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Presence of Antibiotic-Resistant Pathogens in School Cafeteria's Fast Foods in Dhaka City, Bangladesh: A Growing Concern

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Authors' contributions

Author SRA has conceptualized the study and interpreted the data. Author SR collected the samples, analyzed the data, and wrote the first draft of the manuscript. Entire laboratory experiments were carried out by author SR under the supervision of author SRA. Author II helped in literature research and revised the draft critically for important intellectual content. All authors read and approved the final manuscript.

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ABSTRACT

Burden, due to foodborne diseases, particularly *Salmonella* infection, is high in developing countries like Bangladesh. This research aimed at the molecular characterization of *Salmonella* spp., isolated from selected school canteen's fast foods in Dhaka city, Bangladesh, and to evaluate the antibiotic resistance patterns of isolated foodborne pathogens. The school cafeterias were selected by the convenience sampling method. The samples were collected aseptically, and serial dilutions were made. The bacterial colonies were isolated by spread plate technique using appropriate media, and bacterial identification was carried out using gram staining and biochemical tests such as MIU, KIA, Oxidase, and Catalase test. The strain of *Salmonella* spp. was confirmed by molecular characterization employing the 16S rRNA gene sequencing method. The susceptibility of the isolates to various antibiotics was observed by modified Kirby-Bauer disk diffusion method. Most of the samples were found to contain an unacceptable level of a total aerobic count, which ranged from 5.6×10^5 to 6.1×10^7 and 3.4×10^4 to 7.2×10^7 for burger and sandwich samples, respectively. Significant isolates from the pathogenic strains were *Salmonella spp., Shigella, Klebsiella, Proteus, E. coli, Vibrio spp., Clostridium spp., Staphylococcus spp.,* and

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others. The further molecular characterization of isolated *Salmonella spp.* suggests the similarity with *Salmonella enterica* serovar Rissen SeqrSC0091. Most isolates were resistant against Ampicillin (100%), Azithromycin (60.87%), Tetracycline (39.43%), Colistin (32.61%), while were highly sensitive to Gentamycin and Chloramphenicol. The presence of multidrug-resistant foodborne pathogens at this high level in the school cafeteria's fast foods signifies an increased risk for the children's health.

Keywords: Foodborne diseases; fast foods; antibiotic resistance; salmonella.

1. INTRODUCTION

Foodborne diseases, an emerging public health problem, encompass a wide range of illnesses. An estimated 2.2 million death among which 1.9 million being children is attributed to foodborne and waterborne diarrheal diseases annually [1]. Most of the foodborne illnesses are bacterial, *Salmonella* spp.*, Shigella* spp., *Staphylococcus aureus,* and *Bacillus cereus* are the dominant bacteria responsible for causing foodborne illness [2].

In the contemporary world, antimicrobial resistance is one of the grave warnings to global health and lives. The phenomena of antimicrobial resistance result in the failure or ineffectiveness of standard treatments and the persistence of infections, further enhancing the chance of expanding the severity. The microorganisms are developed to resistant strains, which naturally occur when they make erroneous copies of themselves or when resistant genes are swapped in between. The rise of drug-resistant pathogens is stimulated by the uncontrolled use of antimicrobial drugs [3]. Multi-drug resistant bacterial infection in Europe alone causes around 25000 deaths and a loss of €1.5 billion annually [4]. Over and above, there are some causes that accelerate the expansion of antimicrobial resistance. Inappropriate or inadequate infection limiting practices, unhygienic environments, and unsuitable methods of handling foods are significant causes [5,6]. A clinical investigation in Bangladesh revealed that >70% of infecting bacteria were resistant to at least one of the commonly used antibiotics [7]. Moreover, the bacterial antibiotic-resistant gene is transferred to other bacteria, even to other species. This further works in disseminating resistant bacteria from a particular area to different geographical regions [8].

There have been dramatic transitions in population dietary patterns, particularly a substantial increase in the consumption of ultra-processed foods, including fast foods. Fast food consists of pre-cooked meals kept in readiness for a customer's arrival. Some fast-food outlets use mass-produced pre-prepared ingredients such as bagged buns & condiments, frozen beef patties, prewashed and sliced vegetables, etc. Fast food culture is an emerging trend among the younger generation, contributing to a large extent to childhood obesity [9]. The shorter preparation time and longer holding time make the food unsafe for consumption and increase the probability of microbial contamination. Sometimes the leftover foods are served, which poses a substantial risk of food poisoning.

Nowadays, most of the schools in urban areas are facilitated with a canteen. Students spend a great deal of time in educational institutions. Most of these canteens serve fast foods or fried foods such as burgers, sandwiches, vegetable rolls, etc. Moreover, if these foods are contaminated with pathogens, they impose a higher health risk. Therefore, it is pivotal to explore the extent of antibiotic resistance patterns of these pathogenic bacteria. This study was aimed to identify the prevalence of multi-drug resistant pathogenic bacteria present in foods collected from the selected canteens of some private and public schools of Dhaka city, Bangladesh.

2. MATERIALS AND METHODS

2.1 Sample Collection

A total of a hundred Burgers and Sandwiches, ten samples of each food item from five selected school canteens were collected between February 2019 to June 2019. All the samples were collected under aseptic conditions maintaining proper biosafety concerns.

2.2 Preparation of Samples

Ten gram**s** of each sample was taken and mixed with 90 mL peptone water. The flasks were shaken for homogenization of the samples and finally plugged with cotton. After adequately

mixing the sample, 1 ml of the sample solution was pipetted and transferred into sterilized cell culture tubes containing 9 mL of 0.1% peptone water. After that**,** they were mixed thoroughly by shaking by a vortex mixer (XH-C, Taiwan). Serial dilutions were prepared from the initial sample homogenate using the aseptic technique.

2.3 Bacteriological Studies

For bacterial isolation, the spread plate method was performed. Different culture mediums such as Plate count agar (PCA), MacConkey agar, *Salmonella-Shigella* (SS) agar, Eosin-methylene blue (EMB) agar, Thiosulphate citrate bile salt sucrose (TCBS) agar, Cooked meat media were used for the isolation purpose. PCA was used to evaluate the total viable count of the bacteria. MacConkey agar is selective for gram-negative enteric bacteria. SS agar was used for colony characteristics of *Salmonella, Shigella*. TCBS agar is a highly selective medium and was used for the isolation of *Vibrio* spp., and Cooked meat media was used for the growth of *Listeria* and *Clostridium*. An estimated 50µL diluted sample suspension from each test tubes was taken into a sterile petri dish and incubated for 24-48 hours at 37□C. Isolated bacterial colonies of different types grown on these media were collected and maintained in nutrient agar slant. The isolates from the Cooked meat medium were stored in nutrient agar, maintaining an anaerobic condition. Bacterial colonies grown on agar plates/slants were tested instantly for morphological and cultural characteristics.

2.4 Characterization of Isolates

Colonies were observed for size, color, margin, elevation, consistency, and opacity. Morphological characteristics were investigated through Gram-staining and microscopic examination. Cultural, morphological, and biochemical characteristics were observed [10]. Several biochemical tests such as MIU (Motility indole urease), KIA (Kliger iron agar), Catalase, and Oxidase tests were carried out for the identification of the isolated colonies.

2.5 Antibiotic Sensitivity Test

A modified disk diffusion method was performed to see the susceptibility of the isolates to various antibiotics such as Ampicillin, Chloramphenicol, Gentamycin, Tetracycline, Colistin, Azithromycin, Ciprofloxacin, and Levofloxacin. These antibiotics had been chosen randomly. For the antibiotic susceptibility test, Muller-hilton agar and Muller-hilton broth were used.

2.6 Molecular Characterization of *Salmonella* spp.

PCR using universal primers for bacterial 16S rRNA gene and invA gene was done for the molecular characterization of *Salmonella* isolates. The specific primers targeting the invA region of *Salmonella* spp*.* were selected for the molecular identification of the isolates [11]. All the PCR tubes containing the reaction mixtures were heated at 94 ºC for 5 minutes in the thermal cycler to ensure the denaturation of all DNA templates. Then PCR reaction was then continued according to the following program: Denaturation at 94 ºC for 1 minute, annealing at 55 ºC for 1 minute, and extension at 72 ºC for 30 seconds. These three steps were repeated sequentially for 35 cycles with a final extension for 7 minutes at 72 ºC. After completion of the reaction, PCR tubes were stored at -20 ºC until further analysis. For confirmation of the PCR products of 16S rRNA, cycle sequencing was performed using BigDye® Terminator v3.1 Cycle Sequencing Kit (Applied Biosystem, USA). The extension product was purified, followed by capillary electrophoresis using ABI Genetic Analyzer (Applied Biosystems®, USA). Partial sequences of desired genes obtained using specific forward and reverse primers were combined to full-length sequences using the SeqMan Genome Assembler [12] and were compared to the GenBank database through the primary local alignment search tool (BLAST) to identify their close phylogenetic relatives [13].

3. RESULTS

The highest count of bacteria for both sandwich and burger samples was found from the canteen of school A, and the lowest count was found from school C. Apart from the sandwich samples of school C, the total bacterial loads of all other the samples were unsatisfactory as per the International Commission on Microbiological Specifications for Foods [16] (Table 2).

Analysis of the isolates' antibiotic susceptibility pattern showed that 100% of the isolates were resistant against at least one antibiotic, 52% were resistant against two antibiotics, 26% were against three antibiotics, and 22% were resistant against more than three antibiotics. Our studies revealed that the *Salmonella* spp., *E. coli, Pseudomonas, Proteus, Vibrio,* and other

isolates were highly resistant to various antimicrobial agents. Improper hygienic standards and indiscriminate use of antimicrobials could be two of the leading causes for the prevalence of these pathogenic resistance strains (Fig. 1).

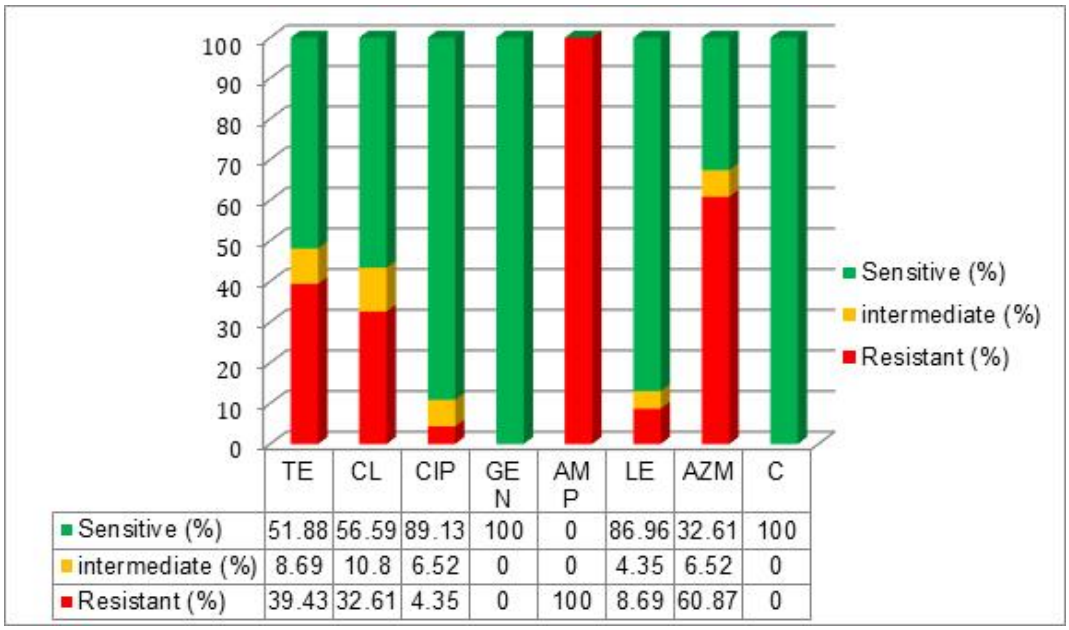
Table 1. Selected primer's sequence for this study

Target gene	Primers	Sequence(5'→ 3')	Amplicon Size (bp)	Annealing Temperature (□C)	Reference
invA	139F	GTGAAATTATCGCCACGTCGGGCAA	284	53	Rahn et al., [14]
	141R	TCATCGCACCGTCAAAGGAACC			
16S rRNA	27F	AGAGTTTGATCMTGGCTCAG	900	55	Lane et al. [15]
	1492R	CCGTCAATTCMTTTRAGTTT			

Table 2. Total count of viable bacteria found in sandwich samples from different school canteens using PCA

Sample	Type of school	Name of school	Mean Total count* (CFU/g)	Remark
Sandwich	Public	School A	7.23×10^{7}	Unsatisfactory
		School B	5.64×10^{6}	Unsatisfactory
		School C	3.41×10^{4}	Acceptable
	Private	School D	5.62×10^{5}	Unsatisfactory
		School E	7.17×10^{6}	Unsatisfactory
Burger	Public	School A	6.12×10^{7}	Unsatisfactory
		School B	1.53×10^{6}	Unsatisfactory
		School C	5.67×10^{5}	Unsatisfactory
	Private	School D	1.33×10^{6}	Unsatisfactory
		School E	8.64×10^{6}	Unsatisfactory

Note: According to International Commission on Microbiological Specifications for Foods, $<10^{4}$ = Good, $<10^{5}$ = Acceptable, $\geq10^{5}$ = Unsatisfactory
**Average count of 10 samples from each school.*



	TE	CL	CIP	GEN	AMP	LE	AZM	C
Sensitive (%)	51.88	56.59	89.13	100	0	86.96	32.61	100
intermediate (%)	8.69	10.8	6.52	0	0	4.35	6.52	0
Resistant (%)	39.43	32.61	4.35	0	100	8.69	60.87	0

Fig. 1. Diagrammatic presentation of the antibiotic resistance pattern of the isolates

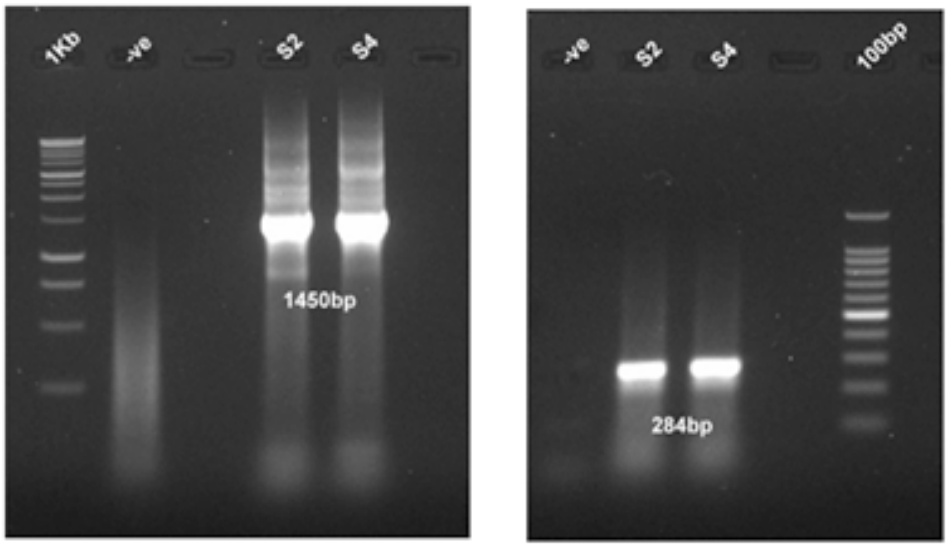


Fig. 2. Agarose gel electrophoresis (on 1% agarose gel) of (left) 16S rRNA gene profile (right) invA gene profile

Fig. 3. Phylogenetic tree of 16S rRNA gene sequences of *Salmonella* isolates and close relative reference isolates retrieved from the database

The Multiple Antibiotic Resistance Index of the 41 Gram-negative isolates was calculated to see the isolate's ability to resist the antibiotics used in the study (Table 3).

Based on biochemical characterization, two isolates coded as S2 and S4 from the presumptive identified *Salmonella* group were selected for the 16S rRNA gene sequencing. PCR product of each isolate was selected for sequence analysis using respective primers (Fig. 2).

Table 3. Multiple Antibiotic Resistance Index of isolated bacteria

Bacterial spp.	MAR %
Salmonella	62.5
E. coli	75
Klebsiella	50
Proteus	37.5
Vibrio	37.5
Pseudomonas	25
Shigella	25
Aeromonas	37.5
Yersinia	37.5

The Phylogenetic tree was constructed in MEGA7 software using the Neighbor-Joining algorithm, and that was found to be similar (98.44%) with *Salmonella enterica* serovar Rissen SeqrSC0091 (Fig. 3).

4. DISCUSSION

The result of the current study indicates unsatisfactory microbial load in the school canteen's food, which can be pernicious to the student's health due to the presence of potentially pathogenic strain.

The fast-food samples were maintained at the same condition as purchased. So, the count represented what the school children were consuming. All the samples were coded to ensure blinding. Sandwiches and burgers contained fresh salad items and mayonnaise as well as were moist inside; these somewhat explain the presence of bacteria in those items. Fresh-cut vegetables or minimally processed ready-to-eat salad samples were highly contaminated with coliforms, *E. coli, Bacillus cereus,* and *Staphylococcus aureus* in several studies [17,18]. The presence of *E.coli*, *Klebsiella, Staphylococcus* in the food samples from school canteens might be because of using these kinds of fresh vegetable products.

As per the CDC statistics, about 1.2 million illnesses, 23,000 hospitalizations, and 450 deaths each year in the USA are attributed to *Salmonella* alone [19]. WHO estimated that diarrheal and invasive infections of all foodborne diseases due to non-typhoidal *S. enterica* infections resulted in the highest-burden causing 4.07 million DALYs, and food is the source for about 1 million of these illnesses [1].*Salmonella* spp., the most notorious group for causing typhoid, paratyphoid, and foodborne toxicity, was found at an alarming number in this study. Both the samples contained a high amount of these pathogenic bacteria. *Salmonella* spp. were found in meat-based fast foods in a similar study in Lebanon [20]. These bacteria are generally transmitted to humans by consuming contaminated food of animal origin, mainly meat, poultry, eggs, and milk. As both the fast-food samples contained ground or whole poultry meat, the sandwich sample also had egg, which could be the most probable route of transmitting this pathogenic species into the children's stomach. The most important sources of *Clostridium* spp. are meat and meat products. A study found a 47% incidence of *Clostridium perfringenes* in ground beef [21]. It can also be found in poultry as both of the food samples contained minced poultry meat. Presence of *Clostridium spp.* indicates an unhygienic environment or lack of proper processing of raw flesh. Despite having similarities in components between burger and sandwich samples, the sandwich samples contain varied and more numbers of bacteria. This could be attributed to fried and moist minced meat in burger and sandwich samples, respectively. No significant statistical difference was found between the burger and sandwich samples.

The present study results also showed a very high level of *Vibrio spp*. A similar study was also found as high as 6×10^6 CFU/g of *V. cholerae* in fast food items in Dhaka city of Bangladesh [22]. The *V. cholerae* genome readily changes, with extensive genetic recombination through lateral gene transfer, resulting in termed shifts and drifts in the genome sequence [23]. This genetic plasticity is reflected in the observation of multiple genetically distinct *V. cholera* strains, which also showed multiple drug resistance. Multidrug-resistant *Vibrio* spp. was isolated from most of the samples.

Our studies revealed that the *Salmonella* spp., *E. coli*, *Pseudomonas, Proteus, Vibrio,* and other isolates were highly resistant to various antimicrobial agents. Improper hygienic standards and indiscriminate use of antimicrobials could be two of the primary causes for the prevalence of these pathogenic resistance strains [24]. The study also showed that 100% of the isolated organisms were resistant to at least one antibiotic, Ampicillin. Multi-drug resistant *Salmonella* spp. and *Vibrio* spp. have been found in poultry in various studies in Bangladesh. In this study, we have found *E. coli, Vibrio* spp., *Salmonella* spp., and *Proteus* spp. to be resistant against Ampicillin, Azithromycin, Colistin, and Tetracycline mainly. A similar study shows that almost 80% of samples were tested for *Salmonella* spp. isolates were resistant to at least one of the tested antimicrobials [20]. In the same study, *Salmonella* spp. were least resistant to Cefotaxime (25.9%) and with moderate susceptibility of 57.1% against both Cefuroxime and Gentamicin, but in our study, the *Salmonella* spp. showed no resistance against Gentamicin but 100% resistance against Ampicillin, Azithromycin, and Colistin.

Ciprofloxacin is a third-generation antibiotic. *E. coli, Vibrio* spp., *Proteus* spp.,and *Salmonella*

spp. were found to be resistant to these antibiotics. The MRI percentage of the individual species ranged from 25% for *Shigella, Pseudomonas*, for *Salmonella* 67.33%, and was highest for *E. coli* (75%). Colistin is considered to be the last resort against several species of multiple drug-resistant bacteria [25]. The present study found several bacteria, including *Salmonella* spp., to be resistant against colistin. Some of the isolated *E. coli* was resistant, and a *Proteus* spp. was found to be intermediately resistant against levofloxacin. *Proteus* spp. are notorious for causing urinary tract infections, and also levofloxacin is used to cure this problem [26]. Horizontal transfer of these resistances to unrelated species is assumed to worsen the situation.

There are several limitations of this current study. The sample size of the study was relatively small. This study should have been done with more samples and throughout the supply chain, particularly in every critical control point of production level to consumer level for finding the source of contamination.

5. CONCLUSION

The result of drug resistance profiling implies that Bangladesh's antibiotic usage pattern is not entirely safe and may impart a significant threat to our lives. In Bangladesh, a regular monitoring system for assessing foodborne illness is unavailable, limiting our knowledge of the actual situation prevailing here. Although numerous studies have been carried out to determine the prevalence of bacterial contamination in street foods, comprehensive studies to comprehend the critical control points causing microbial contamination in school canteen foods are scanty. More research should be done, and data should be published systematically regularly.

CONSENT AND ETHICAL APPROVAL

The study was conducted in accordance with the Declaration of Helsinki. As this current study did not require any human/animal subjects, therefore the institutional ethics committee waived the requirement of ethics approval. A consent form was submitted to the school authority before conducting the study. Though the school authorities provided permission for conducting the research, they did not agree to publish their respective names. Therefore, we had used code names for the schools.

COMPETING INTERESTS AND DISCLAIMER

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

REFERENCES

1. Organization, W.H., WHO estimates of the global burden of foodborne diseases: foodborne disease burden epidemiology reference group 2007-2015. 2015: World Health Organization.
2. Scallan E, et al. Foodborne illness acquired in the United States—major pathogens. Emerging infectious diseases, 2011;17(1):7.
3. Zankari E, et al. identification of acquired antimicrobial resistance genes. Journal of Antimicrobial Chemotherapy. 2012;67(11): 2640-2644.
4. Machowska A, Stålsby C. Lundborg, Drivers of irrational use of antibiotics in Europe. International Journal of Environmental Research and Public Health. 2019;16(1):27.
5. Eltai NO, et al. prevalence of antibiotic resistant *Escherichia coli* isolates from fecal samples of food handlers in Qatar. Antimicrobial Resistance & Infection Control. 2018;7(1):1-7.
6. Sussman G. Antimicrobial resistance relating to wound management and infection. Wound Practice & Research: Journal of the Australian Wound Management Association. 2016;24(4): 224-227.
7. Talukdar PK, et al. Antimicrobial resistance, virulence factors and genetic diversity of *Escherichia coli* isolates from household water supply in Dhaka, Bangladesh. Plos one. 2013;8(4): e61090.
8. Noor R, Munna MS. Emerging diseases in Bangladesh: current microbiological research perspective. Tzu Chi Medical Journal. 2015;27(2):49-53.

9. Alam MR, et al. prevalence and association of different lifestyle factors with overweight and obesity among the children of selected private English Medium Schools from Dhaka City. World. 2019; 7(2):42-47.
10. Holt J, et al. Bergey's manual of determinative bacteriology 9th Edition, Williams and Wilkins. Baltimore, Maryland, USA; 1994.
11. Halatsi K, et al. PCR detection of Salmonella spp. using primers targeting the quorum sensing gene sdiA. FEMS Microbiology Letters. 2006;259(2):201-207.
12. Swindell SR, Plasterer T. Sequence data analysis guidebook. Humana Press New York, NY, USA. 1997;70.
13. Benson DA, et al. GenBank. Nucleic Acids Research. 2005;33(suppl_1):D34-D38.
14. Rahn K, et al. Amplification of an invA gene sequence of Salmonella typhimurium by polymerase chain reaction as a specific method of detection of Salmonella. Molecular and Cellular Probes. 1992;6(4): 271-279.
15. Lane DJ. 16S/23S rRNA sequencing. Nucleic acid techniques in bacterial systematics. 1991;115-75..
16. Mataragas M, Skandamis P, Drosinos E. Risk profiles of pork and poultry meat and risk ratings of various pathogen/product combinations. International Journal of Food Microbiology. 2008;126(1-2): 1-12.
17. Jo MJ, et al. Microbiological quality of fresh-cut produce and organic vegetables. Korean Journal of Food Science and Technology. 2011;43(1): 91-97.
18. Thunberg RL, et al. Microbial evaluation of selected fresh produce obtained at retail markets. Journal of Food Protection. 2002;65(4):677-682.
19. Control CfD. Prevention, surveillance for foodborne disease outbreaks, United States, 2013, annual report. Atlanta, Georgia: US Department of Health and Human Services, CDC; 2016.
20. Harakeh S, et al. isolation, molecular characterization and antimicrobial resistance patterns of Salmonella and Escherichia coli isolates from meat-based fast food in Lebanon. Science of the Total Environment. 2005;341(1-3):33-44.
21. Ladiges W, Foster J, Ganz W. Incidence and viability of Clostridium perfringens in ground beef. Journal of Milk and Food Technology. 1974;37(12):622-623.
22. Hasan T. Microbial quality of selected sandwiches sold at fast food shops in Dhaka city. BRAC University; 2014.
23. Chun J, et al. Comparative genomics reveals mechanism for short-term and long-term clonal transitions in pandemic Vibrio cholerae. Proceedings of the National Academy of Sciences. 2009; 106(36):15442-15447.
24. Hanberger H, et al. Surveillance of antibiotic resistance in European ICUs. Journal of Hospital Infection. 2001;48(3): 161-176.
25. Karaiskos I, et al. Intraventricular and intrathecal colistin as the last therapeutic resort for the treatment of multidrug-resistant and extensively drug-resistant Acinetobacter baumannii ventriculitis and meningitis: a literature review. International Journal of Antimicrobial Agents. 2013; 41(6):499-508.
26. Martin SJ, Jung R, Garvin CG. A risk-benefit assessment of levofloxacin in respiratory, skin and skin structure, and urinary tract infections. Drug Safety. 2001; 24(3):199-222.

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