

ISOLATION AND CHARACTERIZATION OF *SALMONELLA* FROM FOOD HANDLERS

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Accepted 10 March ,2016

Food is an important vehicle for the spread of infectious agents causing disease resulting to appreciable morbidity and mortality. Food handlers play an important role in ensuring food safety. However, in developing countries like Ethiopia the proportion of certified food handlers and their carrier status is not well studied. Salmonellosis is one of such diseases that can be transmitted from chronic asymptomatic salmonella carriers especially the food handlers. The main aim of this paper is to review the isolation and identification method of *salmonella* from food handles. As some study revealed that, isolation rate of *Salmonella* species was obtained and all isolates were resistant at least to one of antimicrobials tested. Accordingly, food handlers could be a source of salmonellosis unless carriers treated after periodic screening and other preventive measures taken. Antimicrobial resistance profile also reflects it would be a serious problem in near future.

Key words: Antimicrobial, Cross sectional study, Food handler, Isolate, *Salmonella*

INTRODUCTION

Food borne diseases are a public health problem in developed and developing countries. The World health organization (WHO) stated that, most of the populations suffer from food borne diseases each year, both in developing and developed countries up (WHO, 2007). Bacteria that cause food-borne diseases include among others are *Salmonella*, *Campylobacter*, *Listeria*, pathogenic *Escherichia coli*, *Yersinia*, *Shigella*, *Enterobacter* and *Citrobacter*. In addition, food-borne diseases can be caused by bacterial toxins. Bacterial toxins are toxins generated by bacteria and may be highly poisonous in many cases. These include toxins from *Staphylococcus aureus*, *Clostridium botulinum* and *Bacillus cereus* (European Union, 2009). *Salmonella* is a bacterium that is widespread in the intestines of birds, reptiles and mammals and is one of the main causes of foodborne disease in humans. There is also concern about increased antibiotic resistance when treating Salmonellosis in humans. It can spread to humans through a variety of different foods of animal origin (Todar, 2005). Salmonellosis is a major cause of bacterial enteric illness in both humans and animals. *Salmonella* are a group of bacteria that can cause diarrheal illness in people. This constitutes a major public health burden and represents a significant cost to society in many countries. One species, *Salmonella enterica* has more than 2,000 serovars with *Salmonella typhimurium* and *Salmonella enteritidis* most commonly encountered globally. *Salmonella* are inhabitants of the feces of many types of animals including poultry, eggs, dairy products and foods prepared on contaminated work surfaces. Therefore, *Salmonella* plays significant role in foodborne disease worldwide (WHO, 2005). *Salmonella* infections can be typhoidal or non-typhoidal. Serotypes such as *S.typhi*, *S.paratyphi A* and *S.paratyphi B*, causes of typhoidal salmonellosis, are highly adapted to humans and do not cause disease in non-human hosts. The vast majority of *Salmonellae* (e.g., *salmonella choleraesuis* and *salmonella enteritidis*), however, are chiefly pathogenic in animals that constitute the reservoir for human infection: poultry, pigs, rodents, cattle, pets (from turtles to parrots), and many others (European Union, 2009). Non-typhoidal salmonellosis is a worldwide disease of humans and animals. Animals are the main reservoir, and the disease is usually food borne, although it can be spread from person to person (Todar, 2005). Typhoid fever is a systemic disease characterized by fever and abdominal pain caused by dissemination of *S. typhi* or *S. paratyphi*. The disease was initially called typhoid fever because of its clinical similarity to typhus. In 1869, given the anatomical site of infection, the term enteric fever was proposed as an alternative designation to distinguish typhoid fever from typhus. However, to this day, the two designations are used interchangeably (Todar, 2005). The *Salmonellae* that cause Typhoid fever and other enteric fevers spread mainly from person-to-person via the fecal-oral route and have no significant animal reservoirs. Asymptomatic human carriers ("typhoid Marys") may spread

the disease. Such infections may occur when food or water contaminated by infected food handlers is ingested (Wikipedia, 2010).

LITERATURE REVIEW

Salmonella is a genus of rod-shaped, Gram-negative, non-spore forming, predominantly motile enterobacteria with diameters around 0.7 to 1.5 μm , lengths from 2 to 5 μm , and flagella which project in all directions (i.e. peritrichous). They are chemoorganotrophs, obtaining their energy from oxidation and reduction reactions using organic sources, and are facultative an-aerobes (Ryan *et al*, 2004). *Salmonella* have a complex antigenic structure. They are classified by heat-stable somatic O (lipopolysaccharide) antigens, heat-labile K (capsular) antigens, and H (flagellar) antigens. In *Salmonella typhi*, the capsular antigens are called Vi antigens (Todar, 2005). *Salmonellae* constitute a genus of more than 2500 serotypes that are highly adapted for growth in both humans and animals and that cause a wide spectrum of disease. A new classification for *Salmonella* has been adopted based on DNA relatedness. This new nomenclature recognizes only two species: *Salmonella bongori* and *Salmonella enterica*, with all human pathogens regarded as serovars within the subspecies of *S. enterica*. For example, the proposed nomenclature would change *S. typhi* to *S. enterica* serovar *Typhi*, abbreviated *S. Typhi*, and *Salmonella enterica* serovar *Enteritidis* would be referred to as *S. Enteritidis* instead of *S. enteritidis* (CDC, 2010). Salmonellosis is an infection with *Salmonella* bacteria transmitted through feco-oral route. Most people infected with *Salmonella* develop diarrhea, fever, vomiting, and abdominal cramps 8 to 72 hours after infection. In most cases, the illness lasts 4 to 7 days and most people recover without treatment. However, in some persons the diarrhea may be so severe that the patient becomes dangerously dehydrated and the *Salmonella* infection may spread from the intestines to the blood stream, and then to other body sites and can cause death unless treated. Salmonellae produce three main types of disease in humans, but mixed forms are frequent. These are enteric fever (typhoid), septicemia and enterocolitis and rarely chronic carriers may develop gall bladder stone (Boyle *et al*, 2007). Some patients may harbor *Salmonella* species in stool or urine for periods of 1 year or longer but remain asymptomatic. Approximately 3% of patients with typhoid fever and 0.2–0.6% of persons with non-typhoid *Salmonella* gastroenteritis will have positive stool cultures for more than 1 year where the organism present in the gallbladder, biliary tract, or rarely the intestine or urinary tract (Vandepitte *et al*, 2003). Mary Mallon (1869 –1938), also known as Typhoid Mary, was the first person in the United States to be identified as a healthy carrier of typhoid fever. Over the course of her career as a cook, she is known to have infected 53 people, three of whom died from the disease (Wikipedia, 2010). *Salmonella enterica* serovar *Typhi* can establish a chronic, asymptomatic infection of the human gallbladder, suggesting that this bacterium utilizes novel mechanisms to mediate enhanced colonization and persistence in a bile-rich environment by forming a biofilm. Bacteria reaching the gallbladder can induce an active local infection (cholecystitis) or exist asymptotically in a chronic carrier state (Crawford *et al*, 2008). The chronic typhoid carrier state can occur following symptomatic or subclinical infections of *Salmonella typhi*. Among untreated cases, 10% will shed bacteria for three months after initial onset of symptoms and 2-5% will become chronic carriers. The chronic carrier state occurs most commonly among middle age women (CDC, 2010). Bacteria shed by asymptomatic carriers contaminate food and water and account for much of the person-to-person transmission of serovar *Typhi* in underdeveloped countries (Vandepitte *et al*, 2003; Crawford *et al*, 2008). In Namakkal, India, screening of *Salmonella typhi* in asymptomatic typhoid carriers among suspected food handlers reported that among 35 samples, 6(17.14%) yielded a positive result. Out of these 4 (20.0%) were women and 2 (13.33%) were men. Five isolates were having the multidrug resistant character for conventional antibiotics: four (66.66%) multidrug resistant isolates were found to have plasmids, while one (16.66%) multidrug resistant isolate had no plasmid and the chromosome encoded the resistance and only one strain (16.66%) showed single antibiotic resistance in the study and had no plasmid DNA (Senthilkumar *et al*, 2005). This study shows that food handlers can be a source of drug resistant strains. In Kyushu, Japan, *Salmonella* were isolated from 0.032% of fecal samples from food handlers in Japan to determine the incidence and features of *Salmonella* serovars among food handlers. *S. enterica* subspecies *enterica* serovar *Infantis* (*S. serovar infantis*) was the dominant serovar (accounting for 48.1%), followed by *S. corvallis*, which showed poor genetic diversity, and *S. enteritidis* among food handlers (Murakami *et al*, 2007). A survey for *Salmonella* carriers in the Chinese army recruits was made. *Salmonella* was detected in 1.83% of the rectal swabs collected from 1,150 recruits and 50 cooks. Among the 22 isolates 5, 1, 5, and 6 strains were identified as *Salmonella* groups: B, C1, D and E1 respectively and 5 strains were ungroupable. *Salmonella* isolates were found to be susceptible to chloramphenicol, kanamycin, Ampicillin and tetracycline (Show *et al*, 1982). A study in Kumasi, Ghana, to determine the prevalence of chronic typhoidal salmonellae among food Vendors, Typhoidal *Salmonellae* were isolated from six people, giving a carriage rate of 2.3%. Three of the *Salmonellae* isolated were *S. typhi* (Feglo *et al*, 2004). Around 12.9% food handlers were suffering from intestinal parasitic infestation, out of which 42.81% were contributed by *Entamoeba histolytica*. Only one person (0.47%) was found to have *S. typhi* in stool sample while 28(13%) were Vi Reactors by agglutination test. The main deficiencies in personal hygiene were poorly kept nails, dirty working clothes, lack of foot-wear, irregular bathing & not brushing teeth

amongst Food Handlers in Amritsar City, India (Mohan *et al*, 2006). A survey of food handlers in a restaurant in Lagos, Nigeria about typhoid fever found more than half (62.2%) washed their hands with water only before eating while 27.7% did not wash their hands always before preparing food. After using toilets, 71.9% washed their hands with soap and water while 28.1% washed their hands with only water (Smith *et al*, 2010). Screening of asymptomatic typhoid carriers from nail samples of roadside foodhandlers in Tamilnadu, India, showed that all the five *S.typhi* isolated were resistant to Ampicillin, four to amoxicillin and one to chloramphenicol but none to gentamycin (Valli *et al*, 2010). In Ethiopia, there have been several studies conducted on salmonellosis which suggest an increase in the antibiotic resistance of *Salmonella* to commonly used antimicrobials in both the public health and veterinary sectors (Gebre-Yohannes, 1985). A study to identify the prevalent serovars and their susceptibility to drugs in Addis Ababa between January 1974 and October 1981 indicates that, of 216 *Salmonella* isolates studied, 54.6% were from stools and 45.4% from invasive sites: blood 34.7%; pus 5.6%; and urine 5.1%. There were 26 different serovars, of which *S. typhi* (48.6%) was the most common, followed by *S. concord* (12.5%), *S. typhimurium* (11.1%) and *S. paratyphi B* (5.6%). The high isolation rate of *S. concord* in Ethiopia is unusual and is in contrast to the other regions in Africa where *S. typhimurium* or *S. enteritidis* are more common (Beyene *et al*, 2008). By 1995, 28.6% of *S. typhi* isolates were resistant to chloramphenicol (Ryan *et al*, 2004) and in 2000, the most recent study, reports that 30.8% of the isolates of *S. typhi* in Jimma were resistant to chloramphenicol, 54% to Ampicillin, and 38% to co-trimoxazole (Mache, 2002). These data show clearly the emergence of a significant resistance problem in the last decade in *S. Typhi* isolated in Ethiopia, especially in Jimma. Fingernail contents of both the hands and stool specimens were collected from all the 127 food-handlers working in the cafeterias of the University of Gondar and the Gondar Teachers Training College, Gondar, Ethiopia. The samples were examined for bacteria and intestinal parasites following standard procedures. Coagulase-negative staphylococci were the predominant bacteria species (41.7%), followed by *Staphylococcus aureus* (16.5%), *Klebsiella* species (5.5%), *Escherichia coli* (3.1%), *Serratia* species (1.58%), *Citrobacter* species (0.8%), and *Enterobacter* species (0.8%). *Shigella* species were isolated from stool samples of four food-handlers (3.1%). None of the food-handlers was positive for *Salmonella* species and *Shigella* species in respect of their fingernail contents (Andargie *et al*, 2008). Among 384 food handlers working in different food services establishments, such as hotels, restaurants and snack bars in Bahir Dar, 158 (41.1%) food handlers had intestinal parasites and 6 (1.6%) were found positive for *S. typhi* where 33.3%, 16.6%, 83.4%, 66.7%, 33.3% and 100% were resistant to chloramphenicol, Norfloxacin, Cotrimoxazole, tetracycline, gentamycin Ampicillin respectively. Of these, 25 (6.5%) were suffering from diarrhea (Abera *et al*, 2010). Among 206 apparently health foodhandlers working in bukkas in Lagos, Nigeria, *Salmonella* species were isolated from 17% of the stool samples obtained from the food handlers in which *S. typhi* (6.8%), *S. enteritidis* (5.3%), *S. choleraesuis* (2.9%), *S. paratyphi A* (1.5%) and *S. arizona* (0.5%). The organisms were completely resistant to tetracycline, Ampicillin and amoxicillin (Smith *et al*, 2009). In general, many studies proved that foodhandlers can be a source of food outbreak infections especially bacterial infections. Salmonellosis is one of the most food and water outbreak infections following contamination of food or water by the organism which can be obtained from symptomatic or asymptomatic carriers. In this study, therefore, the 3.4% isolation rate of *Salmonella* species among apparently healthy foodhandlers serving in AAU students' cafeteria can be a possible source of salmonellosis for the students unless positives treated or other preventive measures taken. Food handlers play a prominent role in food safety and transmission of *Salmonella* and so it is important that the food handlers are well informed about their hygiene status and the causes of salmonellosis transmission and ways by which its spread is prevented. Therefore, foodhandlers at public sectors like universities, hotels, restaurants should be trained and medically checked for possible pathogens like *Salmonella*. All *Salmonellae* positives and most of foodhandlers (82.8%) in students' cafeteria were females. This may also increase the risk of transmission to the students they feed unless intensive preventive measures undertaken. As a whole, foodhandlers in AAU students' cafeteria had a good habit of hand washing with or without soap. It was found that washing hands with soap reduces the chance of *Salmonellae* isolation rate by 93% than washing only with water ($p = 0.003$). Antimicrobial sensitivity of *Salmonella* organisms is crucial for treatment of *Salmonella* infections. Increasing antimicrobial resistance of *Salmonella* with no doubt may result in higher death to case ratios for resistant *Salmonella* infections than for infections with sensitive strains. In whatever way, this study shows that all of the *Salmonellae* isolates had a resistance at least to one of the antimicrobials tested.

CONCLUSION

Finally, we recommended the university to:

- Provide constant & periodic focused medical checkup for *Salmonella* species; preferable if twice a year
- Provide immediate treatment for diagnosed foodhandlers and check again after treatment
- Arrange further training and health education concerning personal and food hygiene
- Provide all food handlers with soap and other sanitary items for maintenance of personal hygiene and aware them to use it always especially after toilet

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