LEVEL OF CONTAMINATION AND ANTIBIOTICS RESISTANCE OF ESCHERICHIA COLI ISOLATED FROM SANDWICHES VENDED IN CAN THO CITY, VIETNAM

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TÓM TẮT

MỨC ĐỘ Ô NHIỄM VÀ TÍNH KHÁNG KHÁNG SINH CỦA ESCHERICHIA COLI ĐƯỢC PHÂN LẬP TỪ BÁNH MÌ THỊT BÁN TẠI THÀNH PHỐ CẦN THƠ, VIỆT NAM

Mục tiêu của nghiên cứu này là đánh giá mức độ nhiễm vi khuẩn coliform và E. coli của bánh mì được lấy tại quận Ninh Kiều, thành phố Cần Thơ, Việt Nam và tính nhạy cảm kháng sinh của vi khuẩn E. coli. Kết quả cho thấy mức độ nhiễm coliform trên bánh mì thịt tại các khu vực trường học, bệnh viện và chợ lần lượt là 5.3±1.3, 5.4±0.8 và 5.3±0.6 log CFU/g; trong khi mức độ ô nhiễm E. coli tại các khu vực này lần lượt là 3.3±1.5, 3.6±1.0 và 3.3±1.0 log CFU/g. Có 17/23 (73,9%) chủng E. coli được phân lập từ bánh mì thịt được nhận thấy là đa kháng kháng sinh. Tỷ lệ kháng cao được quan sát thấy đối với ampicillin (87%), tetracycline (60,9%), cefotaxime (56,5%), chloramphenicol (43,5%) và sulfamethoxazole/trimethoprim (39,1%). Bánh mì là món ăn đường phố phổ biến và được yêu thích, vì thế an toàn và chất lượng của bánh mì cần được kiểm soát để đảm bảo sức khỏe cộng đồng.
Từ khóa: *bánh mì thịt, Escherichia coli, kháng kháng sinh.*

1. INTRODUCTION

Sandwiches are a most famous and favorite street food in Vietnam because they are usually perceived to be inexpensive, convenient and attractive (Anh Ngoc, 2019; Imathiu, 2017). Even though street foods are beneficial, serious concerns raised regarding food safety, the risk of food poisoning outbreaks linked to street foods remains a threat in many parts of the world, with microbiological contamination as a major problem (FAO, 2011). The issues of an increase in foodborne illnesses have become a global concern, foodborne and waterborne diseases kill around 2.2 million people annually (Tritscher et al., 2013; Van Tonder et al., 2007). Besides, some issues related to street foods may cause foodborne diseases by microbial contaminations (Nonato et al., 2016). The main reasons for food poisoning outbreaks are possibly poor quality of raw materials, unhygienic practices during food preparation, processing and preservation and awareness of vendors about food safety (Aluko et al., 2014; Ngoc et al., 2020; Samapundo et al., 2015). The previous studies reported that sandwiches were highly contaminated with pathogens such

as coliforms, *E. coli*, *S. aureus* (Anh Ngoc, 2019). However, scientific information about antibiotics susceptibility of these bacteria from sandwiches is unknown. Antimicrobial resistance is now recognized as one of the most serious global threats to human health (Liu et al., 2016). *E. coli* has also been reported to be a candidate vehicle for transfer of antibiotic resistance gene (Van et al., 2008). Additionally, the report of multi-drug resistance in *E. coli* that is rampant in food sources, environment and human beings is alarming, especially effecting to public health (Dyar et al., 2012; Nhung et al., 2015; Salako et al., 2020; Van et al., 2008). The objective of this study was to evaluate the level of contamination (i.e. coliforms and *E. coli*) and antibiotics susceptibility of *E. coli* collected from the sandwiches in Can Tho city, Vietnam. This study provides useful information about the current food safety status of sandwiches sold by street food vendors and can contribute to developing the regulations for street food sectors.

2. MATERIALS AND METHODS

2.1. Sampling

A total of 37 samples of sandwiches were collected between 7-10 a.m. from different locations such as schools, hospitals and markets in Ninh Kieu district of Can Tho city, Vietnam. The samples were transferred aseptically into stomacher bags (Stomacher bags, BagLight®, France), stored in Styrofoam boxes containing ice packs, and then transported to the Laboratory of Microbiology and Biotechnology of Food Technology Department, Can Tho University for microbiological analysis. All the samples were analyzed for coliforms and *E. coli* within four hours.

2.2. Microbiological analysis

2.2.1. Quantitative analysis of coliforms and E. coli

Twenty-five grams of samples from different portions (including pork meat products, vegetables, bread, pickles, fresh herbs) were taken using sterile scalpels and tweezers and placed in a stomacher bag. Then, 225 mL of Maximum Recovery Diluent (MRD, Merck, Darmstadt, Germany) was added and the mixture was homogenized for three to five minutes. 1 mL of the homogenized sample was aseptically transferred to 9 mL of MRD. Subsequently, a tenfold serial dilution was then made in MRD. Three appropriate and consecutive dilutions were enumerated by pour-plating of 1 mL on Coliform Agar Enhance Selectivity (Coliform Agar ES, Merck, Darmstadt, Germany) and later incubated for 24 h at 37°C after which blue to violet colonies were counted as *E. coli* while pink to red colonies and *E. coli* colonies were counted as coliforms (Lange et al., 2013).

2.2.2. Isolation and confirmation of Escherichia coli

Colonies with blue to violet colors on Coliform Agar ES suspected to be *E. coli* were selected, sub-cultured in Tryptic Soy Broth (TSB, Merck, Darmstadt, Germany) for 18-24 h at 37^{o}C and then streaked on Tryptic Soya Agar (TSA, Merck, Darmstadt, Germany) for 48 h at 37^{o}C to collect the pure colonies and further confirmation by five biochemical tests including "IMViC" (Indole, Methyl red, Voges-Proskauer, Citrate) and Kligler Iron Agar tests (Merck, Darmstadt, Germany). The pure isolates of *E. coli* were stored at -18^{o}C for antibiotics resistance test.

2.3. Antibiotics resistance test

All of the *E. coli* isolates were tested for their resistance to 15 antimicrobial agents by the disk diffusion method (CLSI, 2019). A strain of *Escherichia coli* ATCC 25922 was used as a control. Fifteen antibiotics commercially available and frequently used in the community, aquaculture and animals were tested on *E. coli* as recommended by the Clinical and Laboratory Standards Institute (CLSI). Antimicrobial agents used on the disks include: ampicillin (AMP), 10 µg; meropenem (MEM), 10 µg; gentamicin (GM), 10 µg; tetracycline (TE), 30 µg; chloramphenicol (C), 30 µg; ciprofloxacin (CIP), 5 µg; fosfomycin (FOF), 200 µg (Abtek, United Kingdom);

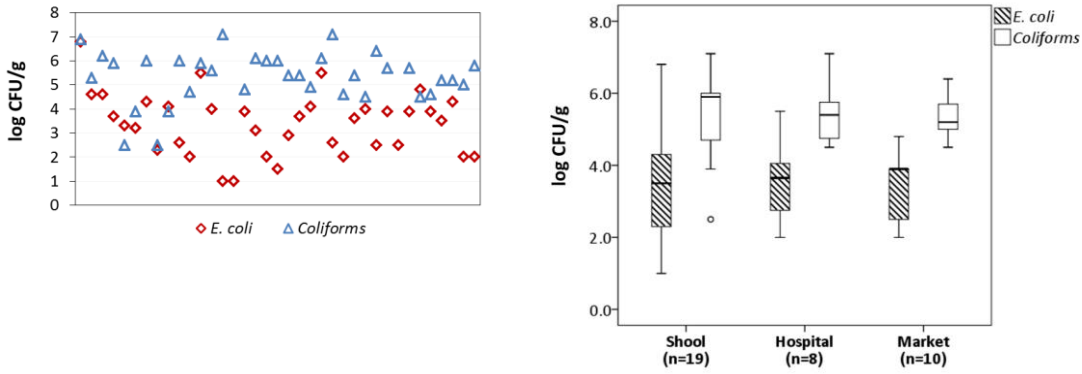
ceftazidime (CAZ), 30 µg; cefotaxime (CTX), 30 µg; cefoxitin (FOX), 30 µg; kanamycin (KM), 30 µg; streptomycin (S), 10 µg; sulfamethoxazole/trimethoprim (SXT), 23.75/1.25 µg; nalidixic acid (NA), 30 µg and colistin (CL), 10 µg (Nam Khoa, Vietnam). The isolates were pre-cultured in TSB broth for 18-24 h at 37°C and then tested for susceptibility to 15 antimicrobial agents as mentioned above (Tong Thi, 2019). The diameters of the inhibition zones were measured and classified as susceptible, intermediate, and resistant according to the zone diameter interpretative standards recommended by CLSI (2019).

2.4. Data analysis

The results were calculated and graphed using Microsoft Excel 2019. The results of the microbial analysis were expressed as log CFU/g and reported as mean value ± standard deviation. The results of the microbial contamination were statistically analysed which based on ANOVA test ($\alpha = 0.05$) using SPSS Statistics version 20 (SPSS Inc., Chicago, U.S.A.).

3. RESULTS AND DISCUSSIONS

3.1. Coliform and E. coli counts of collected sandwiches



(a) *(b)*

Figure 1. E. coli and coliform counts on collected sandwiches (n=37)

Figure 1 shows the results of coliform and *E. coli* counts of 37 sandwiches collected in Ninh Kieu district, Can Tho city (Figure 1a) and at different locations (Figure 1b). Coliforms of sandwiches were 3.4±1.3 log CFU/g whereas *E. coli* were 5.3±1.0 log CFU/g (Figure 1a). Among locations sampled, the contamination of coliforms on sandwich taken at school, hospital and market areas were 5.3±1.3, 5.4±0.8 and 5.3±0.6 log CFU/g, respectively; while the contamination of *E. coli* on sandwiches sampled at these areas were 3.3±1.5, 3.6±1.0 and 3.3±1.0 log CFU/g, respectively (Figure 1b). No difference was found in coliform and *E. coli* count of sandwiches among different locations sampled ($p>0.05$). Anh Ngoc (2019) reported that the level of coliforms and *E. coli* contaminated on the sandwiches vended in Haugiang province from 2.3 to 5.6 and <1 to 5.0 log CFU/g, respectively; while 55.2% (37/67) of the sandwiches samples which *E. coli* contaminated below the detection limit of *E. coli* (<1 log CFU/g). The study of Thanh (2015) also reported that coliform count contaminated on the sandwiches vended in Hochiminh city ranged from 2.5 to 5.1 log CFU/g. The sandwiches are a mixed product of many ingredients such as bread, cooked meat (or pâté), vegetables (cucumber, pickled carrot and white radish, cilantro), etc. Standard's Vietnam Ministry of Health regulations 05/2012/TT-BYT (Vietnam Ministry of Health, 2012) used meat products processed and vegetables for ready-to-eat to be *E. coli* of 1.7 and 2.0 log CFU/g, respectively. In the

current study, 29/37 (78.4%) of the collected sandwiches samples were unsatisfactory for *E. coli* criteria. These results were in agreement with previous studies performed in Haugiang and Hochiminh city, indicating a threat to the safety of sandwiches (Anh Ngoc, 2019; Thanh, 2015). Coliforms and *E. coli* is hygiene indicator and their presence indicates poor hygiene practices during or after food production (Kothe et al., 2016). Therefore, it is suggested that food vendors should be good hygiene practices during food preparation, storage and preservation to avoid contamination and ensure the safety of sandwiches as well as other street foods.

3.2. Antibiotics resistance of *E. coli* isolated from collected sandwiches

Antimicrobial susceptibility of the *E. coli* isolates collected from sandwiches samples was determined against 15 antibiotics (Figure 2).

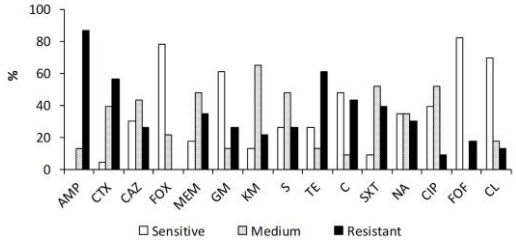


Figure 2. Antibiotics resistance of E. coli isolates (n=23)

Notes: AMP: ampicillin, CTX: cefotaxime, CAZ: ceftazidime, FOX: cefoxitin, MEM: meropenem, GM: gentamicin, KM: kanamycin, S: streptomycin, TE: tetracycline, C: chloramphenicol, SXT: sulfamethoxazole/trimethoprim, NA: nalidixic acid, CIP: ciprofloxacin, FOF: fosfomycin, CL: colistin

E. coli was resistant to 14 antibiotics tested except for Cefoxitin. The high resistance was observed to ampicillin (87%) followed by tetracycline (60.9%), cefotaxime (56.5%), chloramphenicol (43.5%), sulfamethoxazole/trimethoprim (39.1%) and other antibiotics (8.7-34.8%). Van Sy et al. (2017) stated that ampicillin was widely used in farms or veterinary due to its broad-spectrum applicability and reasonable cost. According to Van et al. (2008), 84% of *E. coli* isolates from raw meat and shell fish in Vietnam is resistant to ampicillin, tetracycline, etc. Singh (2017) stated that *E. coli*, animal intestinal tract's microflora, might be a major sources of antibiotic resistance dissemination; mostly resistant to ampicillin, tetracycline, and sulphonamide/trimethoprim. Likewise, *E. coli* isolated from sandwiches were resistant to sulfamethoxazole/trimethoprim and nalidixic acid; those antibiotics have known to be importance in human medicine (Heuer et al., 2009). These findings were in agreement with a previous study of Campos et al. (2015) who reported that resistant *E. coli* isolates were found in food handlers and hamburgers, hotdog samples in Portugal. Furthermore, raw vegetables, a component of sandwiches, can be contaminated with multi-antibiotic resistant *E. coli* due to poor hygiene practices during preparation and production (Campos et al., 2015; Kothe et al., 2016).

Table 1 shows the patterns of multi-antibiotic resistance of *E. coli*. Three out of 23 *E. coli* isolates (13%) was no resistance to all antibiotics tested (data not shown). 17/23 (73.9%) of *E. coli* isolates were multi-antibiotics resistance (resistance 3-11 antibiotics). Multi-antibiotic resistant *E. coli* can be transferred their resistant genes to other human pathogenic strains; consequently, the failures of treatments for pathogens diseases in humans can occur (Liebana et al., 2013). Therefore, contamination of multi-antibiotic resistant *E. coli* on the sandwiches might pose a potential health risk to the consumers who consumed street food on daily basis.

Especially, the sandwiches are ready-to-eat food, it is a great factor for acquiring pathogenic organisms, including antibiotic resistance bacteria. It is suggested that potential spread of relevant antibiotic resistant *E. coli* through food chains particularly street food should be controlled, as this might become a serious threat to public health. As far as we know, this is one of the first report in Vietnam, highlighting the presence of multi-antibiotic resistant *E. coli* in sandwiches.

Table 1. The antibiotic resistance patterns (n=20) of E. coli isolates

Number of resistant antibiotics	No. isolates (%)	AMP	CAZ	CTX	FOX	MEM	GM	S	KM	TE	C	NA	SXT	CIP	FOF	CL
1	1 (4.35)	R	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2	1 (4.35)	R	-	R	-	-	-	-	-	-	-	-	-	-	-	-
2	1 (4.35)	R	R	-	-	-	-	-	-	-	-	-	-	-	-	-
3	1 (4.35)	R	-	R	-	-	-	-	-	R	-	-	-	-	-	-
4	1 (4.35)	R	-	-	-	-	-	-	R	R	R	-	-	-	-	-
4	1 (4.35)	R	-	R	-	R	-	-	-	-	-	R	-	-	-	-
4	1 (4.35)	R	-	-	-	-	-	-	-	R	R	-	R	-	-	-
5	1 (4.35)	R	-	-	-	-	-	-	R	R	R	-	R	-	-	-
5	1 (4.35)	R	-	R	-	R	-	-	-	-	-	R	-	-	R	-
5	1 (4.35)	R	-	R	-	-	R	-	-	R	-	-	-	-	-	R
5	1 (4.35)	R	R	R	-	R	R	-	-	-	-	-	-	-	-	-
6	1 (4.35)	R	-	-	-	-	-	-	R	R	R	-	R	-	R	-
6	1 (4.35)	R	-	R	-	-	-	-	R	R	R	R	-	-	-	-
6	1 (4.35)	R	R	R	-	R	-	-	-	R	-	-	R	-	-	-
6	1 (4.35)	R	-	-	-	-	R	R	-	R	R	-	R	-	-	-
8	1 (4.35)	R	R	R	-	R	-	R	-	R	R	-	R	-	-	-
9	1 (4.35)	R	-	R	-	R	-	R	-	R	R	R	R	R	-	-
10	1 (4.35)	R	R	R	-	R	R	R	-	R	-	R	R	-	R	-
10	1 (4.35)	R	R	R	-	R	R	R	-	R	R	R	-	-	-	R
11	1 (4.35)	R	-	-	-	-	R	R	R	R	R	R	R	R	R	R

Notes: R Resistance - No resistance

AMP: ampicillin, CTX: cefotaxime, CAZ: ceftazidime, FOX: cefoxitin, MEM: meropenem, GM: gentamicin, KM: kanamycin, S: streptomycin, TE: tetracycline, C: chloramphenicol, SXT: sulfamethoxazole/trimethoprim, NA: nalidixic acid, CIP: ciprofloxacin, FOF: fosfomycin, CL: colistin

4 CONCLUSIONS

It can be concluded that level of contamination of coliform and *E. coli* counts on sandwiches collected in Ninh Kieu district, Can Tho city, Vietnam ranged from 2.1 to 7.5 and <1 to 6.8 log CFU/g, respectively. There were multi-antibiotic resistances of *E. coli* isolated from the sandwiches. The high resistance was observed to ampicillin (87%) followed by tetracycline (60.9%), cefotaxime (56.5%), chloramphenicol (43.5%), and sulfamethoxazole/trimethoprim (39.1%). It is a preliminary study on multi-antibiotic resistances of *E. coli* isolated from street food. Therefore, it highly suggested that the elaborate study about multi-antibiotic resistances of *E. coli* and other pathogens contaminated on street foods should be further investigation.

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The authors declare no conflict of interest.

REFERENCES

1. Aluko, O.O., Ojeremi, T.T., Olaleke, D.A., and Ajidagba, E.B., 2014. Evaluation of food safety and sanitary practices among food vendors at car parks in Ile Ife, southwestern Nigeria. Food Control. 40: 165-171.

2. Anh Ngoc, T.T., 2019. Microbial contamination of street foods in Hau Giang province, Vietnam. The 5th conference on quality management and food safety – QMFS. 2019: 41-50.

3. Campos, J., Gil, J., Mourão, J., Peixe, L., and Antunes, P., 2015. Ready-to-eat street-vended food as a potential vehicle of bacterial pathogens and antimicrobial resistance: An exploratory study in Porto region, Portugal. International Journal of food Microbiology. 206: 1-6.

4. CLSI, 2019. Clinical and Laboratory Standards Institute, 29th Edition, M100 - Performance Standards for Antimicrobial Susceptibility Testing, Clinical and Laboratory Standards Institute. National Commitee for Clinical Laboratory Standards, Wayne, PA, January 2019.

5. Dyar, O.J., Hoa, N.Q., Trung, N.V., Phuc, H.D., Larsson, M., Chuc, N.T., and Lundborg, C.S., 2012. High prevalence of antibiotic resistance in commensal Escherichia coli among children in rural Vietnam. BMC infectious diseases. 12(1): 1-8.

6. FAO, 2011. The place of urban and peri-urban agriculture (UPA) in national food security programmes. Food Agriculture Organization of the United Nations. Technical Cooperation Dept Rome.

Heuer, O.E., Kruse, H., Grave, K., Collignon, P., Karunasagar, I., and Angulo, F.J., 2009. Human health consequences of use of antimicrobial agents in aquaculture. Clinical Infectious Diseases. 49(8): 1248-1253.

7. Imathiu, S., 2017. Street Vended Foods: Potential for Improving Food and Nutrition Security or a Risk Factor for Foodborne Diseases in Developing Countries? Current Research in Nutrition and Food Science Journal. 5(2): 55-65.

8. Kothe, C.I., Schild, C.H., Tondo, E.C., and da Silva Malheiros, P., 2016. Microbiological contamination and evaluation of sanitary conditions of hot dog street vendors in Southern Brazil. Food Control. 62: 346-350.

9. Lange, B., Strathmann, M., and Oßmer, R., 2013. Performance validation of chromogenic coliform agar for the enumeration of *Escherichia coli* and coliform bacteria. Letters in applied microbiology. 57(6): 547-553.

10. Liebana, E., Carattoli, A., Coque, T.M., Hasman, H., Magiorakos, A.-P., Mevius, D., Peixe, L., Poirel, L., Schuepbach-Regula, G., and Torneke, K., 2013. Public health risks of enterobacterial isolates producing extended-spectrum β-lactamases or AmpC β-lactamases in food and food-producing animals: an EU perspective of epidemiology, analytical methods, risk factors, and control options. Clinical Infectious Diseases. 56(7): 1030-1037.

11. Liu, Y.-Y., Wang, Y., Walsh, T.R., Yi, L.X., Zhang, R., Spencer, J., Doi, Y., Tian, G., Dong, B., and Huang, X., 2016. Emergence of plasmid-mediated colistin resistance mechanism MCR-1 in animals and human beings in China: a microbiological and molecular biological study. The Lancet Infectious Diseases. 16(2): 161-168.

12. Ngoc, T.T.A., Hang, N.T.M., Thanh, D.K., and Hoa, L.V., 2020. Evaluation of microbial safety knowledge, attitudes and practices of street food vendors and consumers in Can Tho City, Vietnam. Food Research. 4(5): 1802-1814.

13. Nhung, N.T., Cuong, N.V., Campbell, J., Hoa, N.T., Bryant, J.E., Truc, V.N.T., Kiet, B.T., Jombart, T., Trung, N.V., Hien, V.B., Thwaites, G., Baker, S., and Carrique-Mas, J., 2015. High levels of antimicrobial resistance among *Escherichia coli* isolates from livestock farms and synanthropic rats and shrews in the Mekong Delta of Vietnam. Applied and environmental microbiology. 81(3): 812-820.

14. Nonato, I., Minussi, L.d.A., Pascoal, G., and De-Souza, D., 2016. Nutritional issues concerning street foods. Journal of Clinical Nutrition & Dietetics. 2(1): 1-7.

15. Salako, D.A., Trang, P.N., Ha, N.C., Miyamoto, T., and Ngoc, T.T.A., 2020. Prevalence of antibiotic resistance escherichia coli isolated from pangasius catfish (*Pangasius hypophthalmus*) fillet during freezing process

at two factories in mekong delta Vietnam. Food Research. 4(5): 1785-1793.
16. Samapundo, S., Climat, R., Xhaferi, R., and Devlieghere, F., 2015. Food safety knowledge, attitudes and practices of street food vendors and consumers in Port-au-Prince, Haiti. Food Control. 50: 457-466.
17. Singh, O.V., 2017. Foodborne pathogens and antibiotic resistance, Foodborne Pathogens and Antibiotic Resistance. https://doi.org/10.1002/9781119139188.
18. Thanh, T.N.C., 2015. Food safety behavior, attitudes and practices of street food vendors and consumers in Vietnam, MSc Thesis, Ghent University.
19. Tong Thi, A.N., 2019. Assessment of antibiotic resistance and bacterial contamination of ice sold in Cantho city, Vietnam. Vietnam Journal of Science Technology. 57(3B): 49-58.
20. Tritscher, A., Miyagishima, K., Nishida, C., and Branca, F., 2013. Ensuring food safety and nutrition security to protect consumer health: 50 years of the Codex Alimentarius Commission. Bulletin of the World Health Organization. 91: 468-468.
21. Van Sy, N., Harada, K., Asayama, M., Warisaya, M., Sumimura, Y., Diep, K.T., Thang, N.N., Hoa, T.T.T., Phu, T.M., and Khai, P.N., 2017. Residues of 2-hydroxy-3-phenylpyrazine, a degradation product of some β-lactam antibiotics, in environmental water in Vietnam. Chemosphere. 172: 355-362.
22. Van Tonder, I., Lues, J.F., and Theron, M.M., 2007. The personal and general hygiene practices of food handlers in the delicatessen sections of retail outlets in South Africa. Journal of Environmental Health. 70(4): 33-38.
23. Van, T.T.H., Chin, J., Chapman, T., Tran, L.T., and Coloe, P.J., 2008. Safety of raw meat and shellfish in Vietnam: an analysis of Escherichia coli isolations for antibiotic resistance and virulence genes. International Journal of Food Microbiology. 124(3): 217-223.
24. Vietnam Ministry of Health, 2012. Circular No. 05/2012/TT-BYT, promulgating national technical standards for microbial contamination in foods. Available from: https://m.thuvienphapluat.vn/van-ban/the-thao-y-te/thong-tu-05-2012-tt-byt-quy-chuan-ky-thuat-quoc-gia-o-nhiem-vi-sinh-vat-138722.aspx.