Microbiological quality and safety of Ready- to- eat foods from restaurants and food establishments in Yirgalem town Southern, Ethiopia

Tsegaye Shamebo Arficho (tsegayeshamebo0@gmail.com)
Yirgalem Hospital Medical College
Asefa Hamato Kebede
Yirgalem Hospital Medical Colleg

Research article

Keywords: Microbial quality, Yirgalem town, Southern Ethiopia

Posted Date: August 30th, 2019

DOI: https://doi.org/10.21203/rs.2.13735/v1

Abstract

Background: Foodborne illnesses are considered as one of the most important public health problems particularly in developing countries like Ethiopia. This study aimed to determine the microbiological quality and safety of ready-to-eat foods in Yirgalem town, southern Ethiopia from November 2016 to August 2017. Methods: The collection of ready-to-eat food samples and laboratory-based microbiological analysis was used as the study design. A total of 160 food samples comprising of 40 'Injera firfir', 40'Bayeaynet', 40 Vegetables and 40 Spaghetti were collected and analyzed for microbial contamination following standard microbiological methods. Ten grams of each food sample was transferred into 90 ml of buffered peptone water and homogenized for 5 minutes using a vortex mixer. The homogenates were serial diluted up to 10-7 and a volume of 0.1ml aliquot was spread plated on pre-solidified media of Aerobic plate count agar, MacConkey agar, Mannitol salt agar, and Salmonella-Shigella agar and incubate at 35-37oc for 24 hrs. Also, Potato Dextrose Agar was used for the isolation of fungi. Data were entered into Microsoft Excel and analyzed using SPSS version 20.0. Results: All the collected food samples were subjected to total aerobic mesophilic bacteria, Coliform bacteria, Enterobacteriaceae, Staphylococcal, Yeasts, and Molds counts. Accordingly, the mean counts expressed as log10 CFU/g of food for each group of the organism were 7.90 ± 0.71, 4.31±1.30, 4.32 ± 1.30, 6.70 ± 0.34 and 4.5 ± 1.01, respectively. The highest bacterial load 162(28.9%) was detected in 'Injera firfir' whereas the lowest 108(19.2%) case was investigated in Spaghettis. Regarding the food safety issue, the frequency of S. aureus, E. coli and Salmonella spp in the food samples were 54.4%, 43.8%, and 0.6%, respectively. Conclusion: The high microbial load and existence of foodborne pathogens in ready-to-eat foods in Yirgalem town, Southern Ethiopia is calling for the creation of awareness among restaurant and food establishment owners and food handlers concerning the hygienic practice. Keyword: Microbial quality, Yirgalem town, Southern Ethiopia

Background

The fight against foodborne diseases is facing new challenges due to the globalization of the food market, climate change and changing patterns of human consumption [1]. The foodborne disease remains a major source of morbidity and mortality in the general population, mainly in susceptible groups, such as infants, elderly and the immunocompromised people [2]. According to the World Health Organization (WHO), 2007 report, up to 1.5 billion cases of diarrhea and more than 3 million deaths that occur in children every year are as a result of food and water contamination[3]. In the United States of America (USA) it is estimated that foodborne diseases result in 76 million illnesses, 325,000 hospitalizations and 5,000 deaths each year [3,4].

Although foodborne illnesses cause substantial morbidity in developed countries, the main burden is borne by developing nations. In Southeast Asia, approximately 1million children below the age of five years die each year from diarrheal diseases due to contaminated food and water [5]. Several devastating foodborne outbreaks have been reported from the African continent; for example, in 2004, Kenya experienced an acute aflatoxicosis outbreak which was attributed to maize whereas, in 2007, Angola registered 400 cases of bromide poisoning, associated with the use of sodium bromide as cooking salt [6].

Foodborne illness is also one of the common problems in Ethiopia because of the prevailing poor food handling and sanitation practices, inadequate food safety laws, weak regulatory systems, lack of financial

resources to invest in safer types of equipment, and lack of education for food-handlers [7]. National Hygiene and Sanitation Strategy program [8] reported that about 60% of the disease burden was related to poor hygiene and sanitation in Ethiopia. Studies conducted in different parts of the country have shown that the poor sanitary conditions of catering establishments and the presence of pathogenic organisms like *Campylobacter*, *Salmonella*, *Staphylococcus aureus*, *Bacillus cereus* and *Escherichia coli*, [9, 10, 11, 12, 13] make foodborne illness more worsen. More aggravated situations and challenges prevailing in the country where food safety issues are not well understood and have received little attention. Therefore, this study aimed to assess the microbiological quality and safety of ready-to-eat foods from restaurants and food establishments in Yirgalem town, Southern Ethiopia.

Methods

Study area and period

The study was conducted at Yirgalem town, located 275 km south of Addis Ababa; the capital city of Ethiopia. The town's geographical coordinates are 7°41'N latitude, 36°50'E longitude, and an average altitude of 1, 780 m above sea level. It lies in the climatic zone locally known as "Woyna Daga" (1,500-2,400 m above sea level) which is considered ideal for agriculture as well as human settlement. The town is generally characterized by a warm climate with a mean annual maximum temperature of 30°C and a mean annual minimum temperature of 14°C. The annual rainfall ranges from 1138-1690 mm. The maximum precipitation occurs during the three months from June through August, with minimum rainfall occurring in December and January. The study was conducted between November 2016 to August 2017.

Sample collection

A total of 160 food samples comprising of 40 Bayeaynet, 40 Injera firfir, 40 Vegetables, and 40 Spaghetti, were collected from different restaurants and food establishments in Yirgalem town, southern Ethiopia between November 2016 and August 2017. Food samples were collected using food serving utensils and placed into a sterile aluminum plate at a time between 5 PM and 6 PM after a brief description of the study purpose for the restaurant and food establishment owners. The samples collected were transported to the Yirgalem Hospital Medical College, Microbiology Laboratory in the date of collection using icebox and were kept in the refrigerator at 4°C until microbial analysis was conducted. The microbial analysis was conducted within one to three hours of collection.

Sample processing

Twenty-five grams of each food sample was cut into small pieces using food cutter and was added into 225 ml of 0.1% sterile peptone buffer water and homogenized for 2 minutes using a stomacher. One ml of the resultant homogenate was added into 9 ml of sterile 0.85% saline in a test tube and serially diluted up to 10^{-7}.

Microbial analysis

Approximately 0.1 ml aliquot portions of the dilutions were spread onto duplicate sterile plates of plate count Agar, MacConkey Agar, Salmonella-Shigella Agar and Mannitol salt Agar, for total aerobic plate count,

Coliform count, Enterobacteriaceae count, isolation of Salmonella and Shigella and isolation of *Staphylococcus aureus,* respectively. Also, Potato Dextrose Agar was used for the isolation of fungi. All cultures were incubated at 37^{o}c for 24-48 hrs except that of Potato Dextrose Agar which was incubated for 3 to 5 days at 25^{o}c. All the culture plates were checked for microbial growth when the appropriate time of the incubation was achieved. Thereafter, colonies between 30-300 were counted using colony counter (Gallenkamp, England) and the counts were expressed as colony-forming units per gram (CFU/g) of food. Cultures were purified by repeated plating and maintained on appropriate slants at 4^{0}C (14). Finally, the pure isolates were identified through gram staining and biochemically using (Catalase test, Oxidase test, coagulase test, Triple sugar iron agar, Lysine iron agar, Urea agar, Simmons Citrate agar, and SIM media) following standard microbiological methods (15, 25). Moreover, fungal isolates were identified based on their macroscopic and microscopic features. Reference was made standard identification keys and atlas.

Coliform test

Presumptive test: One gram of each food sample was transferred to sterile McCartney bottles containing Lactose broth and inverted Durham tube. Then, the tubes were incubated at 37^{o}c for 24-48 hrs. Tubes showing gas production and/or color change of dye were streaked on Eosin Methylene Blue plates. The plates were incubated at 37^{o}c and 44^{o}c for 24 hrs for **a confirmatory test**. Colonies grown on the Eosin Methylene Blue plates were picked and inoculated into tubes containing lactose broth for a **complete test** and onto Nutrient Agar slants for further characterization. The inoculated slants and lactose broth tubes were incubated at 37^{o}c for 24 hrs.

Statistical Analysis

The data obtained were analyzed using SPSS software version 20.0. The significance of differences was considered at a 95% confidence interval ($P < 0.05$).

Ethical Consideration

The study was approved by the Institutional Review Board of Yirgalem Hospital Medical College and an official permission letter was obtained from the Yirgalem town Hotel and tourism office. Written informed consent was also obtained from restaurant and food establishment owners after a brief explanation of the objectives and benefits of the study.

Data quality control

Aseptic techniques were employed in every step of the study. Samples were transported in an icebox and were processed without delay and in replicates. To ensure sterility, materials and media were autoclaved at 121^{o}c for 15 to 20 minutes. The media were checked for sterility by incubating 5% of the batch at 37^{o}c for 18-24 hrs.

Operational definitions

Ready-to-eat foods: Foods that are available directly for consumption without further processing

Microbiological quality of food: a load of microbes existing in a certain food item

Microbiological safety of food: Safeness of foods ready for consumption from foodborne pathogens

Results

A total of 160 different food samples comprising of 40 Injera firfir, 40 Bayeaynet, 40 Vegetables, and 40 Spaghetti were collected from restaurants and food establishments in Yirgalem town southern Ethiopia and subjected to microbiological analysis. Accordingly, total Aerobic mesophilic bacteria count (TAMC), total coliform bacteria count (TCC), total Enterobacteriaceae count (TEC), total Staphylococcal count (TSC), total Yeast (TYC) and Mold counts (TMC) were carried out. The results have shown that the mean counts (expressed as log10 CFU /g of food) were 7.90 ± 0.71, 4.31±1.30, 4.32 ± 1.30, 6.70 ± 0.34, 4.50 ±1.01 and 4.50 ±1.01, respectively (Table 1).

A total of 560 AMB were isolated from the collected food samples. The isolates were included *Staphylococcus* spp 279(49.82%), EB 125(22.32%), Coliform70 (12.50%), *Micrococcus* spp 32(5.71%), *Pseudomonas* spp 27(4.82%) and *Acinetobacter* spp 27(4.82%) (Table 2). The highest isolates were detected in Injera firfir followed by Bayeaynet.

The overall prevalence of *S. aureus* was 54.37% which comprises; Injera firfir; 87.5%, Bayeaynet; 70%, and Vegetables; 40% and Spaghettis 20%. The total frequency of *Salmonella* spp was 0.63% which consisted of 2.5% in Injera firfir, with Salmonella-free Bayeaynet, Vegetables and Spaghettis. The total prevalence of *E. coli* was 43.75% with 65% in Injera firfir, 42.5% in Bayeaynet, 55% in Vegetables and 12.5% in Spaghettis (Table 3).

Discussion

Foodborne illnesses caused by *Salmonella* spp, *S. aureus*, and *E. coli* represent a major public health problem worldwide. These pathogens are transmitted mainly through the consumption of contaminated food and the presence of these organisms in ready to eat foods has relevant public health implications [16]. In the present study, it was found out that almost all the food samples collected from restaurants and food establishments in Yirgalem town southern Ethiopia had harbored various pathogenic and indicator microorganisms. This finding was in agreement with the study reported from Gondar, Ethiopia where over 82.8% of the food samples analyzed for microbial contamination had contained various pathogenic microorganisms [17].

In this study, a total of 560 AMB were isolated from the collected food samples. The isolates were included *Staphylococcus* spp 279(49.82%), EB 125(22.32%), Coliform70 (12.50%), *Micrococcus* spp 32(5.71%), *Pseudomonas* spp 27(4.82%) and *Acinetobacter* spp 27(4.82%). The presence of coliform in ready to eat foods can be linked to contamination resulted from inappropriate processing, incomplete heating, use of contaminated water during preparation and washing or secondary contamination via contact with contaminated types of equipment such as chopping boards, knives, and serving wares[17].

In this study, *Escherichia coli* were detected in 43.8% of the food samples. This detection rate was consistent with the previous study carried out in Amravati city and Gondar, Ethiopia where 41.0% and 46.3% of the food samples analyzed were found contaminated with *E. coli*, respectively [17, 18]. The presence of *E. coli* in ready to eat foods might attribute to the heat processing failure or post-processing contamination, fecal contamination and poor hygienic practice of food handlers [19].

In the current study, the prevalence of *S. aureus* in the whole food samples was 54.4%. This result was greater than the study result of [22] where 56.2% of 'Bonbolino' samples were contaminated with *S. aureus*. However, *S. aureus* frequency in this study was consistent with the findings of [18] from Gondar, Ethiopia where 53.7% of the street vended food samples were contaminated with *S. aureus.* The presence of *S. aureus* is an indication of contamination from the skin, mouth or nose of food handlers through coughing and sneezing during food handling and processing [22].

In this study *Salmonella*, spp was detected in 0.6% of the food samples. This is in agreement with previous work done on 'Sambusa' and 'Macaroni' in Ethiopia [23]. The presence of *Salmonella* spp in foods ready to eat might be an indication of the existence of fecal contamination.

In the present study, 'Injera firfir' and 'Bayeaynet' were highly contaminated food items with bacteria and fungi. This could be due to the method of handling and preparation of the foods. 'Injera firfir' and 'Bayeaynet' are more frequently handled by bare hands when compared with other food groups. Hence, food handlers may be the source of food contamination either as carriers of a pathogen or through poor hygienic practices. All food handlers have a basic responsibility to maintain a high degree of personal cleanliness and observe hygienic and safe food handling practices [19, 24].

Limitations

Even though the present study tried to address an important issue concerning food contamination, it has some limitations. Antimicrobial sensitivity for the isolates was not done because of the inaccessibility of the appropriate disc during the study period. Due to a shortage of enrichment and selective media for isolation, we did not other foodborne pathogens.

Conclusions

The results of this study demonstrated that the ready to eat foods in Yirgalem town southern Ethiopia were contaminated with different pathogenic bacteria. The existence of pathogenic bacteria such as *S. aureus*, *E. coli*, and *Salmonella* spp in foods could induce potential health problems for consumers. Therefore, concerned bodies should give health education to restaurant and food establishment owners and food handlers to improve their hygienic conditions during the preparation, handling, storing and serving foods.

Further studies should be conducted in detail by considering a large sample size and antimicrobial sensitivity patterns of the isolates.

Abbreviations

AMB, Aerobic mesophilic bacteria; LAB, lactic acid bacteria; S.D, standard deviation; WHO, World Health Organization

Declarations

Acknowledgments

The authors would very grateful to those restaurant and food establishment owners for their willingness to participate in the study. The authors would also like to acknowledge Yirgalem Hospital Medical College for funding the study.

Authors' contributions

TS conceptualized, designed the study, collected, analyzed and interpreted data, and wrote the manuscript; AH reviewed the content. Both authors read and approved the final manuscript

Funding

The study was funded by Yirgalem Hospital Medical College with Ref. No YH, SR: 2016/005. But the funder has no role in the study design, analysis or publication of the manuscript.

Availability of data and materials

All available data that served for the drawing of the conclusion was included in the result section of the manuscript.

Ethics approval and consent to participate

Ethics approval for the study was granted by Yirgalem Hospital Medical College Institute Review Board (YHMC-IRB) committee with minute No. IBR/03/2016 and Ref. No.YHMCDO/62/01/2016. Official permission letter was granted from the cultural and tourism office of the Yirgalem Town, Southern Ethiopia. The written consent was obtained from the restaurant and food establishment owners after explaining the aim of the study.

Consent for publication

Not applicable

Competing interest

The authors declare that they have no competing interests.

Author Details

[1]Department of Microbiology, Immunology, and Parasitology, Yirgalem Hospital Medical College Yirgalem, Ethiopia. [2]Department of Microbiology, Immunology, and Parasitology, Yirgalem Hospital Medical College Yirgalem, Ethiopia.

References

1. Schelin J, Wallin-Carlquist N, Cohn TM, Lindqvist R, Barker CG, Rådström P. The formation of *Staphylococcus aureus* Enterotoxin in food environments and advances in risk assessment. Special Focus Review: Foodborne Infection. 2011; 2: 580-592.
2. World Health Organization estimates the global burden of foodborne diseases, Geneva, Switzerland. 2011.
3. World Health Organization. Food Safety and Foodborne Illness. Fact sheets No. 237. Geneva, Switzerland. 2007.
4. Mead PS, Slutsker L, Dietz V, McCaig FL, Bresee SJ, Shapiro C *et al*. Food-Related Illness and Death in the United States. *Emerg Infect Dise*. 1999; 5: 607-625.
5. World Health Organization, Foodborne diseases; a focus for health education.53rdWorld Health Assembly. Geneva, Switzerland. 2000.
6. World Health Organization. Food safety issues in low and middle-income countries. Geneva, Switzerland. 2005.
7. World Health Organization. The global burden of foodborne disease: taking stock and charting the way forward: WHO consultation to develop a strategy to estimate the global burden of foodborne Geneva, Switzerland. 2006.
8. Kalekidan T, Behailu K, Rediet H. The Ethiopian perception on the food safety system. *Adv in Food Sci and Technol*. 2014; 2:260-268.
9. Zeru K, Kumie A. Sanitary conditions of food establishments in Mekelle town, Tigray, north Ethiopia. *J. Health Dev.*2007; 21:1-9
10. Ayana Z, Yohannis M, Abera Z. (Food-Borne Bacterial Diseases in Ethiopia. *Academ J Nutri*. 2015; 4: 62-76.
11. *Molla B, Alemayehu D, Sala W.* Sources and distribution of *Salmonella* serotypes isolated from food animals, slaughterhouse personnel and retail meat products in Ethiopia: 1997-2002. *J.Health Dev*. 2003; 17:63-70
12. Tefera W, Daniel A, Girma Z. Prevalence of Thermophilic *Campylobacter* species in carcasses from sheep and goats in an abattoir in must be given trainings and refreshment courses in Debre Zeit area, Ethiopia. Ethiopian J. Health Dev. 2009; 23: 230.
13. Mekonnen H, Habtamu T, Shewit K. Study on food safety knowledge and practices of abattoir and butchery shops in Mekelle City, Ethiopia (unpubl). 2011.
14. Acco M, Ferreira FS, Henriques J A, Tondo E C. Identification of multiple strains of *Staphylococcus aureus* colonizing nasal mucosa of food handlers. *Food Micrbiol*. 2003; 20: 489-493

15. Johnson TR, Case CL. Laboratory experiments in microbiology. (8thed.). San Francisco: Pearson Education. USA. 2007.
16. Sousa CP. The Impact of Food Manufacturing Practices on Foodborne Diseases. *Braz arch biol Technol*.2008; 51:815-823
17. Asefa A, Bimerew M, Tadesse G, Gizaw Z, Adane T. Bacteriological quality assessment of selected street foods and their public health importance in Gondar town, North West Ethiopia. *Global Veterinaria.* 2016;17(3):255–64.
18. Derbew G, Sahle S, Endris M. Bacteriological assessment of some street vended foods in Gondar, Ethiopia. *Int J Food Saf.* 2013;15:33–8.
19. Tambekar DH, Kulkarni RV, Shirsat SD, Bhadange DG. Bacteriological Quality of Street Vended food Panipuri: A Case Study of Amravati City (MS) India. *Bioscience Discovery.*2011; 2:350-354.
20. Eley AR. Infective Bacterial Food Poisoning. In: Eley AR Microbial Food Poisoning. London: Chapman & Hall. 1992; pp 47.
21. Suneetha C, Manjula K, Depur B. Quality Assessment of Street Foods in Tirumala. *Asian J. Exp. Sci*. 2011; 2:207-211.
22. Sandel MK, McKillip JL. Virulence and recovery of *Staphylococcus aureus* relevant to the food industry using improvements on traditional approaches. *Food Control*. 2004; 15:5–10.
23. Muleta D, Ashenafi M. Bacteriological profile and holding temperatures of street-vended foods from Addis Ababa. *J. Environ. Health Res*. 2001a; 11:95–105.
24. Ashenafi, M. Bacteriological profile and holding temperature ready -to –serve food items in open market in Awassa, *Trop.geogr.med*. 1995; 47:1-4.
25. Shamebo T, Bacha K, Ketema T. The antimicrobial susceptibility patterns and growth potential of *Salmonella* species and *Staphylococcus aureus* isolated from mobile phones of food handlers and health care workers in Jimma Town, Southwest Ethiopia. *J. Microbiol. Res.* 2016: 10(8), 254-259.

Tables

Table 1: Microbial counts (log CFU/g) of ready to eat food samples collected from restaurants and food establishments in Yirgalem town, Southern Ethiopia, 2017

Microbial group	Mean	S.D.	Min	Max
AMB	7.9	0.71	6.10	9.20
TC	4.31	1.30	2.10	7.60
Enterobacteraceae	4.32	1.30	2.00	6.80
Staphylococci	6.70	0.34	5.70	7.30
Yeasts & Moulds	4.5	1.01	2.50	7.00

AMB, Aerobic mesophilic bacteria; TC, total coliforms; S.D, standard deviation, Min; Minimum, Max; Maximum

Table 2: Frequency distribution of mesophilic bacteria in food samples collected from restaurants and food establishments in Yirgalem town, southern Ethiopia, 2017

Food type	No. of isolates %	*Staphylococcus* spp. *%*	*Micrococcus* spp. %	Coliform %	EB %	*Pseudomonas* spp. %	*Acinetobacter* spp. %
Injera firfir	162(28.9)	81(50.00)	11(6.79)	22(13.58)	31(19.14)	8(4.43)	9(5.55)
Bayeaynet	154(27.5)	73(47.40)	9(5.84)	25(16.23)	34(22.08)	6(3.89)	7(4.54)
Vegetables	136(24.2)	67(49.30)	7(5.15)	15(11.03)	32(23.53)	9(6.62)	6(4.41)
Spaghettis	108(19.2)	58(53.70)	5(4.63)	8(7.41)	28(25.93)	4(3.70)	5(4.63)
Total	560(100)	279(49.82)	32(5.71)	70(12.50)	125(22.32)	27(4.82)	27(4.82)

Where: EB, Enterobacteriaceae; Numbers in the parenthesis are a percentage of the total isolates of respective species

Table 3: Prevalence of *Salmonella* spp, *E. coli*, and *S. aureus* in ready to eat food samples in Yirgalem town, southern Ethiopia 2017

Food type	Prevalence		
	S. aureus	*Salmonella Spp*	*E. coli*
Injera firfir	35(87.5%)	1(2.5%)	26(65.0%)
Bayeaynet	28(70.0%)	-	17(42.5%)
Vegetables	16 (40.0%)	-	22(55.0%)
Spaghettis	8(20.0%)	-	5(12.5%)
Total	87(54.37%)	1(0.63%)	70 (43.75%)