

## An Outbreak of *Salmonella Enteritidis* Food Poisoning: The Role of PulseNet International

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Received: 03 November 2017; Accepted: 02 December 2017

**Citation:** Elshafie Sittana Shamseldin, Abraham Joji, Al-Bakir Wasan. An Outbreak of *Salmonella Enteritidis* Food Poisoning: The Role of PulseNet International. *Microbiol Infect Dis*. 2017; 1(2): 1-6.

### ABSTRACT

**Background:** *Salmonella* spp reach food in many different ways; directly from slaughter animals to food, and from human excreta transferred to food through hands, utensils, equipment, flies etc. Foods commonly involved are animal derived foods such as poultry, meat and meat products, milk and milk products, eggs and egg products.

We investigated a hospital outbreak of *Salmonella enteritidis* food poisoning due to contaminated chicken and compared the strains isolated from the patient's samples and contaminated chicken with those isolated from the community outbreaks. Through PulseNet International our strains were compared with the strains of the country of origin of the chicken.

**Methods:** An increased number of *Salmonella* Group D was noted in the community during January, June, September, November, and December 2006. The December 2006 cluster was mainly due to infection of the Asian Games Drivers (24 out of 28 reported cases). A surge of infections were also noticed during March, April, and June 2007 in the community as well as in the hospital. Nine inpatients from Hamad Medical Corporation (HMC) developed infection with *Salmonella* Group D eight of them had food poisoning symptoms following therapeutic feeds (Pureed, diabetic and gastric diets) prepared from chicken breast from the same supplier. Patient number 9 had no symptoms of food poisoning and *Salmonella* Group D was isolated from his blood only. All isolated *Salmonella* strains were preserved in CryoBank by the Microbiology laboratory and notified to the Public Health Department (PHD), Supreme Council of Health (SCH).

As part of the investigation samples were collected from catering staff, therapeutic feeds, environment of Catering Department, and raw chicken from Catering Department, and Material Management Department Store (same batch).

**Results:** *Salmonella* Group D was isolated from Chicken breast and chicken legs samples collected from the Catering Department and Material Management Store. To confirm their genetic relatedness, *Salmonella* Group D isolated from Asian Games Drivers, from the community, from HMC patients and from the Chicken were sent to Naval Medical Research Unit # 3 (NAMRU-3) for full identification and genetic analysis by PFGE. All strains were found to be *Salmonella enteritidis* and they are all genetically related. With the help of PulseNet International the strains were found to be genetically identical to the isolates of the country of origin of the chicken.

**Conclusion:** Based on our investigation, the hospital outbreak may be due to the contamination of the chicken breast during processing /packing at the company's premises. Prolonged stay of patients feed outside the refrigerator enabled multiplication of the organisms which escaped the cooking process. The source of infections in the Asian Games Drivers and other community outbreaks could not be identified since food samples were not available for testing.

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## Keywords

Outbreaks, PulseNet International, *Salmonella*.

## Introduction

Non-typhoidal *Salmonella spp* is one of the four key global causes of diarrheal diseases and food poisoning in humans. *Salmonellae* are widely distributed in domestic and wild animals. They are prevalent in food animals such as poultry, pigs, and cattle; and in pets, including cats, dogs, birds, and reptiles such as turtles. They can pass through the entire food chain from animal feed, primary production, and all the way to households or food-service institutions [1-4].

Salmonellosis in humans is generally contracted through the consumption of contaminated food of animal origin (mainly eggs, meat, poultry, and milk), although other foods, including green vegetables contaminated by manure, have been implicated in its transmission. Person-to-person transmission can also occur through the fecal-oral route. Human cases also occur where individuals have contact with infected animals, including pets. People who are carriers of the *Salmonella spp* contaminate the food [4,5]. A heavy dose up to 10,000 -1,000,000 organisms per gram of food is required to cause infection [6].

*Salmonella enteritidis* is the predominating serotype and continues to cause large numbers of human illnesses in spite of adopted preventive measures [7-11]. The *Salmonellae* are killed by temperatures attained in commercial pasteurization. They can remain alive in moist earth for one year and in dry earth for 16 months, They are not destroyed in carcasses or offal maintained at chilling or freezing temperatures.

The Division of Microbiology provides clinical microbiology service to all hospitals under HMC. It also provides referral service for other hospitals in the country. *Salmonella spp* isolated by the laboratory were identified to the group level only. Further identification and serotyping will be referred to external referral laboratories. As part of the communicable diseases notification policy, all *Salmonella spp* isolated from patients will be notified to the SCH, PHD for contact tracing and outbreak investigation.

In 2006 the Microbiology Laboratory noticed an increased number of *Salmonella* Group D compared to previous years. This increase was considered by the PHD to be due to the increased number of recruited workers from endemic areas as part of the preparation for the Asian Games.

Immediately after the end of the Asian Games in December 2006, 24 of the Drivers who worked for the Games experienced symptoms of diarrhea illnesses following consumption of leftover food from the games. All 24 drives had *Salmonella* Group D isolated from their stool samples. In March and April 2007, eight elderly bed ridden inpatients developed vomiting and diarrhoea of whom two also had septicemia, *Salmonella* Group D was isolated from their stool and from the blood of the 2 septicemic patients. A ninth patient admitted to the surgical intensive care unit with

no symptoms of food poisoning had *Salmonella* Group D isolated from his blood.

We describe the action plan of the Infection Control Committee to investigate and control the hospital outbreak, define the relationship between the hospital and community outbreaks, and the role of PulseNet International in outbreak confirmation and trace back of source of the organism.

## Methods

Between 28th of March 2007 and 19th of April 2007, the Microbiology laboratory notified the Infection control Team following isolation of *Salmonella* Group D from nine patients admitted in the different hospitals of HMC. In response, the Chair of the Infection Control Committee organized a subcommittee including representative from Catering Department, Infection Control Team, Material Management Department, Nursing Department, Microbiology Laboratory, Public Health Laboratory (PHL), and PHD to investigate the outbreak.

Action plan and data collection tool developed. Case finding was done by interviewing patients on therapeutic feeds from March 25 to April 21/2007. The Material Management Department Stores and the hospital Catering Department were inspected and the catering procedures were reviewed.

As part of the investigation samples were collected from therapeutic feeds, environment of Catering department, equipment, random samples from various food items and raw chicken from Catering and Material Management stores. Catering staff involved in therapeutic diet section & butchery section were also screened.

To confirm the outbreak and define whether we were dealing with one outbreak or multiple outbreaks, *Salmonella* Group D strains isolated from the patients, from Asian Games Drivers and from the community clusters in 2006 and 2007 were sent to Naval American Institute Unit # 3 (NAMRU 3) in Cairo for further serotyping and genetic analysis using Pulse Field Electrophoresis (PFGE).

## Results

### The Hospital outbreak

A total of nine patients developed *Salmonella* food poisoning. Eight patients were elderly bed ridden who were solely on pureed diet prepared from chicken breast in the same kitchen. Patient number 9 was admitted to the Surgical Intensive Care Unit following car accident and who was on normal diet with whole grilled chicken. He had no symptoms of food poisoning and *Salmonella* Group D was isolated from his blood culture only (Table 1).

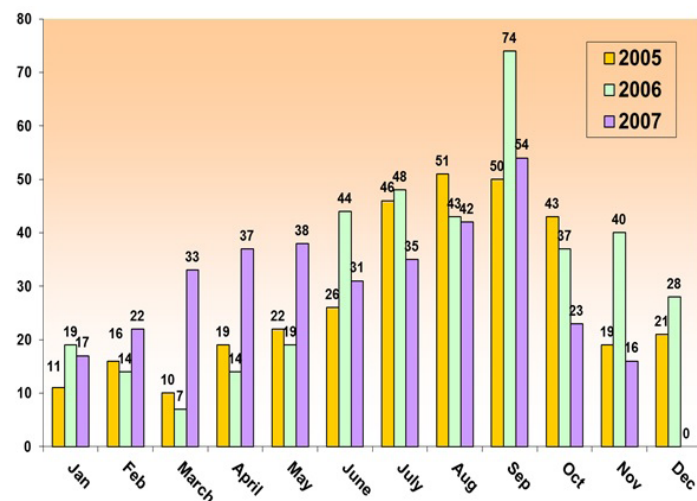
### Outbreaks in the community

Our analysis highlighted that outbreaks of salmonellosis occurred frequently in Qatar without being fully investigated. Outbreaks occurred in January, June, September, November and December 2006 as well as in January, February, March, April and June 2007. 24 of the Asian Games Drivers were affected and *Salmonella* Group D isolated from their stool samples (Figures 1 and 2). No

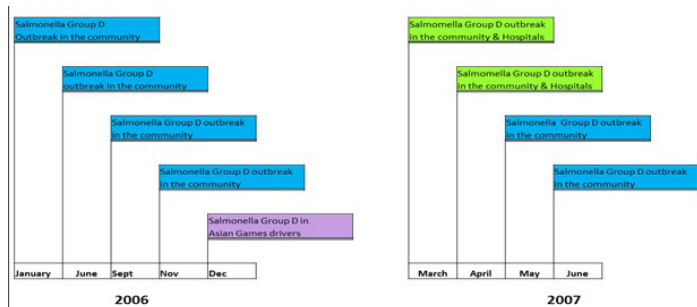
food items were available for testing for all these outbreaks.

#	Unit/ward	Onset of symptoms	Date of culture	Specimen type	Result	Type of diet
1	4S1	28/3/07	28/3/07	Stool & blood	<i>Salmonella</i> Group D	Therapeutic
2	5N1	28/3/07	28/3/07	Stool & blood	<i>Salmonella</i> Group D	Therapeutic
3	5N1	28/3/07	28/3/07	Stool	<i>Salmonella</i> Group D	Therapeutic
4	MRU-3	28/3/07	31/3/07	Stool	<i>Salmonella</i> Group D	Therapeutic
5	MRU-1	30/3/07	30/3/07	Stool	<i>Salmonella</i> Group D	Therapeutic
6	3N3	2/4/07	2/4/07	Stool	<i>Salmonella</i> Group D	Therapeutic
7	FRU-3	2/4/07	2/4/07	Stool	<i>Salmonella</i> Group D	Therapeutic
8	AAH	5/4/07	8/4/07	Stool	<i>Salmonella</i> Group D	Therapeutic
9	SICU	No symptoms of food poisoning	19/4/07	Blood	<i>Salmonella</i> Group D	Normal

**Table 1:** Patients with *Salmonella* food poisoning.



**Figure 1:** *Salmonella* Group D in the State of Qatar; 2005-2007.



**Figure 2:** The distribution of the outbreaks 2006 & 2007.

Specimens collected from the catering staff, catering environment, whole raw chicken, freshly prepared therapeutic diet, and other dietary components were all negative for *Salmonella* Group D, while raw chicken thighs and chicken breast from the kitchen and

from Material Management Stores were all positive for *Salmonella* Group D (Table 2).

Specimens	Date of culture	Result
5 samples from pureed diet freshly prepared	10/04/07	No <i>Salmonella</i> isolated
Raw whole chicken from the kitchen	10/04/07	No <i>Salmonella</i> isolated
Raw chicken breast from the kitchen	10/04/07	<i>Salmonella</i> Group D isolated
Raw chicken thighs from the kitchen	10/04/07	<i>Salmonella</i> Group D isolated
Cooked vegetable	10/04/07	No <i>Salmonella</i> isolated
Cooked meat	10/04/07	No <i>Salmonella</i> isolated
Grilled chicken; kitchen	10/04/07	No <i>Salmonella</i> isolated
Swabs from the kitchen environment, cutting boards, blender machine, utensils and sink	15/04/07	No <i>Salmonella</i> isolated
Raw whole chicken from the Material Management store	18/04/07	No <i>Salmonella</i> isolated
Raw Chicken thigh from Material Management store	18/04/07	<i>Salmonella</i> Group D isolated
Raw chicken breast from Material Management store	18/04/07	<i>Salmonella</i> Group D isolated
Stool and finger prints from food handlers	15/04/07	No <i>Salmonella</i> isolated

**Table 2:** Result of Culture of raw chicken, pureed diet and the Kitchen environment.

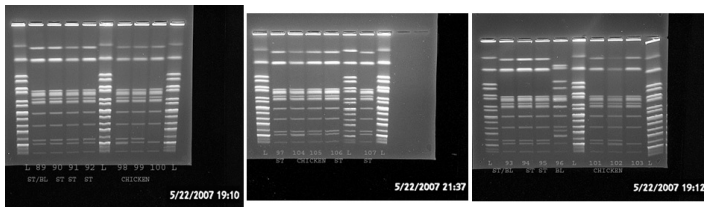
### Problems identified

- 1) Not all chicken supplies entering the country were checked by the PHL before release for consumption. The policy of Municipality was to test the first 3 batches of food from each new supplier and if all tests were negative, further testing will be done randomly. The brand involved in the outbreak was not tested.
- 2) Food was stored at patient bed side for variable periods before consumption.
- 3) In Material management Department monitoring of the temperature freezers and refrigerators used for storing food supplies including chicken was not done regularly and no record available. Recording of temperature was done by Engineering Department and record was kept by them and not shared with other departments.
- 4) Not all Catering staff were screened after returning from vacation. 3 consecutive stool samples were not sent for microbiology analysis according to infection control guidelines (noncompliance with policy).
- 5) Not all Catering Department staff was certified by Municipality.

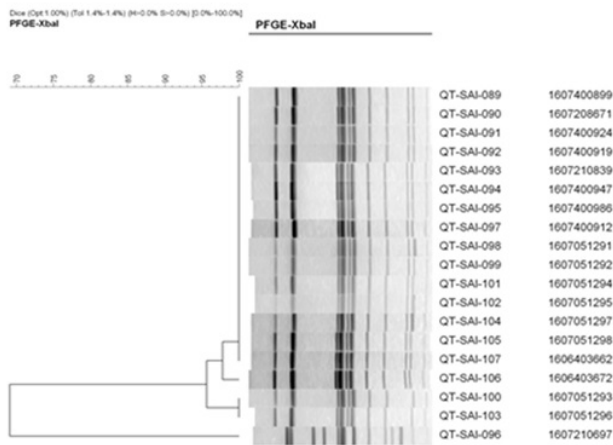
### Identification and Typing of the Strains

A question was raised whether we were dealing with one outbreak or multiple outbreaks throughout the years 2006 and 2007. To address this question *Salmonella* isolated from Asian Games Drivers, from the community, from HMC patients and from the Chicken breast and legs were sent to NAMRU-3 for full identification and genetic analysis. All isolated strains were identified as *S. enteritidis* and

all except one, were genetically related based on PFGE results (Figures 3 and 4). The PFGE identified 3 patterns: a) “Cluster pattern” = match human/chicken; b) Chicken 100 & 103 = only chicken (missing 1 band); and c) Human 96 = very different pattern. This confirms it was probably one intermittent common source outbreak.



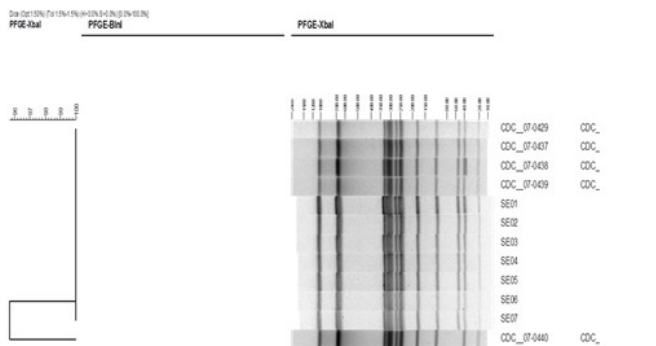
**Figure 3:** PFGE pattern of *Salmonella enteritidis* isolated from patients, the community, Asian Games Drivers and chicken thighs and breast.



**Figure 4:** Qatar isolates Dendrogram.

The source of the outbreak was most likely the chicken thighs and breast. Patient number 9 (pattern number 96), who had completely different pattern, was the SICU patient who was fed on whole grilled chicken and had no symptoms of food poisoning.

*S. enteritidis* strains of the outbreak and chicken breast and legs were sent to Centers for Diseases Control and Prevention (CDC) Atlanta, Georgia, USA to compare with the strains circulating in the country of origin of the chicken through Pulse Net International. *S. enteritidis* of the outbreak was found to be identical to the *Salmonella enteritidis* strain of the country of origin (Figure 5).



**Figure 5:** Dendrogram with strains of the country of origin.

### Contributing factors to the outbreak

Contamination during processing/packing of breasts and thighs at the company’s premises was the most likely source of contamination of the chicken breast and legs as whole chicken tested negative for *S. enteritidis*. Some *Salmonellae* which were present in the chicken may have escaped the cooking temperature and multiplied during the interim period of serving to the patient. In HMC and according to our survey, normally food reach in time, but it took a longer time (more than 1 hour) before the food was consumed. Prior reports of outbreaks of salmonellosis have suggested the presence of low level contamination and low infectious dose particularly for elderly and immunocompromised patients [12,13].

### Action taken to prevent recurrence of outbreak included

Action taken by the Infection Control Committee included reinforcement of infection control practices for Catering department, staff education, installation of alcohol based hand rub due to lack of hand washing facilities ,ongoing monitoring of Infection control practices related to hand hygiene and food safety, freezer and temperature monitoring and documentation. Food warmer trollies were provided to ensure food was, and provision of kept at the appropriate temperature during transportation.

The supply of the involved chicken brand was stopped and local supplier notified by Municipality to withdraw the brand from the market.

To maintain the improvement various measures were taken including update all related infection control and food safety policies and guidelines; improve hand hygiene and hand hygiene facilities and monitor staff compliance. All food coming to Catering Department must be checked by the PHL before consumption. Education in food hygiene practices reinforced as well as periodic screening of Catering staff and contracted workers according to Infection Control policy. Random sampling of 5 to 10% of stock chicken was sent to the PHL before releasing for consumption. Samples of daily menu will be hold for 48hrs before being discarded; and notification of infection control and PHD for any diarrheal illness.

In the community The Ministry of Municipality regularly inspects restaurants, hotels and food handlers to ensure their compliance with licensing requirements of the Ministry of Environment and the SCH. *Salmonella* serotyping and PFGE was introduced in Qatar; together with National Food safety committee to set up policies and guidelines for outbreaks investigation, and train epidemiologist on outbreak investigation.

### Discussion

One in every 10 individuals worldwide become ill due to microbial contamination of food with 550 million cases and 230000 death yearly [14,15] *Campylobacter* and *Salmonella* are the most common causes of bacterial foodborne infections and are responsible for 96 and 80 million every year respectively [15].

*Salmonella* food poisoning remains a challenge for healthcare authorities in Qatar as well as in other countries. In many countries e.g. USA the incidence substantially increased for some serotypes and decreased for others [16-18] *Salmonella enteritidis* the cause of our outbreak is mainly linked to eggs and chicken [19-21].

Investigation of outbreaks play key role in identifying source of outbreak and the food implicated for preventive measures to be implemented. Both epidemiological and laboratory data are essential to verify the source of the outbreak [22]. Hence the Microbiology Laboratory of HMC regularly reports all food borne pathogens to the PHD of the SCH to identify source of outbreak and implement preventive measures to stop further outbreaks.

An outbreak is defined as  $\geq 2$  cases of similar illness resulting from the consumption of a common food item. A *Salmonella spp* is considered the confirmed etiological agent if the same serotype is isolated from  $\geq 2$  ill persons and epidemiologically linked to similar food [23].

Our hospital outbreak fulfils these criteria. All cases had food poisoning characterized by vomiting and diarrhoea, 2 cases also had symptoms of septicemia. *S. enteritidis* was isolated from their stool and from the chicken breast used for preparing their feeds. The exception was the SICU patient who had no symptoms of food poisoning and who had completely different strain of *S. enteritidis*.

To confirm the source of the outbreak full serotyping and PFGE is necessary. The use of PFGE recognizes outbreaks more easily and improves timely detection and control. CDC, USA helped us through PulseNet International to confirm the identity of the *S. enteritidis* strain isolated from our patients and from the chicken breast and legs and compare with *S. enteritidis* strain of the country of origin of the chicken breast and legs (Figures 3, 4 and 5).

PulseNet International is a global laboratory surveillance network that performs molecular typing of foodborne disease-causing bacteria. The method of typing was PFGE that creates DNA fingerprints. The DNA fingerprints are shared electronically nationally and internationally with a laboratory network from Africa, Asia Pacific, Canada, Europe, Latin America and Caribbean, The Middle East and the United States of America. Qatar is a member of the PulseNet Middle East [24].

The role of PulseNet International is to detect food borne diseases clusters, facilitates early identification of common source outbreaks, assists investigation of transmission chain, Links two or more outbreaks together and Links apparently sporadic cases. It will also assess the intervention methods, the distribution of pathogens and how this distribution changes, and the emergence of new pathogens. The use of standardized protocols, quality control and quality assurance program, training and certifications of member laboratories make International comparison possible [25].

The *S. enteritidis* strains isolated from the different community outbreaks and from the Asian Games drivers according to

serotyping and PFGE results match those isolated from the patients during the hospital outbreak and the chicken thighs and breast. This raised the possibility of one intermittent common source outbreak. However since the implicated food was not available for testing, we are not sure whether these outbreaks were also caused by chicken breast and thighs or through other route. More epidemiological data needed to confirm the source of the outbreak. There is also a possibility that the *S. enteritidis* strain introduced in Qatar and is circulating through carriers.

## Conclusion

Despite the use of improved food production and processing technologies poultry contaminated with *Salmonella* continues to be a challenge and a risk particularly for immunocompromised and elderly people.

To our knowledge this is the first foodborne outbreak in Qatar to be traced to the source of food contamination using PFGE and PulseNet International. The introduction of PFGE in the Public Health laboratory will meet the needs of real-time surveillance together with proper epidemiological investigation.

The use of PulseNet Middle East and PulseNet international will facilitate timely identification of the source of food contamination and notification of other countries to avoid similar outbreaks. Network should be set up and connected to allow rapid communication to other parts of the world. Since PulseNet international is moving towards whole genome sequence which is more accurate than PFGE, this test should be considered in Public Health Laboratory.

## Acknowledgement

The authors would like to thank the Infection control Team HMC and the Subcommittee for the full investigation of the outbreak, HMC Microbiology Laboratory staff for identification of the strains from the patients, the PHL staff for identifying the strains from chicken, Staff of NAMRU 3 Cairo; Egypt who did the serotyping and PFGE; and the CDC Atlanta Georgia, USA for facilitating comparison with country of origin through PulseNet International.

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