Food Research 4 (4) : 1351 - 1356 (August 2020)
Journal homepage: http://www.myfoodresearch.com

FOOD RESEARCH

FULL PAPER

Microbiological analysis of different categories of food items in Dhaka city, Bangladesh

*Uddin, M.A., Jabin, T., Siam, E.A., Afreen, M.T., Uddin, S.S. and Sayeda, U.

Department of Microbiology, Stamford University Bangladesh, 51 Siddeswari Road, Dhaka 1217, Bangladesh

Article history:
Received: 5 March 2020
Received in revised form: 2 April 2020
Accepted: 8 April 2020
Available Online: 25 April 2020

Keywords:
Microbiological quality,
Pathogens,
Foods,
Antibiogram

DOI:
https://doi.org/10.26656/fr.2017.4(4).099

Abstract

Pathogenic microorganisms have been so far reported to contaminate a wide range of foods triggering food borne infections or intoxications including the enteric complications, abdominal pain, fever, bloodstream infection etc. The current study was attempted to observe the microbiological quality of some popular foods collected from different places of Dhaka city, Bangladesh. Conventional cultural, microscopic and biochemical tests were followed for the detection and enumeration of bacterial isolates associated with these food samples. The investigation encompassed detection of total viable bacterial count (TVBC) and presumptive identification of other pathogenic bacteria from these samples. Higher counts of TVBC, coliform, *Staphylococcus* spp. and fungal load were recorded as 1.46×10^7 CFU/g (yogurt 1), 4.5×10^6 CFU/g (yogurt 1), 5.6×10^5 CFU/g (raw meat) and 2.9×10^3 CFU/g (sea fish), respectively. Fecal coliform was detected only in one out of ten samples. On the contrary *Salmonella* spp., *Vibrio* spp., *Shigella* spp. and *Pseudomonas* spp. were completely absent. The antibiogram study showed that all the isolates were sensitive against Kanamycin and Azithromycin. Better sensitivity was also observed against Gentamycin and Ciprofloxacin. Varying degree of antibiotic resistance was also detected against Cefixime, Amikacin and Neomycin. Our study emphasizes the need for continuous monitoring of the various categories of food samples for the safety of public health.

1. Introduction

Food is the basic and utmost need for people and animals to survive. Sources of foods are traditionally from animals and plants. It is consumed by living beings for the constant supply of energy (Forbes, 2007). Foods are generally contaminated with various bacterial species and fungi during harvesting; processing; and through the use of associated machines, utensils, water, etc. (Frazier and Westhoff, 2007; Alam *et al.*, 2015). The analysis of food products for the presence of pathogenic microorganisms is one of the fundamental procedures to maintain the safety and quality of food. Though a number of advancement have been observed recently in the field of food technology, foodborne diseases are still considered as a major concern for human health, in developing as well as in developed countries. Everybody, especially very young or old and immunologically weak people are vulnerable to foodborne diseases and some severe complications can happen as a result of infection (Sherin *et al.*, 2015). Foodborne illnesses are occurred due to the action of microorganisms and/or their toxins, fungi with their related toxins, and chemical contaminants (Khamis and Hafez, 2011). The World Health Organization (WHO) listed some hazards like potentially harmful bacteria, viruses, toxins, parasites and chemicals that may be found in food. According to the WHO, one person out of 10 people becomes ill and 420,000 people die every year as a result of consuming contaminated food (World Health Organization (WHO), 2018). The International Commission on Microbiological Specifications for Food (ICMSF), 2005 brought in the concept of Food Safety Objectives (FSOs) as the maximum frequency and/or concentration of a microbial hazard (micro-organisms or toxins) in a food measured tolerable for consumer safety.

Another current health related alarming issues is the threat and existence of multidrug resistant pathogens in foods. Antibiotic resistance is a feature of a microorganism (CDC, 2018). The WHO defines antimicrobial resistance as a microorganism's resistance to an antimicrobial drug that was once able to treat an infection caused by those microorganisms (WHO, 2014).

*Corresponding author.
Email: *aftab_mb12@yahoo.com*

eISSN: 2550-2166 / © 2020 The Authors. Published by Rynnye Lyan Resources

FULL PAPER

Antibiotic resistance is a global issue and the origin of resistance is often found in the world's tropical or hottest regions (Collignon *et al*., 2018). As per the antibiotic resistance report 2019, more than 2.8 million antibiotic-resistant infections occur in the U.S. each year, and more than 35,000 people die as a result (Edwards *et al.*, 2019).

Along these lines of considerations, the current study focused on the presumptive detection of some common food borne pathogens from the daily consumable food samples (fish, meat, yoghurt, milk, ice cream, juice). The antibiogram profile of the suspected bacterial isolates from the food samples was also tested in this study.

2. Materials and methods

2.1 Sample collection and processing

A total of eighteen food samples of various categories such as fish, meat, yoghurt, ice cream, milk and juice samples were collected from some popular super shops located in various areas of Dhaka city such as Mouchak, Shantinagar, and Badda areas. Collection of samples was done in different time intervals and taken to the laboratory as quickly as possible according to the method suggested by FDA, 2013. Before calculating the bacterial and fungal load, samples were subjected to serial dilutions up to10^{-4}.

2.2 Microbiological analyses

For the estimation of total viable bacterial count (TVBC), 0.1 mL sample of each dilution was introduced onto the nutrient agar (NA) plates following spread plate technique. The nutrient agar plates were kept for incubation at 37°C for 24 hrs. For the detection of specific bacteria, from 10^{-2} and 10^{-3} dilutions of each sample, 0.1 mL of suspension was spread onto MacConkey agar, Mannitol salt agar (MSA) and Pseudomonas agar media for the enumeration of Coliform, *Staphylococcus* spp. and *Pseudomonas* spp., respectively (Cappuccino and Sherman, 2014; Afroz *et al.*, 2015; Uddin, 2018). All the plates were incubated at 37^{o}C for 24 hrs. The characteristic pink colonies on MacConkey agar, yellow colonies on MSA and colonies with greenish fluorescent pigmentation on Pseudomonas agar was indicative for the presumptive isolation of coliform, *Staphylococcus* spp. and *Pseudomonas* spp., respectively.

2.3 Enrichment procedure.

For the identification of *Salmonella* spp. and *Vibrio* spp., enrichment culture technique was followed. In this procedure, 1 mL of the sample was inoculated into 9 mL of selenite cystein broth (SCB) and alkaline peptone water (APW) and incubated at 37^{o}C for 6 hrs. Following incubation, the samples were diluted up to 10^{-3} and then 0.1 mL of samples from 10^{-2} and 10^{-3} dilutions were spread over Salmonella-Shigella agar (SS) and Thiosulfate-Citrate-Bile-Sucrose Agar (TCBS) from the respective enrichment media (Cappuccino and Sherman, 2014; Tabassum and Uddin, 2016). The appearance of small blackish colonies after incubation for 24 hrs at 37^{o}C was an indication of the presence of *Salmonella* spp., while the large (2-41 mm) and slightly flattened, yellow colonies on the TCBS agar referred to the presence of *Vibrio* spp.

2.4 Biochemical tests for the confirmative identification

Biochemical tests Identification of the isolates was carried out by major biochemical tests, for example-Triple Sugar Iron (TSI), Motility Indole Urease (MIU), Methyl-Red (MR), Voges-Proskauer (VP) and Citrate Utilization were performed following the standard methods (Cappuccino and Sherman, 2014).

2.5 Study of antibiogram

After the isolation of the bacterial isolates, antibiotic susceptibility assay was carried out against different groups of antibiotics *in vitro* by the Kirby-Bauer method (Bauer *et al*., 1966). Drug resistance was observed against Neomycin (30 µg), Cefixime (5 µg), Kanamycin (30 µg), Gentamycin (10 µg), Amikacin (30 µg), Ciprofloxacin (5 µg), and Azithromycin (25 µg). From overnight culture plate, a small portion of a fresh colony was transferred to Muller-Hinton broth and incubated at 37^{o}C for 4 to 5 hrs until the growth reached to the equivalent turbidity standard of McFarland (0.5 standards). Muller-Hinton agar plates were seeded properly by spreading the inoculate using sterile cotton swab. Discs (OXOID, UK) were placed gently at a proportionate distance from each other using a sterile needle. The plates were then incubated overnight at 37^{o}C and zones of inhibition (if any) were measured and interpreted as susceptible, intermediate and resistant categories by referring the recommended interpretative standards (CLSI, 2006).

3. Results and discussion

Foodborne diseases are the leading global problem causing considerable morbidity and mortality each year (Hanson *et al.*, 2012). The most frequent known causes of foodborne diseases are pathogenic bacteria. In this study, the goal was to analyze the microbiological quality of different popular food items consumed by the people of Dhaka metropolitan, Bangladesh. Therefore, the current study attempted to check the presence of microorganisms in such categories food items in terms of total viable count as well as in the finding of different

 © 2020 The Authors. Published by Rynnye Lyan Resources

pathogenic organisms such as *Vibrio* spp., *Salmonella* spp. and *Shigella* spp. along with the drug resistance properties of the assumed bacterial isolates.

3.1 Isolation and enumeration of microorganisms

3.1.1 Total viable bacterial count (TVBC)

In this study, the total viable bacterial counts ranged from 2.0×10^3 CFU/g to 1.64×10^7 CFU/g which was found in commercial juice-4 and yoghurt-1 samples respectively (Table 1). This finding was quite similar to the study done by Oranusi *et al.* (2013) and Uddin, 2018. According to FDA guideline (2013) the acceptable limit is $5x10^4$–10^5 CFU/g in case of total viable bacteria. So, 14 out of 18 studied samples were very much within the acceptable range as per FDA guideline.

3.1.2 Coliform, fecal coliform, Salmonella spp. and Shigella spp. count

Coliform was detected in eight out of eighteen samples of which the highest count as 4.5×10^6 CFU/g in yoghurt-1 whereas the lowest count was 1.1×10^2 CFU/g in ice cream-1 (Table 1). In this study, the detection of coliform bacteria, especially *E. coli* and *Klebsiella* spp. in these samples showed the possibility of the presence of fecally contaminated microorganisms as also suggested by Adams and Moss (2008). The lack of hygiene of the food handlers may be a parameter for the possible presence of coliforms in food items. In this study, fecal coliform was noticed in a sea fish sample. Fortunately, *Salmonella* spp., *Shigella* spp., *Vibrio* spp. and *Pseudomonas* spp. were totally absent in the selected food items. But this result was contradictory to the findings of Tambekar *et al.* (2008) who identified these organisms in some ready to eat street vendor food items.

3.1.3 Total Staphylococcal count

Staphylococcus spp. count was observed within the range from 1.1×10^2 CFU/g – 4.5×10^6 CFU/g which was found in ice cream-1 and yoghurt sample-1 respectively (Table 1). The existence of *S. aureus* in food is a sign of poor hygiene practices. *S. aureus* in food is connected with cross contamination occurring during processing and storage or through the contamination of raw ingredients (Akindele and Ibrahim, 2016).

3.1.4 Total fungal load

Fungus presence is an indication of the availability of various Mycotoxins in the food samples that may render severe threat to human health (Habiba *et al.*, 2015). A study conducted by Șumălan *et al.* (2011) showed variable fungal growth in between 3.8×10^4 - 5.9×10^4 CFU/g in the food samples. Overall fungal growth was observed in only two samples which were much lower than the previous report (Table 1). Fungi are far and wide disseminated and seen wherever moisture is present with sufficient nutrients that can prolong their growth. Fungi are the foremost spoilage of foods and feedstuffs. The propagation of various fungi in

Table 1. Microbiological analysis of the various categories of food items

Sample no	Sample	TVBC (CFU/g or CFU/mL)	TCC (CFU/g or CFU/mL)	TSC (CFU/g or CFU/mL)	TFC (CFU/g or CFU/mL)	Fecal coliform (CFU/g or CFU/mL)
1	Fish (raw)	4.6×10^4	1.6×10^4	0	0	0
2	Fish (frozen)	1.30×10^5	0	1.30×10^3	0	0
3	Sea fish (raw)	3.1×10^3	8.3×10^3	2.2×10^3	2.9×10^3	2×10^1
4	Fish (shrimp frozen)	1.94×10^6	8.1×10^4	2.6×10^3	0	0
5	Meat (raw)	7.2×10^4	1.5×10^4	5.6×10^5	0	0
6	Meat (frozen)	1.76×10^6	0	7.5×10^3	0	0
7	Sausage	8.2×10^2	1.2×10^2	4.5×10^1	4×10^1	0
8	Yogurt-1	1.64×10^7	4.5×10^6	2.2×10^5	0	0
9	Yoghurt-2	1.0×10^4	0	0	0	0
10	Yogurt-3	1.3×10^5	0	0	0	0
11	Ice cream-1	1×10^5	1.1×10^2	3.8×10^3	0	0
12	Ice cream-2	2×10^4	2.3×10^2	6.0×10^2	0	0
13	Pasteurized milk	4.4×10^5	0	0	0	0
14	Raw milk	8.24×10^6	2.5×10^5	4.8×10^4	0	0
15	Commercial juice-1	3.2×10^3	2×10^3	0	0	0
16	Commercial juice-2	3.7×10^3	0	0	0	0
17	Commercial juice-3	4.5×10^3	0	0	0	0
18	Commercial juice-4	2.0×10^3	0	0	0	0

TVBC: Total viable bacterial count, TCC: Total coliform count, TSC: Total staphylococcal count, TFC: Total fungal coliform. *Salmonella* spp., *Shigella* spp., *Vibrio* spp., *Pseudomonas* spp. were completely absent in all the samples being studied.

 © 2020 The Authors. Published by Rynnye Lyan Resources

agricultural products leads to lessening in yield and quality with noteworthy economic losses. The level of contamination will vary with geographic location, agricultural practices and the vulnerability of goods to the incursion of fungi during storage and processing periods (Adeyeye, 2016)

3.1.5 Antibiogram of the Isolates

Antibiotic resistance has augmented worldwide causing to failures in the treatment of human infectious diseases. Resistance against antibiotics by pathogenic bacteria is a major threat in the anti-infective therapy of both humans and animals. The Kirby-Bauer disk diffusion method was used in this experiment to resolve whether the isolated organisms were susceptible or resistant towards a pool of commonly prescribed antimicrobial agents. Antibiotic resistance pattern of *E. coli*, *Klebsiella* spp. and *Staphylococcus* spp. has been shown in Table 2. Both *E. coli* and *Klebsiella* spp. showed a high degree of sensitivity against Neomycin, Kanamycin, Gentamycin, Amikacin and Azithromycin. 40% of the *Klebsiella* spp. isolates were found to be resistant against cefixime. On the contrary, *Staphylococcus* spp. isolated from various samples exhibited 100% resistance against Cefixime. Similar type of resistance patten *in S. aureus* isolates was also detected by Taj *et al.* (2010). Researchers have found that bacterial resistance to various antibiotics is encoded by over 100 different genetic mechanisms, a number of which are readily moveable to other bacteria through the process of conjugal elements, transposons, plasmids etc (Bhowmik *et al.*, 2016).

4. Conclusion

It is obligatory that foods should be free of contaminations as much as possible. The existence of *E. coli, Staphylococcus* spp. and *Klebsiella* spp. indicates a potential health risk as these organisms are pathogenic and have been implicated in food borne diseases. Based on FDA guideline (2013), the level of contaminations was within unacceptable microbiological limits for four samples; this could be due to improper processing, poor handling practices and post-cross contamination which can produce a risk to the health of the consumers. Foodborne illness can be avoided by good hygiene practices such as the use of Hazard Analysis Critical Control Point (HACCP) application in the sequence of food production, processing and storage. Ensuring proper supervision and education to the food handlers/ food vendors on food safety practices and stringent control of various categories foods sold to the busy city residents should be properly observed by the pertinent authorities to ward off the food borne illness related epidemics within the Dhaka city, Bangladesh. Besides recurrent use of antibiotics should be prohibited as antibiotic defiant strains are incessantly mounting.

Conflict of Interest

The authors declare no conflict of interest.

Acknowledgments

All the authors gratefully acknowledge Department of Microbiology, Stamford University Bangladesh for financial and technical support.

References

Adams, M.R. and Moss, M.O. (2008). Bacterial agents of foodborne illness. In Food Microbiology, 3rd ed. Cambridge, United Kingdom: The Royal Society of Chemistry.

Adeyeye, S.A.O. (2016). Fungal mycotoxins in foods: A review. *Cogent Food and Agriculture*, 2(1), 1-11. https://doi.org/10.1080/23311932.2016.1213127

Afroz, H., Ahmed, T. and Uddin, M.A. (2015). Microbiological analysis and antibacterial activity of pear samples. *Stamford Journal of Microbiology*, 5 (1), 1-4. https://doi.org/10.3329/sjm.v5i1.26910

Akindele, P.O. and Ibrahim, K.A. (2016).

Table 2. Antibiogram profile of the suspected bacterial isolates

Name of Antibiotic disc	Disc Content	Zone of inhibition (mm) against test bacteria					
		E. coli (N = 10)		*Klebsiella* spp. (N = 10)		*Staphylococcus* spp. (N = 10)	
		R%	S%	R%	S%	R%	S%
Neomycin	30 µg	0	100	10	90	3	70
Cefixime	5 µg	0	100	40	60	100	0
Kanamycin	30 µg	0	100	0	100	0	100
Gentamycin	10 µg	10	90	0	100	0	100
Amikacin	30 µg	15	85	0	100	10	90
Ciprofloxacin	5 µg	10	90	5	95	0	100
Azithromycin	25 µg	0	100	0	100	10	90

R% = Resistant percentage, S% = Sensitive percentage

 © 2020 The Authors. Published by Rynnye Lyan Resources

Microbiological analysis of ready-to-eat-foods obtained from bukaterian within the Ekiti State University and environment, Ado-Ekiti, Nigeria. *Journal of Advances in Microbiology,* 1(2), 1-8. https://doi.org/10.9734/JAMB/2016/30948

Alam, M.S., Feroz, F., Rahman, H., Das, K.K. and Noor, R. (2015). Microbiological contamination sources of freshly cultivated vegetables. *Nutrition and Food Science*, 45 (4), 646-658. https://doi.org/10.1108/NFS-04-2015-0032

Bauer, A.W., Kirby, W.M., Sherris, J.C. and Tierch, M. (1966). Antibiotic susceptibility testing by a standardized single disc method. *American Journal of Clinical Pathology,* 45(4), 493-496. https://doi.org/10.1093/ajcp/45.4_ts.493

Bhowmik, S., Chowdhury, R.A. and Uddin, M.A. (2016). Microbiological analysis and detection of anti-bacterial activity of *Centella asiatica* and *Aloe vera* samples collected from different areas of Dhaka city, Bangladesh. *Stamford Journal of Microbiology*, 6(1), 39-43. https://doi.org/10.3329/sjm.v6i1.33518

Cappuccino, J. and Sherman, N. (2014). Microbiology. A laboratory manual. London, United Kingdom: Pearson Education.

Centers for Disease Control and Prevention (CDC). (2018). Antibiotic Prescribing and Use. Retrieved April 29, 2015 from CDC website: https://www.cdc.gov/antibiotic-use/index.html

Clinical Laboratory Standards Institute (CLSI). (2006). Performance standards for antimicrobial disks susceptibility tests: Approved standard-9th ed. CLSI document. M2-A9, 26:1. Wayne, USA: CLSI.

Collignon, P., Beggs, J.J., Walsh, T.R., Gandra, S. and Laxminarayan, R. (2018). Anthropological and socioeconomic factors contributing to global antimicrobial resistance: a univariate and multivariable analysis. *Lancet Planet Health*, 2(9), 398–405. https://doi.org/10.1016/S2542-5196(18)30186-4

Edwards, M., Hamilton, R., Oliver, N., Fitzgibbon, S. and Samarasekera, R. (2019). Antibiotic Resistance: Modelling the Impact on Mortality and Morbidity. United Kingdom: Institute and Faculty of Actuaries.

Food and Drug Administration (FDA). (2013). Revised guidelines for the assessment of microbiological quality of processed foods. Retrieved from Food and Drug Administration Philippines website: https://ww2.fda.gov.ph/attachments/article/17218/FC2013-010.pdf

Forbes, J.M. (2007). Voluntary food intake and diet selection in farm animals. United Kingdom: CABI Publishing. https://doi.org/10.1079/9781845932794.0000

Frazier, W.C. and Westhoff, D.C. (2007). Food Microbiology, 4th ed. New Delhi, India: Tata McGraw-Hill.

Hanson, L.A., Zahn, E.A., Wild, S.R., Döpfer, D., Scott, J. and Stein, S. (2012). Estimating global mortality from potentially foodborne diseases: An analysis using vital registration data. *Population Health Metrics*, 10, 5. https://doi.org/10.1186/1478-7954-10-5

ICMSF. (2005). Microorganisms in foods. 6. Microbial ecology of food Commodities. 2nd ed., p. 25-40. New York, USA: Kluwer Academic/Plenum Publishers.

Khamis, N. and Hafez, M. (2011): A microbial survey of food served to oncohematology patients at a university hospital. *International Journal of Infection Control,* 7(1), 1-6. https://doi.org/10.3396/ijic.v7i1.005.11

Oranusi, S.U., Oguoma, O.I. and Agusi, E. (2013). Microbiological quality assessment of foods sold in student's cafeterias. *Global Research Journal of Microbiology*, 3(1), 1 –7.

Șumălan, R.M., Doncean, A., Șimonea, L., Șumălan, R., Camen, D. and Beinsan, C. (2011). Study on fungal load of pome fruits, hazardous factor for quality maintaining and ensuring the consumer safety. *Journal of Horticulture, Forestry and Biotechnology,* 15(2), 147- 151.

Sherin, A.S., Masry, E.L., Rashad, S.S., Hadia, H.B. and Sorour, T.R. (2015). Survey of the Microbiological Quality of Food Served at a University Hospital in Egypt. *International Journal of Current Microbiology and Applied Sciences,* 4(9), 10-21

Tabassum, S. and Uddin, M.A. (2016). Microbiological Analysis of Different Flavoured Milk Samples Collected from Various Areas of Dhaka city, Bangladesh. *Bangladesh Journal of Microbiology,* 33(1 and 2), 11-15. https://doi.org/10.3329/bjm.v33i1.39597

Tambekar, D.H., Jaiswal, V., Dhanorkar, D., Gulhane, P. and Dudhane, M. (2008). Identification of microbiological hazards and safety of ready-to-eat food vended streets of Amravati City, India. *Journal of Applied Bioscience,* 7, 195-201.

Taj, Y., Abdullah, F.E. and Kazmi, S.U. (2010). Current pattern of antibiotic resistance in *Staphylococcus aureus* clinical isolates and the emergence of vancomycin resistance. *Journal of College of Physicians and Surgeons Pakistan,* 20(11), 728-732. doi: 11.2010/JCPSP.728732.

Uddin, M.A. (2018). Microbiological analysis of ready to eat foods collected from different places of Dhaka

 © 2020 The Authors. Published by Rynnye Lyan Resources

city, Bangladesh. *Stamford Journal of Microbiology*, 8(1), 30-33. https://doi.org/10.3329/sjm.v8i1.42436

World Health Organization (WHO). (2014). Antimicrobial resistance Fact sheet. Retrieved March 7, 2015 from WHO Website: https://www.who.int/news-room/fact-sheets/detail/antimicrobial-resistance

World Health Organization (WHO). (2018). Food Safety. Retrieved from WHO website: http://www.who.int/news-room/facts-in-pictures/detail/food-safety.

 © 2020 The Authors. Published by Rynnye Lyan Resources