

Characterization of Bacteria from Different Street Foods and Their Antibiotic Resistance Profile from different Selected Area in Gazipur, Bangladesh

Md. Abdul Khalek¹, Moriom Akter², Nazmi Ara Rumi³, Md. Shiblee Sadik Sabuj⁴,
Mahmudul Hasan⁵, Md. Aoulad Hosen^{6*}

¹Department of Microbiology, Gono Bishwabidyalay, Dhaka, Bangladesh

²Department of Food Processing and Preservation, Hajee Mohammad Danesh Science & Technology University, Dinajpur, Bangladesh

³Department of Microbiology, Hajee Mohammad Danesh Science & Technology University, Dinajpur, Bangladesh

⁴Department of Microbiology, Hajee Mohammad Danesh Science & Technology University, Dinajpur, Bangladesh

⁵Department of Microbiology, Gono Bishwabidyalay, Dhaka, Bangladesh

^{6*}Department of Microbiology, Hajee Mohammad Danesh Science & Technology University, Dinajpur, Bangladesh

E-mail: ¹abkhaledgonomicro@gmail.com, ²eng.moriom11@gmail.com, ³rumi_dvm@yahoo.com, ⁴shibleesadik.vet@gmail.com, ⁵mhmasud1993@gmail.com, ^{6*}aoulad1992@gmail.com

ABSTRACT

Bacterial contamination of retorted foods has become an important public health issue that foods is sold by street vendors. In Bangladesh, forborne illness is very common and is mainly occurred by consumptions of contaminated street foods. This study was conducted to bend the microbiological standard of various types of street foods (chotpoti and fuchka) sold by street vendors of different areas at Gazipur in Bangladesh. Total 32 food samples from 2 different types as chotpoti and fuchka were collected from different areas. Bacterial isolation, identification, colony count and antibiotic sensitivity testing were performed by using the succeeding level of bacteriological techniques. Total viable count of bacteria is calculated with CFU/g units range from 9×10^5 CFU/g to 5.3×10^7 CFU/g. For enumeration and identification of test organisms all food samples were inoculated into different types of selective media including Eosin Methylene Blue agar (EMB), Salmonella-Shigella agar (SS) and Mannitol salt agar (MS). From different street foods at gazipur district we isolate the highest frequent of *E. coli* 15(37.5%) succeeding by *Staphylococcus* spp 10 (25%), *Klebsiella* spp 8(20%), *Salmonella* spp 5(12.5%) and *Shigella* spp 2(5%). *E. coli* isolates were resistant to ampicillin, penicillin G, chloramphenicol and cephalexin where sensitive to gentamicin and ciprofloxacin. *Salmonella* spp were sensitive only ciprofloxacin and remaining antibiotic disc were resistant. In case of *Shigella* spp gentamicin and ciprofloxacin were sensitive where remaining disc were resistant. *Klebsiella* spp. isolates were resistant to ampicillin, cephalexin and sensitive to gentamicin, neomycin and ciprofloxacin. Gram positive *Staphylococcus* spp. were resistant to ampicillin, cephalexin and vancomycin and sensitive to were resistant to ampicillin, vancomycin, and cephalexin and sensitive to gentamicin and ciprofloxacin. Ciprofloxacin shown 100% sensitivity and cephalexin shown 100% resistant in all tested bacterial isolates. This study confirmed multidrug resistance bacteria from different local street foods in Gazipur City that causes foodborne illness and impact on public health hazard. Recommendation of this study are proper handling of food, training on safety practices and regular inspection.

Keywords:

street foods, contamination, antibiotic sensitivity, total aerobic count, isolation

1.Introduction

There is a very high demand of the street foods in Gazipur District of Bangladesh. Street foods are ready-to-eat foods and beverages prepared and/or sold by vendors and hawkers, particularly in streets and other similar public places [FAO, 1989]. This type of food plays an important role in meeting the food needs of urban dwellers in many developing countries' cities and towns. It feeds millions of people with a diverse range of foods that are relatively inexpensive and easily

accessible [Tambekar DH, et.al:2008]. The convenience and distinctive flavors of street-vendor foods can be attributed in *Edition to the population's nutritional status. In many developing countries, street foods ensure a greater number of people's survival and food security for low-income urban residents [Rane, S. 2011] despite the numerous benefits offered by street vendors, they have been reported to pose serious safety and health concerns to consumers and food handlers. This is due to their diversity, a lack of food safety knowledge and practice, a lack of basic hygiene, and a lack of public awareness [C. Muyanja.et.al: 2011, S. Rane. 2016]. As a result, they are unaware of proper food handling and their role in pathogen transmission [Mensah PD, 1999]. Knowing the microbiological safety of sold foods is an important factor in appreciating the safety problems associated with street vendors, so that concerned organizations can take appropriate steps to improve safety and sanitation in this sector [Muleta D, 2001]. There are several risk factors at play, including the possibility that the water will become contaminated as it is left out in the open. You never know if the hands used to fill the fuchka are always clean. During this season, a large number of people are becoming victims of water-borne diseases. I like the fuchka, chotpoti with the sour tamarind. Be wary of overindulging in this delectable street food, as it is high in calories and saturated fat. These may cause an upset stomach and a queasy feeling if they are high in oil. Acidity and heartburn are also common side effects to consider before diving into this treat. Microbial contamination of street food causes the foodborne illnesses, which are regarded as a major public health issue [Al Mamun et.al: 2013, Biswas et.al: 2010]. More than 250 different types of bacteria, viruses, parasites, metals, toxins, and prions cause foodborne diseases in humans [Tambekar DH, et.al:2008]. Bacterial agents cause foodborne infections, which can result in hospitalizations and death. [FAO, 2012]. In most cases, water is not available at vending locations for washing hands and utensils; hand and utensil washing is usually done in a single bucket—sometimes without soap. Bacterial agents in food are the most common cause of serious and fatal foodborne illnesses. More than 90% of food-poisoning illnesses are caused by Staphylococcus, Salmonella, Clostridium, Campylobacter, Listeria, Vibrio, Bacillus, and enteropathogenic bacteria. Escherichia coli [Schmidt R, et. al.; 2003]. Thus, contamination of food by Escherichia coli, Salmonella typhi, Pseudomonas species, Staphylococcus aureus, Proteus species, and other species during preparation, post-cooking, and various handling stages poses potential health risks (Ghosh M, et.al.; 2007 and Hanashiro A, et.al.;2005).

Furthermore, multi-drug resistance of food-borne microorganisms made the food safety situation in public health more vulnerable. [Khairuzzaman M,et.al; 2014]. According to the Globe Health Organization's report on worldwide surveillance of antimicrobial resistance, the ability to treat common infections in the community and hospitals is jeopardized due to antibiotic resistance across the world, which is not a forecast for the future but is occurring right now. [WHO, 2014]. Antibiotic resistance may be a natural feature of the organism or it may be obtained. Mutations in extra genes coding for a resistance mechanism in the bacterial genome result in acquired resistance to an antimicrobial agent. The bacteria's defensive mechanism is changed as a result of these genetic modifications, which result in a change in membrane permeability. [Chadwick, D.J. & Goode, J. A. 2008; Poole, K. 2002].

2. Materials And Method

The research study is split into three parts. The first section covers the total viable counts (TVC) of the collected samples from various regions. The second portion consists of isolating and

identifying pathogenic bacteria from isolated samples using cultural, morphological, and biochemical analysis. The third section involves an appraisal of the antibiotic sensitivity test.

2.1 Sample collection

Our research experiment was started during the period from January to June in 2016 and whole research work was done in the bacteriology laboratory of the department of microbiology, Gono Bishwabidyalay, Savar, Dhaka. Total 32 food samples (each of 16 samples) were randomly chosen in this study and samples were collected from different street vendors in the local area included Kaliakoir bus stand, Chandura bus stand, Shafipur bus stand, and Joydebpur rail station at Gazipur district. Food samples including chotpoti and fuchka were collected by using zipperlock polybag with 250 gm and immediately transfer all food samples to the microbiology laboratory of Gono Bishwabidyalay. Food samples were analyzed within 24 hours from sample collection.

2.2 Sample preparation

To dislodge adhered bacteria, a 10 gm food sample was homogenized in 90 ml of buffered peptone water and vigorously shaken with a vortex (Kiiyukia C.; 2003). Homogenized 1 ml sample added into 90 ml of buffer solution and make 10⁻² to 10⁻⁵ dilution for total aerobic count (TAC), coliform bacteria and enterobacteriaceae counts (Bridson, EY 2009).

2.3 Prediction of total viable count (TVC)

After preparation of samples from each dilution 50 µl of diluted food samples were transferred into a plate count agar with a micropipette and immediately spread for determination of total bacterial load. After spreading the plates were transferred in incubator at 37°C for 24 hours for proper growth of bacteria. After 24 hours calculate the bacterial count by colony counter and the results were mentioned as the colony forming units (CFU).

2.4 Separation of bacteria by cultural test

After preparation of food samples aerobic colony count was determined by pouring of 1 ml of liquid sample into nutrient agar plates and then keep incubator for bacterial growth at 30-35 °C for 48 hours. For detection of coliform bacteria 1 ml liquid sample was poured on Eosin Methylene blue agar and incubated at 30-35°C for bacterial growth. By following the same procedure detection of enterobacteriaceae family poured 1 ml liquid sample on MacConkey agar incubated at 35 °C for 24-48 hours (Bridson EY, 1998). Remaining food sample were poured on Manitol salt agar, TCBS agar, Xylose Lyse Deoxicolate (XLD) agar and incubate same temperature for 24-48 hours. Then suspected colonies were again subcultured for further confirmation and pure colonies formation at 35°C for 24-48 hours.

2.5 Confirmation of test bacteria

According to the standard method of International Commission on Microbiological Specifications for Foods (ICMSF, 1985). Bacteriological test of street food samples such as chotpoti and fuchka was done for cultural examination. For bacterial identification gram-staining and different types of biochemical tests such as catalase, oxidase, methyl red- voges proskauer, simon citrate, indole, urease, motility, coagulase, Klinglar iron agar, H₂S gas production and mannitol fermentation were used (Bridson EY, 1998, Cheesebrough M, 2002).

2.6 Antibiotic Susceptibility Test

Different types of commercially available antibiotics disc were used to determine the resistance patterns of test organisms. According to the guidelines of clinical and laboratory standards institute (CLSI, 2007) using Mueller hinton agar (difco) by Kirby-Bauer disc diffusion technique for the determination of antibiotic resistance patterns. After spread test organism on plate and overnight incubate at 37°C then recorded the diameter of zone of inhibition and categorized as sensitive, intermediate and resistance following the company guidelines (Cappuccino and Carpenter, 2005).

3. Result And Discussion

3.1 Total viable count of chotpoti and fuchka

Different street foods sample (fuchka and chotpoti) were collected from different vendors shop and calculate total viable counts which was shown in table 1. Results showed that in chotpoti highest number of bacterial counts was observed and the number was 6.2×10^7 CFU/g and followed fuchka sample was 5.3×10^7 CFU/g. Highest number of bacteria found in chotpoti sample from chanduraarea.

Table number 1.Total viable count (TVC) of Bacteria

Place name and foods	Dilution factor	Colony count	Total viable count(TVC)
Kaliakoir(Chotpoti)	10^{-1}	More than300	TNTC TNTC
	10^{-2}	More than300	9×10^5 cfu/g 7.5×10^6
		90	cfu/g 6.0×10^7 cfu/g
	10^{-3}	75	
	10^{-4}	60	
	10^{-5}		
Chandura (Chotpoti)	10^{-1}	More than300	TNTC TNTC
	10^{-2}	More than300	8.2×10^5 cfu/g 7.0×10^6
	10^{-3}	82	cfu/g 6.2×10^7 cfu/g
	10^{-4}	70	
	10^{-5}	62	
Shafipur (Fuchka)	10^{-1}	More than300	TNTC TNTC
	10^{-2}	More than300	8×10^5 cfu/g 6.7×10^6
	10^{-3}	80	cfu/g 5.5×10^7 cfu/g
	10^{-4}	67	
	10^{-5}	55	

Joydeppur railway station (Fuchka)	10-1	More than 300	TNTC TNTC
	10-2	More than 300	8.2 x 10 ⁵ cfu/g
	10-3	82	6.6 x 10 ⁶ cfu/g
	10-4	66	5.3 x 10 ⁷ cfu/g
	10-5	53	

3.2 Results of bacterial count and identification from different vendor shop

For isolation and identification of different bacteria we collected different food samples such as chotpoti and fuchka from different street food vendors in gazipur district and we identified total 32 bacterial species such as *Escherichia Coli*, *Salmonella spp*, *Klebsiella spp*, *Shigella spp* and *Staphylococcus spp*. In chotpoti sample we isolated *Escherichia Coli* for 9(36%), *Salmonella spp* for 4(16%), *Staphylococcus spp* for 6(24%), *Klebsiella spp* for 4(16%) and *shigella spp* were found 2(8%). In fuchka samples we identified *Escherichia Coli* for 6(40%), *Salmonella spp* for 1(6.6%), *Staphylococcus spp* for 4(26%), *Klebsiella spp* for 4(26%). Both 2 samples such as chotpoti and fuchka we found *Escherichia Coli* was highest positive number that was mentioned in table 2.

Table number 2: Results of bacteria in different food sample

Isolates	Chotpoti	Fuchka	Total	Percentage (%)
<i>E. coli</i>	9 (36%)	6 (40%)	15	37.5%
<i>Salmonella spp.</i>	4 (16%)	1 (6.66%)	5	12.5%
<i>Shigella spp.</i>	2 (8%)	0 (0%)	2	5%
<i>Klebsiella spp.</i>	4 (16%)	4 (26%)	8	20%
<i>Staphylococcus spp.</i>	6 (24%)	4 (26%)	10	25%
Total bacteria identified	25 (100%)	15 (100%)	40	100%

3.3 Results of cultural test from isolated bacteria

Cultural characteristics of isolated bacteria from different street food were included their size, shape, and characteristics of colony experiment in various cultural media. Cultural characteristics confirmed by using staining, pure culture technique and different selective media which are showed in below table 3.

Table 3: Observation of isolated bacteria in culture media

Sl. no	Isolates name	Culture media	Observation
1	<i>Escherichia. Coli</i>	Eosin methylene blue Agar	Metallic sheen (greenish black) - colony
2	<i>Salmonella spp.</i>	Salmonella –shigella agar	Non lactose fermented colony
3	<i>Shigella spp.</i>	Salmonella –shigella agar	Small colony with non-lactose

4	<i>Klebsiella spp.</i>	MacConkey agar	Lactose fermented colony with large, mucoid and bright pink.
5	<i>Staphylococcus spp.</i>	Mannitol Salt Agar	Pure yellowish colony

3.4: Results of morphological characteristics

After cultural test in media all isolates were morphologically characterized by using gram staining method and the cultural character of *E.coli*, and *Staphylococcus spp* were near similar to other authors (Sharada et. al., 1999; Tomas et al, 2005; Konuko et al, 2012) and the test results were mentioned in below table 4.

Table 4: Results of gram staining properties

Gram staining properties			
Name of isolates	Gram staining	Shape	Arrangement
<i>Escherichia. Coli</i>	negative	Short rods	Single pair or short chain
<i>Salmonella spp.</i>	negative	Rod shape	Single or pair
<i>Shigella spp.</i>	negative	Rod shape	Single or pair
<i>Klebsiella spp</i>	negative	Rod shape	Single or cluster
<i>Staphylococcus spp.</i>	positive	Cocci shape	Grape like cluster

3.5: Biochemical test results

In our research study we confirmed the isolates by using different biochemical tests with different characteristics. In case of catalase test *Escherichia. Coli*, *Salmonella spp.*, *Shigella spp.* *Klebsiella spp* and *Staphylococcus spp* were gave positive results where all isolates were oxidase negative, except *Escherichia. Coli* all isolates were indole negative. *Escherichia. Coli*, *Salmonella spp.*, *Shigella spp.* gave positive results for MR test and negative in VP test. All biochemical test results mentioned in table 5 and this results were similar to (Tomas, 1998; Konuku et al, 2012).

Table 5: Results of biochemical test

Serial No.	Identification	Ca	Ox	In	Ci	MR	VP	MIU	Spore	TSI
01	<i>Escherichia. Coli</i>	+	-	+	-	+	-	+	-	Yellow butt and yellow slant, Gas=(+ve) H ₂ S=(-ve)
02	<i>Salmonella spp.</i>	+	-	-	+	+	-	+	-	Slant alkaline Butt acidic H ₂ S=(+v) Gas=(-ve)

03	<i>Shigella spp.</i>	+	-	-	+	+	-	-	-	Slant alkaline Butt acidic H2S=(-v) Gas=(-ve)
04	<i>Klebsiella spp.</i>	+	-	-	+	-	+	-	-	Yellow butt and yellow slant, Gas= (+ve) H2S=(-ve)
05	<i>Staphylococcus spp.</i>	+	+	-	-	+	+	-	-	Slant and Butt both acidicH2S=(- ve) Gas=(- ve)

Legends: Ca: Catalage test, Ox: Oxidase test, In: Indole test, Ci: Citrate utilization test, MR: Methyl red test, VP: Voges proskaeur test, MIU: Motility indole urease test, TSI; Triple sugar iron, H2S: Hydrogen sulphide, (+): Positive and (-): Negative

3.6: Antibiotic sensitivity test results

Total 40 bacterial isolates were isolated from food samples and 7 different antibiotics were used for sensitivity test which were mentioned in table 7. *E. coli* isolates were resistant to ampicillin, penecilin G, chloramphenicol and cephalixin where sensitive to gentamicin and ciprofloxacin. *Salmonella spp* were sensitive only ciprofloxacin and remaining antibiotic disc were resistant. In case of *Shigella spp* gentamicin and ciprofloxacin were sensitive where remaining disc were resistant. *Klebsiellaspp.* isolates were resistant to ampicillin, cephalixin and sensitive to gentamicin, neomycin and ciprofloxacin. Gram positive *Staphylococcus spp.* were resistant to ampicillin, cephalixin and vankomycin and sensitive to were resistant to ampicillin, vancomycin, and cephalixin and sensitive to gentamicin and ciprofloxacin and the results were more or less similar to (Lin and Modarressi,2011; Singh et al,2011; Tagoe et al, 20011). Gentamicin and ciprofloxacin were highly sensitive to all bacterial isolates.

Table 7. Antibiotic Sensitivity test

Isolates	Sensitive	Resistance
<i>Escherichia. Coli</i>	GEN CIP	AMP CN CL P
<i>Salmonella spp.</i>	CIP	GEN AMP P CN
<i>Shigella spp.</i>	GEN CIP	AMP CN N
<i>Klebsiella spp.</i>	GEN CIP N	CN AMP
<i>Staphylococcus spp.</i>	GEN CIP	AMP CN V

4. Conclusion

Most of the isolated bacteria are antibiotic resistant. Our research findings are pathogenic bacteria presence in street foods that may cause diarrhea, vomiting and other problems in the human body. Most important fact of microbial contamination which creates infection is unconscious about maintaining sterile condition during preparing and selling foods. Safety measure should be taken during making and preparation of foods and handover to the customer. Good hygienic practices can reduce the contamination.

Acknowledgements

Our research experiment was fully supported by the department of microbiology, Gono Bishwabidyalay, Savar, Bangladesh. The author specially wants to thank bacteriology laboratory of Gono Bishwabidyalay for providing lab facilities and financial supports.

References

- [1] Al Mamun, M., Rahman, S. M. M., Turin, T. C. (2013) Microbiological quality of selected street food items vended by school-based street food vendors in Dhaka, Bangladesh. *Int. J. Food Microbiol.* 166 (3): 413- 418.
- [2] Biswas, S., Parvez, M., Shafiquzzaman, M., Nahar S., Rahman, M. (2010) Isolation and characterization of *Escherichia coli* in ready-to-eat foods vended in Islamic University, Kushtia. *J. Bio-Sci.* 18: 99-103.
- [3] Bridson EY. Oxoid. The Oxoid Manual. Hampshire, England 1998. p. 1-371.
- [4] C. Muyanja, L. Nayiga, N. Brenda, and G. Nasinyama, "Practices, knowledge and risk factors of street food vendors in Uganda," *Food Control*, vol. 22, no. 10, pp. 1551–1558, 2011.
- [5] Cappuccino N and D Carpenter, 2005. Invasive exotic plants suffer less herbivory than non-invasive exotic plants. *Biol. Lett.*, 1: 435–438.
- [6] Chadwick, D. J., Goode, J. A. (2008) Antibiotic resistance: origins, evolution, selection and spread. John Wiley & Sons. Vol. 207.
- [7] Cheesebrough M. District laboratory practices in tropical countries. Cambridge, UK: Cambridge University Press; 2002.
- [8] Clinical and Laboratory Standards Institute (CLSI, formerly NCCLS) 2007: Performance standards for antimicrobial susceptibility testing. 17th Informational Supplement document M100-S17: 1. Wayne, Pennsylvania. pp: 32-50.
- [9] FAO (Food and Agricultural Organization). Street foods. Rome, Italy. 1989.
- [10] Food and Agriculture Organization (2012) The state of food insecurity in the world 2012. <http://www.fao.org/docrep/016/i3027e/i3027e00.htm>.
- [11] Ghosh M, Wahi S, Kumar M, Ganguli A. Prevalence of enterotoxigenic *Staphylococcus aureus* and *Shigella* spp. in some raw street vended Indian foods. *International Journal of Environmental Health Research*. 2007;17(2):151-6.
- [12] Hanashiro A, Morita M, Matte G.R, Matte M.H and E.A.F.S. Torres. Microbiological

- quality of selected street foods from a restricted area of São Paulo city, Brazil Food Control, 2005;16:439-444.
- [13]Huws SA, Edwards JE, Kim EJ, Scollan ND 2007: Specificity and sensitivity of eubacterial primers utilized for molecular profiling of bacteria within complex microbial ecosystems. *Journal of Microbiological Methods* 70 565–569.
 - [14]Khairuzzaman M, FM Chowdhury, S Zaman, AA Mamun and ML Bari, 2014. Food safety challenges towards safe, healthy and nutritious street foods in Bangladesh. *Int. J. Food Sci.*, Article ID 483519, 9 pages.
 - [15]Kiyyukia C. *Laboratory Manual of Food Microbiology for Ethiopian Health and Nutrition Research Institute.* 2003.
 - [16]Konuku S, Rajan MM, Muruhan S 2012: Morphological and biochemical characteristics and antibiotic resistance pattern of *Staphylococcus aureus* isolated from grapes. *International Journal of Nutrition, Pharmacology, Neurological Diseases* 2 70-73.
 - [17]Lin KT, Modarressi S 2011: Antimicrobial resistant genes associated with *Salmonella* from retail meats and street foods. *Food Research International* 44 2641-2646.
 - [18]Mensah PD, Yeboah-Manu K, Owusu-Darko A, Ablordey FK, Nkurumar H, Kamiya. The role of street food vendors in the transmission of enteric pathogens. *Ghana Med J*, 1999; 33:19-29.
 - [19]Muleta D, Ashenafi M. Bacteriological profile and holding temperatures of street-vended foods from Addis Ababa. *Int. J. Environ. Health Res*, 2001; 11:95-105.
 - [20]NSW Food Authority. Microbiological quality guide for ready-to-eat foods: A guide to interpreting microbiological results, July 2009.
 - [21]Poole, K. (2002) Mechanisms of bacterial biocide and antibiotic resistance. *J. Appl. Microbiol.* 92 (s1): 55S-64S.
 - [22]Rane, S. (2011) Street vended food in developing world: hazard analyses. *Indian J. Microbiol.* 51 (1): 100- 106.
 - [23]S. Rane, “Street vended food in developing world: hazard analyses,” *Indian Journal of Microbiology*, vol. 51, no. 1, pp. 100–106, 20116
 - [24]Schmidt R, MG Ren’ee, L Douglas, Archer and RS Keith, 2003. General Overview of the Causative Agents of Food bomeIllness,|| This document is FSHNO33, one of a series of the Food Science and Human Nutrition Department, Florida Cooperative Extension Service, WAS, University of Florida
 - [25]Sharada R, Krishnappa G, Raghavan R, Sreevinas G, Upandra HA 1999: Isolation and serotyping of *Escherichia coli* from different pathological conditions in poultry. *Indian Journal of Poultry Science* 34 366- 369.
 - [26]Singh V, Chandel R, Chauhan P K, Bala I, and Thakur K 2011: Prevalence and antibiogram pattern of bacteria isolated from food product (Burger) of street food vendors of Paonta Sahib. *International Journal of Institutional Pharmacy and Life Sciences* 86-90.

- [27] Tagoe DNA, Nyarko H, Arthur SA, Birikorang E 2011: A Study of antibiotic susceptibility pattern of bacterial isolates in sachet drinking water sold in the cape coast metropolis of Ghana. *Research Journal of Microbiology* 6 153-158.
- [28] ambekar DH, Jaiswal VJ, Dhanorkar DV, Gulhane PB, Dudhane MN. Identification of microbiological hazards and safety of ready-to-eat food vended in streets of Amravati City, India. *Journal of Applied Biosciences*. 2008; 7:195-201.
- [29] Tambekar, D. H., Jaiswal V. J., Dhanorkar, D. V., Gulhane, P. B., Dudhane, M. N. (2008) Identification of microbiological hazards and safety of ready-to-eat food vended in streets of Amravati City, *Indian J. Appl. Bio-Sci.* 7: 195-201.
- [30] Thomas AR, Bruce AD, Stacy A, Genagon NM, Warholc UM, Patrick D, Pawlicki JM, Beannan RO, Burce AH, Paul RK 2005: *Escherichia coli* virulence factor hemolysin induces neutrophil apoptosis and necrosis/lysis in vitro and necrosis/ lysis and lung injury in a rat pneumonia model. *American Journal of Physiology-Lung cellular and Molecular Physiology* 289 207-261.
- [31] Thomas CGA 1998: Gram-negative Bacilli. In: *Medical Microbiology*. 6th edn. Bailliere Tindall, Oxford, UK pp. 273-274. World Health Organization (2014). *Antimicrobial resistance: global report on surveillance.*