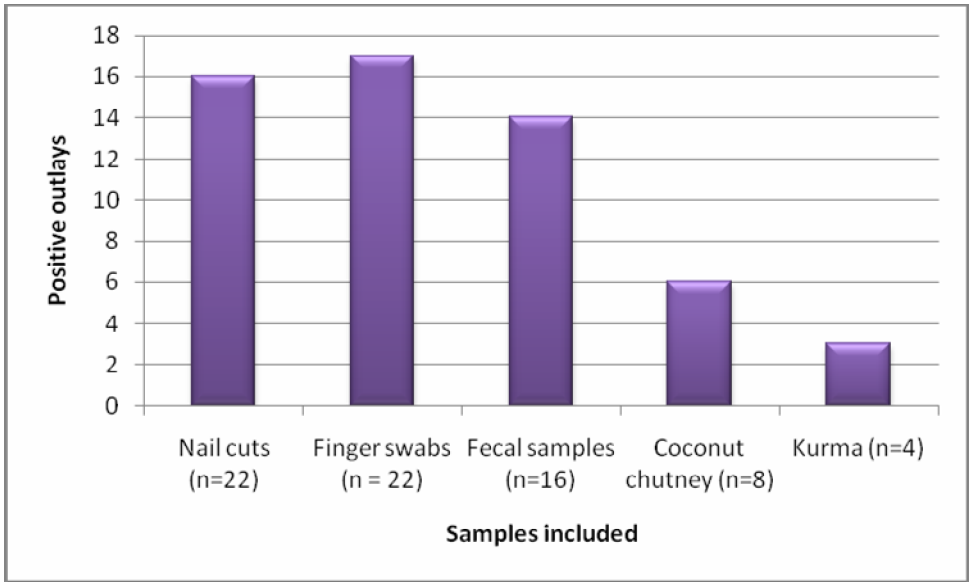
Table.1 Univariate analysis of breakfast menu included in this study

Item	Cases	Controls	Odds ratio	Confidence intervals	P value
Sambar	12	6	0.66	0.0 – 4.3	0.70
Onion chutney	11	1	8.00	0.7 – 386.2	0.10
Idly	14	2	6.12	0.8 – 69.9	0.05
Coconut chutney	21	3	42.00	2.8 - 2017	0.0007
Kurma	19	3	19.00	1.8 - 243	0.003
Coconut milk	19	3	19.00	1.8 - 243	0.003
Water	17	4	4.00	0.5 – 28.4	0.11

Table.2 Stratification analysis

Coconut chutney adjusted for	Odds ratio	Confidence interval	P value
Onion chutney	30.00	2.2 – 405	0.006
Sambar	42.00	2.1 - 825	0.009
Kurma adjusted for			
Onion chutney	13.50	1.4 – 123	0.02
Sambar	18.00	1.2 – 255	0.03
Onion chutney adjusted for			
Coconut chutney	2.20	0.1 – 28.1	0.50
Kurma	2.22	0.1 – 28.8	0.50
Sambar adjusted for			
Coconut chutney	1.00	0.0 – 13.0	0.72
Kurma	1.08	0.0 – 14.4	0.70

Figure.1 Results of *S. typhimurium* isolates among the samples included



During interview, we got the information that they are provided with leave even they are sick. However the health officials must take some necessary action to overcome this issue.

Among the 22 samples included for finger swab and nail cuts, the culture showed positive to 16 (72.7%) and 17 (77.2%) respectively. In the Hektoen enteric agar, bluish-green with black centre colonies found and this agar medium is designed for the detection of *S. typhimurium* (Rosenberger *et al.,* 2000). Fecal samples taken from symptomatic diners yielded a total of 14 (87.5%) positive results for *S. typhimurium*, with 2 (12.5%) persons found to be negative.

The 8 and 4 samples of coconut chutney and kurma were found to have a pH range of 5.12 and 5.16 respectively and showed culture positive to *S. typhimurium*. Among the 8 coconut chutney samples included, 6 (75%) showed culture positive, whereas 3 (75%) showed positive to kurma included (Figure 1). *Salmonella* was not isolated from any of the swabs taken from plates and spoons. The possible reason is the plates and spoons were dipped and cleaned with hot water.

The results from the epidemiological investigation suggest that the most likely cause of this outbreak was contaminated coconut chutney and kurma served in the road side hotels and restaurants. This type of infection is most commonly associated with *S. typhimurium* and is not endemic. The odds ratios for both the coconut chutney and kurma were extremely high, indicating a strong association. The strength of the association remained following adjustment for the possible confounding. However the two items are usually served and could not be separated with stratification. Though the two items could not be statistically separated, the environmental investigation suggested that the coconut chutney provided a more plausible explanation for the infection.

With the emergence of high level ciprofloxacin resistance and potential horizontal transfer it has necessary to determine the minimum inhibitory concentration for preventing therapeutic failure. Empirical therapy of ciprofloxacin should be discouraged. One of the major limitations of this study was the small sample size and disproportionate numbers of cases to controls. This may be affected the results leading to an erroneous exclusion of other foods possible sources of the infection. However, this would seem less likely given the supporting environmental evidence and higher attack rates among persons eating coconut chutney and kurma. An illness between illness and consumption of coconut chutney was strong enough to be detected in this group of consumers. As mentioned previously, there is strong evidence of confounding in these results.

This investigation concluded the most likely cause of this study was consumption of uncooked coconut chutney putatively contaminated with Salmonella. This showed that the restaurant people have to serve freshly prepared coconut chutney (not the stored of late prepared one). This self limited action may have averted future infections, as well as increased general awareness about appropriate procedures for the distribution of such routinely consumed food materials. The incident also led to improvements in hygiene and food storage procedures at the hotels and restaurants and serves to highlight the need for further education of food handlers in relation to the preparation of the plates,

spoons, etc. This demonstrates the importance of the exemplary hygiene and food storage practices in hotel and restaurant settings as a means of reducing the risk of food related salmonellosis (Amalie *et al*., 2009; Roberts *et al.,* 2009; Mitchell *et al.,* 1989). In addition to highlighting the importance of effective hygiene measures within commercial kitchen settings in India, this also suggests the need for the more stringent regulation for the food preparation. Health authorities should also consider prohibiting commercial outlets servings for the reduction of the risk to the public of *Salmonella* infection.

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