academicJournals

Vol. 11(16), pp. 631-636, 28 April, 2017
DOI: 10.5897/AJMR2016.8382
Article Number: A36EC6164035
ISSN 1996-0808

http://www.academicjournals.org/AJMR

African Journal of Microbiology Research

Full Length Research Paper

Prevalence and antibiotics resistance patterns of *Salmonella* isolated from kitchen sponges at Jimma town, Ethiopia

Tesfaye Wolde[1*] and Ketema Bacha[2]

[1]Department of Biology, College of Natural and Computational Sciences, Wolkite University, P.O. Box 07, Wolkite, Ethiopia.
[2]College of Natural and Computational Sciences, Jimma University, P.O. Box 378, Jimma, Ethiopia.

Received 14 November, 2016; Accepted 15 March, 2017

It is identified that through the cleanout practice of utensils, dishes, etc. in kitchens, the before washing and after washing activities are done with the use of sponges to remove food remains. These food residues along with the wetness in the sponges tender an encouraging environment for microbial proliferation. Sponges and tea towels used in cleaning equipments and utensils have been known as possible agents in the spread of microbes and it has been pragmatic that bacteria stick to these vehicles. Evaluation on the prevalence of *Salmonella* spp. from kitchen sponges was conducted from October, 2010 to June, 2011. The sponges used on a daily basis in food establishments were studied for the incidence of *Salmonella* spp. A total of 201 sponge samples from restaurants, hotels, cafeterias and pastry shops were included in the study. Antibiotic resistance patterns of *Salmonella* isolates were done using nine antibiotics selected on the basis of accessibility and present use in Ethiopia. The results show that 11.9% of the kitchen sponges were found to have *Salmonella*. Frequencies of isolation of *Salmonella* differed among the establishment types and it varied from 10 (restaurants) to 12.8% (cafeterias). Noteworthy, deviation in prevalence of *Salmonella* among restaurants, hotels, pastry shops and cafeterias (p=0.023) were statistically significant. Ampicillin and nalidixic acid were the most resisted drugs. Five drug resistance patterns were distinguished among *Salmonella* isolates. These results demonstrate the risk posed by the daily use of kitchen sponges in food establishments' vis-à-vis *Salmonella*. Awareness creation training on basic hygienic practices to personnel's working in food establishment, frequent change of sponges being used in kitchens, and monitoring of safety practices of the establishments are recommended.

Key words: *Salmonella* sp., prevalence, antibiotic resistance, kitchen sponges, Jimma town.

INTRODUCTION

It is identified that through the cleanout practice of utensils, dishes, etc. in kitchens, the before washing and after washing activities are done with the use of sponges to remove food remains. Due to this practice, fraction of

*Corresponding author. E-mail: tesfalem2002@gmail.com.

the food remains sticks to the sponge's exteriors (Speirs et al., 1995). These food remains along with the wetness maintained in the sponges give an encouraging situation for microbial growth. Previous studies on microbial contamination in the kitchen were done in the late 1960s, assessing bacterial load of hand towels and the sanitary conditions of household tea towels and dishcloths (Speirs et al., 1995). Such cloths were seriously contaminated with bacteria and supposed as one of the main vectors for spreading of the bacteria in the kitchen (Speirs et al., 1995). The existing interest on bacterial contamination in the kitchen was on track in the 1970s. Preceding investigations have recommended that although raw material is most likely the main source of contamination in the kitchen, the area near the kitchen could also be sources of free living bacteria (Scott and Bloomfield, 1990). Sponges and dishcloths have been recognized as potential agents in the spread of microorganisms, it has been observed that bacteria persist in these vehicles (Speirs et al., 1995; Scott and Bloomfield, 1990).

According to the proclamation stated in Codex Alimentarius Commission, states that "sufficient, secure, sound and nutritious food is a very important part for the success of adequate standards of living, and that the right to a typical of living enough for the health and welfare of the individual and his family is stated in the Universal Declaration of Human Rights of the United Nations (UNHR). Regular food establishments' checkup and control is a task of the Ethiopian Health and Food Regulatory Authority. Regardless of this, it is likely that there is a threat of Salmonellosis associated with during food served at food establishments in Jimma town. It is anticipated that there are 22 million new cases of enteric fever yearly, with 200,000 deaths (Crump et al., 2004). It is one of the main causes of human gastro-intestinal diseases. There are factors contributing to its pathogenicity. These factors comprise of adaptive ability of pathogen, altering features of the population, alarmingly increase in globalization and change in the life style of the consumer (Hald, 2015)

A study based in the United States (Ackers et al., 2002) shows an increase in the number of multi-drug resistance (MDR) and nalidixic acid resistant *Salmonella* internationally, though all isolates remained sensitive to ceftriaxone and ciprofloxacin. There has been a reported decrease in MDR isolates with no corresponding increase in sensitive strains in Bangladesh (Rahman et al., 2002). For strains imported to United Kingdom (Threlfall and Ward, 2007) and Bangladesh (Asna et al., 2003), there has been an increase in MIC toward ciprofloxacin. The prevalence of *Salmonella* species was 17.3% among butcher shop premises and utensils in Gonder town (Legesse et al., 2015), the current study was relatively better with respect to the prevalence of this microbe. This may be due to the difference in sample source as well as sample processing methods. These observations with variations in the sensitivity patterns reported for *Salmonella* shows the importance of continuous examination of antibiotic sensitivity patterns to supply appropriate strategy for treatment. Generally, irrational use of antimicrobials in animal and human medicine has been known as a contributing factor in emergence of antimicrobial resistant pathogens and evolves MDR strain. The alarmingly increasing level of drug resistant *Salmonella* has become a problem in many countries, tending to have high level of resistant *Salmonella* (Wolde et al., 2016; Hald, 2015). Ethiopia is socio-economically underprivileged county where both personal and community hygiene are negligible. According to records of public and private hospitals, enteric fever is major infectious disease occurring at high unpredictable frequency.

The aim of this study was to evaluate microbiological safety of kitchen sponges used in food establishments in terms of *Salmonella* prevalence and antibiotics resistance patters. Hence, the study would be used as primary source of information for other related studies.

MATERIALS AND METHODS

Sampling

This study was conducted from 1st October, 2010 to 1st June, 2011 in Jimma Town (Ethiopia). The sample size was determined using the principle taking $Z\alpha / 2$ with a confidence level of 95%. In total, two hundred and one synthetic sponges used in daily household use were collected at erratically selected food establishments in the city (hotels, 101; restaurants, 20; cafeterias, 47; and pastries, 33).

The samples (201 sponges) packaged in sterile polyethylene bags were identified by label and placed directly in a cold chain for transportation. These samples were immediately forwarded to the research laboratory of college of Natural Science, Jimma University. Their contents were analyzed in one to three hours after their arrival in the laboratory.

Sample preparations and microbiological analysis

In the laboratory, 25 mm^3 (determined using detection limit) of each sample was aseptically transferred into sterile flask containing 225 ml buffered peptone water (BPW) (Oxoid, England), mixed for 5 min and incubated at 37°C for 24 h for revival and growth of cells which might be ill-treated during processing or to make number of target organisms grow to measurable level. After crucial enhancement using BPW, secondary enrichment broth namely Rappaport Vassiliadis (Oxoid, England) enrichment broth were used due to the fact that the selective property of this broth lies in its ability to inhibit non-targeted microorganisms and permit the rapid multiplication of *Salmonella*. Accordingly, after pre enrichment in buffered peptone water, 1 ml of culture from the buffered peptone water was transferred into 10 ml of Rappaport Vassiliadis broth and was incubated at 43°C for 48 h.

Salmonella-Shigella agar, xylose lysine desocholate (XLD) agar and Brilliant Green Modified agar were used for plating purpose. A loopful of culture from Rappaport Vassiliadis broth was streaked onto each of the solid medium and incubated at 37°C for 18 h. Typical colonies from each discriminatory agar were picked, auxiliary purified and biochemically tested.

Table 1. Prevalence of *Salmonella* spp. from kitchen sponges of different establishments type.

Establishments type	Sample size	Number of *Salmonella* positive	*Salmonella* positive (%)	P value
Restaurant	20	2	10	
Hotels	101	12	11.9	
Pastry shops	33	4	12.1	p= 0.023
Cafeteria	47	6	12.8	
Total	201	24	11.9	

All supposed non-lactose fermenting bacterial colonies, selected from Salmonella-Shigella (SS) agar, xylose lysine desocholate (XLD) agar or Brilliant green modified agars were inoculated into the subsequent biochemical tubes for identification: Simmon's Citrate agar, urea agar, lysine iron agar, triple sugar iron agar, SIM medium and fermentation of mannitol, sucrose and glucose (Oxoid, England).

Antimicrobial susceptibility testing

The antibiotics resistance patterns of isolates were determined according to Kirby Bauer disc diffusion method as depicted by National Committee for Clinical Laboratory Standard (NCCLS) (Cheesbrough, 2006). Nine standard antibiotics used included: ampicillin (10 µg), chloramphenicol (30 µg), gentamycin (10 µg), streptomycin (10 µg), kanamycin (30 µg), nalidixic acid (30 µg), ciprofloxacin (5 µg), tetracycline (25 µg) and norfloxacin (10 µg). The test antibiotics were selected based on availability and current use in Ethiopia. Mueller-Hinton agar was inoculated with overnight culture adjusted to 0.5 McFarland standards. Six antibiotic discs per plate were aseptically laid on the surface of the agar using sterile tweezers. Plates were incubated for 24 h at 35°C. Zones of inhibition were measured using transparent ruler. Standard strains used as reference were kindheartedly got from Ethiopian Health and Nutrition Research Institute (EHNRI).

Statistical analysis

Data entrance and scrutiny were done by the program statistical analysis using SPSS, version 16.0. Descriptive statistical method was applied to determine the *Salmonella* prevalence. Frequency and percentages were computed to describe the relevant variables (prevalence, duration of kitchen sponge usage and food establishment types). P-value of 0.05 was considered as limit for statistical significance to evaluate difference in prevalence of *Salmonella* among the four food establishment types.

RESULTS

Prevalence of *Salmonella*

From the 201 kitchen sponge samples examined, 24 (11.9%) kitchen sponges from food establishments were found to be positive for *Salmonella*. *Salmonella* were isolated from 2 (10%) kitchen sponges of restaurants, 12 (11.9%) kitchen sponges of hotels, 4 (12.1%) kitchen sponges of pastry shops and 6 (12.8%) cafeterias kitchen sponges. Frequencies of isolation of *Salmonella* varied among the food establishment types and ranged from 10 (restaurants) to 12.8% (cafeterias). Statistical scrutiny showed the presence of noteworthy dissimilarity in prevalence of *Salmonella* among kitchen sponges of restaurants, cafeterias, hotels and pastry shops (p= 0.023) (Table 1).

Antibiotics resistance of *Salmonella*

Totally, 24 isolates were evaluated against nine frequently used antibiotics. Amongst all antibiotics tested, high resistance was recorded for ampicillin and nalidixic acid followed by kanamycin, tetracycline and chloramphenicol. On the other hand, norfloxacin, gentamycin and ciprofloxacin showed strong activity against the test Salmonella and were the only antibiotics not resisted by any isolates (Table. 2).

Five drug resistance outlines were noticed amongst the *Salmonella* isolates from kitchen sponges. Out of 24 isolates, 10 (41.7%) were resistant to 3 antimicrobials, 8 (33.3%) were resistant to 4 antimicrobials, while 4 (16.7%) were resistant to 2 antimicrobials, only 1 (4.2%) isolate was resistant to only 1 antibiotic, and 1 (4.2%) was resistant to 5 antimicrobials (Table 3).

DISCUSSION

There are no published literatures on microbiological safety problems associated with kitchen sponges applied in food establishments of Jimma town preceding this study. Consequently, the results of this study are argued, compared and contrasted to related studies in other countries.

Epidemics of food poisoning recurrently occur because of unseemly food preparation in which cross-contamination in combination within sufficient storage or cooking is concerned with many occasions (Olsen et al., 2000). Sponges were documented as a probable source for spreading of microbes and it was experimental that bacteria existed in these vehicles (Rusin et al., 1998). From the results of the present study, about 12% of the sponges from food establishments of Jimma town have *Salmonella* spp. This indicates that the prevalence is very

Table 2. Antibiotic resistance of *Salmonella* isolates of kitchen sponges by catering establishment types (n=24).

Antibiotic disc	Number (%) of resistant isolates from kitchen sponges (24)				
	Total isolate (n=24)	Restaurant's (n=2)	Hotel's (n=12)	Pastry shop's (n=4)	Cafeteria's (n=6)
AMP	24 (100)	2 (100)	12 (100)	4 (100)	6 (100)
STR	2 (8.3)	0 (0)	2 (16.7)	0 (0)	0 (0)
NOR	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
TET	7 (29.2)	0 (0)	5 (41.7)	1 (25)	1 (16.7)
KAN	16 (66.7)	1 (50)	8 (66.7)	3 (75)	4 (66.7)
GEN	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
CHL	6 (25)	0 (0)	3 (25)	1 (25)	2 (33.3)
CIP	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
NAL	21 (87.5)	1 (50)	11 (91.7)	4 (100)	5 (83.3)

AMP: Ampicillin, CIP: Ciprofloxacin; CHL: Chloramphenicol; GEN: Gentamycin; KAN: Kanamycin; NAL: Nalidixic Acid; NOR: Norfloxacin; STR: Streptomycin; TET: Tetracycline.

Table 3. Multi drug resistance pattern of *Salmonella* from kitchen sponges of food establishments.

MDR pattern	Resistance pattern	Number of isolates	Percent (%)
One	Amp	1	4.2
Two	Amp, Nal	3	12.5
	Amp, Str	1	4.2
Three	Amp, Kan, Nal	7	29.2
	Amp, Tet, Nal	1	4.2
	Amp, Chl, Nal	1	4.2
	Amp, Str, Chl	1	4.2
Four	Amp, Tet, Kan, Nal	1	4.2
	Amp, Tet, Kan, Nal	4	16.8
	Amp, Kan, Chl, Nal	3	12.6
Five	Amp, Tet, Kan, Chl, Nal	1	4.1

AMP: Ampicillin, CIP: Ciprofloxacin; CHL: Chloramphenicol; GEN: Gentamycin; KAN: Kanamycin; NAL: Nalidixic Acid; NOR: Norfloxacin; STR: Streptomycin; TET: Tetracycline.

high because washing materials are used until worn-out. This is one reason that makes kitchen sponges unsafe in terms of the microorganisms found there.

In terms of *Salmonella* prevalence in kitchen sponge's samples from four food establishment in this study showed that there was considerable dissimilarity. Comparatively, high prevalence of *Salmonella* was identified from cafeterias and pastry shops. This might be because of the extended utilization of kitchen sponge in contrast with other establishment types. In actuality, the hygienic conditions of the kitchen among pastry shops during sample collection were better.

A study conducted in India identified bacteria isolated from kitchen's air in rural areas known to be virulent with disease causing ability. In other words, bacteria from kitchens in urban areas were commonly safe and caused food spoilage apart from *Acinetobacter* spp. (Shruti et al., 2011). The disparity in prevalence of *Salmonella* spp. amongst the four food establishment types in this study may be due to variation in extent of using kitchen sponges. This is much lower than the report from kitchen sponges reported elsewhere (Enriquez et al., 1997). A study by Shruti et al. (2011) showed that, probably detrimental pathogens simply exist in every individual through contaminated premises present in kitchens (Enriquez et al., 1997). This is in agreement with the report of Scott et al. (1982), De Boer and Hahne (1990) and Josephson and Rubino (1997). Bacterial profile of sponges usually used in kitchens were also reported (Suaad, 2007). Thus, the detection of *Salmonella* spp. in the kitchen sponges sample investigated in this study could indicate inappropriate sanitizing practice or post

sanitizing/washing contamination due to improper handling. Contamination may occur in kitchen washing water. Therefore, the presence of *Salmonella* spp. in kitchen sponges could indicate the possible public health risk from using utensils washed by kitchen sponges due to cross contamination from sponges to utensils (Erdogrul and Erbilir, 2006).

In a laboratory test of kitchen, *Salmonella* spp. were reportedly transferred during the handling of chickens to food contact surfaces and cleaning cloths (Cogan et al., 2002; Gorman et al., 2002). Reports have shown that *Salmonella* spp. are generally only detected on cleaning cloths/kitchen sponges if they are sampled during or directly after food preparation (Beumer et al., 1996). Since, the food establishments enrolled in this study had used kitchen sponges without sanitizing every day, despite this, they harbored this bacterium. In a study by Christison et al. (2006), cleaning cloths were sampled during or directly after ready to eat food preparation and 8% were subsequently positive for *Salmonella* spp. Prevention and managing Salmonellosis in the food business by different means such as enhanced bio-security and the beginning of original immuno-potentiators with narrow achievement has required the use of antimicrobial chemotherapy (Zhao et al., 2007).

The situation in Jimma town may be embellished by effortless ease of access to antimicrobials at every pharmaceutical shop without physician's prescription and their extensive use. An additional key hinder might be the strength and eminence of produced antimicrobial drugs; for instance, there are over 80 variety of the flouroquinolones and ciprofloxacin (Hart and Kariuki, 1998). As a result, there is extensive accessibility and indiscriminate utilization of antibiotics by the community.

The appearance and perseverance of antimicrobial resistance is driven by various factors including the unsystematic use of antibiotics and inconsistent drug efficacy presenting a major threat to the control of infectious diseases (Omulo et al., 2015). In this study, 100% of the isolates were resistant to ampicillin followed by nalidixic acid (87.5%), kanamycin (66.7%), tetracycline (29.2%), chloramphenicol (25%) and streptomycin (8.3%). All the tested isolates were susceptible to ciprofloxacin, norfloxacin and gentamycin. Whereas, nalidixic acid resistance is similar with the prevalence of 92-96% reported in India (Lakshmi et al., 2006). Other commonly used aminoglycoside antibiotics such as gentamycin showed good activity against Gram-negative bacteria in Europe, but exhibited only 48% to enterobacteriaceae in India (Lakshmi et al., 2006).

Aminoglycoside has been found to be ineffective *in vivo* against *Salmonella* despite its activity *in vitro*; therefore, it is not recommended for treatment of *Salmonella* infections such as typhoid fever (CLSI). The high antibiotics resistance pattern of the *salmonella* isolates to some drugs in this study might be due to indiscriminate use of different antibiotics in the study area.

Chloramphenicol sensitivity was approximately 75%. The high prevalence of resistance to nalidixic acid among the isolates (87.5%) was also mentioned in France (Cailhol et al., 2005). Among flouroquinolones, ciprofloxacin resistance was found to be relatively lesser in this study as it contrasted with 9.6% at Austria (Mayrhofer et al., 2004), 10.2 to 16.8% at Germany (Malorny et al., 2003) and 35% resistance at USA (Cui et al., 2005). The undetected ciprofloxacin resistance among *Salmonella* isolates (0%) from this study may be due to the less accessibility of this antibiotic in the study area as well as in the country (Ethiopia).

In general, the presence of microbial groups on cleaning tools is undesirable as the cleaning tools may disseminate bacterial pathogens throughout the food establishments. Therefore, kitchen sponges may potentially act as reservoirs for the contamination of foods in food establishments. There is a necessity to alert dining establishment workers, managers, customers, and all others who in one way or another utilize sponges in food establishments about the hygienic and proper treatment of these materials.

Conclusions

The increased prevalence of *Salmonella* spp. isolates shown in this study showed that kitchen sponges used in establishments on a daily basis have been known as probable agents in the spread of microbes, and it has been pragmatic that bacteria stick to these vehicles. Kitchen sponges should not be used for prolonged period of time, unless they are sanitized properly. The antibiotic resistance pattern on *Salmonella* spp. isolated from kitchen sponges also showed high resistant patterns in some presently approved drugs and all the tested isolates were susceptible to ciprofloxacin, norfloxacin and gentamycin. Practice of arbitrary use of drugs should be controlled. Additional studies that could add to isolation of *Salmonella* spp. should be conducted to assess the forthcoming danger caused by microbes from kitchen sponges and their antibiotic resistance patterns. There had been higher prevalence of *Salmonella* spp. obtained from pastry shops; this is mainly due to prolonged use of kitchen sponges.

CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest.

ACKNOWLEDGEMENT

The authors appreciate Jimma University for funding this research.

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