Iraqi Journal of Veterinary Sciences, Vol. 36, Supplement I, 2022 (85-89)
Proceedings of the 7th (1st International) Scientific Conference, College of Veterinary Medicine, University of Mosul

Iraqi Journal of Veterinary Sciences

www.vetmedmosul.com

Detection of extended spectrum beta lactam producing *Escherichia coli* isolated from *Cyprinus carpio* in Mosul city

E.N. Mahmmoud and S.Y. Al-Dabbagh

Department of Microbiology, College of Veterinary Medicine, University of Mosul, Mosul, Iraq

Article information

Article history:
Received August 30, 2022
Accepted November 14, 2022
Available online November 30, 2022

Keywords:
ESBLs *E. coli*
Fish
CTX-M
SHV genes

Correspondence:
E.N. Mahmmoud
sumayaaldabbagh2018@gmail.com

Abstract

Extended-spectrum b-lactamase (ESBL) producing *Escherichia coli* constitute an emerging health problem globally, fish act as a potential reservoir for ESBLs *E. coli* and serve as a vehicle of transmission of ESBL resist genes to others strains of bacteria. A total of 75 samples of *Cyprinus carpio* were collected from the local fish market of Mosul city during the period between) October 2021 to February 2022). each sample was placed separately in a sterile plastic bag and transported directly under cooling conditions to the microbiology lab, College of Veterinary Medicine. ESBLs producing *Escherichia coli* were isolated and characterized using MacConkey agar medium supplemented with 2 mcg/ml cefotaxime. Twenty-six isolates 34% of fish gut samples were obtained. A polymerase chain reaction was carried out to confirm the results of the isolation using special primers for *E. coli* (ECO223-f, ECO223-r). Resistance genes assay were performed using the primers Cefotaxamase (CTX-M) and sulphydryl variable (SHV). All isolates showed that possessed the CTX-M gene 100%, while none of the isolates possessed the SHV gene. This study showed that fish play a major role in the transmission of broad-spectrum beta-lactam-resistant *Escherichia coli* to humans as a result of handling or marketing it, or by consuming contaminated or infected fish.

DOI: 10.33899/ijvs.2022.135604.2497,

Introduction

Fish is considered an important source of proteins, vitamins, essential amino acids, and contain rare minerals that are not found in other meats (1). Fish diseases are constituting one of the major problems in the fish industry because of the economic losses of fish due to fish mortality, cost of treatment and causing of zoonotic diseases for the fish consumer by handling (2). Contamination of rivers by fecal bacteria as a result of poor hygienic conditions and some bad people activities in addition to the deposition of untreated sewages causing a problem that concerns the fishes' quality, Moreover, unhygienic conditions of the storage and marketed places lead to increasing problems related to consumer safety of fishes (3). *Escherichia coli* is widely spreading in the environment as a dominant gut normal flora of humans and animals. Fish found in the natural aquatic environment harbored the pathogenic Enterobacteriaceae including *E. coli* (4). Therefore, it's considered as a potential vehicle of foodborne bacterial infections, and contributed to a threat to human public health. *E. coli* acquired pathogenicity via virulence factors or through the spread of antibiotic resistant genes, farther that *E. coli* containing antibiotic resistant genes increase potentially the threat of transferring resistance of antibiotics to other strains (5). fish Contamination with extended spectrum beta lactam -bacteria may be demonstrate the risk of the persistence of these bacteria in the gut of fish (6). Recent studies indicated that *E. coli* that developed resistance to antibiotics particularly in fish may be transmitted to humans (7). *Escherichia coli* can produce extended spectrum B-lactamase (ESBL) enzymes that hydrolyze a broad spectrum of b-lactam drugs such as

monobactams and cephalosporins (8). The main (ESBLs) genes are blaCTX-M, SHV, and blaTEM which induce such type of resistance (8). The increase of b-lactamase enzymes activity is derived from mutations in CTX-M, TEM and SHV genes that located on bacterial chromosomes or plasmids (9). These genes can easily be transferred horizontally, from one bacterial strain to another. ESBL-producing *E. coli* particularly those producing cefotaximase (CTX-M) enzymes have emerged as important pathogens causing healthcare- and community-associated infections worldwide (10,11). In the last decades, studies were increased in different countries about foods as potent sources of *E. coli* that produce ESBLs, such as meat, poultry, milk and other animal products. in Iraq, many reports documented these bacteria, especially in poultry meat and milk with no attention to fish as a source of ESBL-producing *E. coli* (12,13).

So, this study aimed to detect of ESBL-producing *E. coli* from the gut of *Cyprinus carpio*.

Materials and methods

Ethical approve

All samples were collected according to authority given from an Institutional Animal Care and Use Committee, University of Mosul, College of Veterinary Medicine according to authority no. UM.VET.2021.061.

Fish samples

A total of 75 samples of fish (*Cyprinus carpio*) were collected from the local fish market of Mosul city during the period between October 2021 to February 2022. each sample was placed in a sterile plastic bag and transported directly to the microbiology lab, College of Veterinary Medicine, under cooling conditions.

Isolation and identification of ESBLs *E. coli*

The surface of fish samples was washed with sterile saline, then the fishes were opened from the dorsal side using a sterile surgical blade then a longitudinal incision was made along the gut. Following, opened the incision and the loopful of gut contents were streaked on MacConkey agar supplemented with 2 µg/ml cefotaxime (MA+) (14). The inoculated plates were incubated for 24 h at 37°C. This medium is selective for the bacteria that resist to cefotaxime. All suspected colonies of ESBL *E. coli* were subcultured on (MA+) and Eosin methylene blue agar (EMBA) for purification. Biochemical tests including indole, methyl red, Voges- Proskauer, citrate utilization, H_2S production and urease tests, were used for identification of *E. coli* (15).

Extraction of DNA

According to the manufactured company these ESBL *E. coli* isolates were subjected to genomic DNA extraction (Jena Bioscience, Germany). Fresh colonies of *E. coli* that cultivated on Brain Heart Infusion Agar (BHIA) for 24h at 37°C were suspended in an Eppendorf tube for the Lysis of cells, followed by a protein precipitation step. The supernatants were separated in a 1.5 ml Eppendorf microcentrifuge with 300 µl Isopropanol 99%. Then the tubes were centrifuged and discarded the supernatant, then draining tubes. The small pellets of DNA were washed using washing buffer by inverting it several times before being centrifuged. Then the supernatant was discarded, the tubes were dried at room temperature, added 100 µl of Hydration solution for DNA hydration, and incubated at 65 °C for one hour. The extracted DNA was stored under -20°C for the following use (12).

Detection of *E. coli and* their ESBL gene by PCR reaction method

All samples were screened for *E. coli* using specific species primers 232bp, ECO223- f (5′ ATCAACCGAGATTCCCCCAGT ′3) and ECO445-r (5′ TCACTATCGGTCAGTCAGGAG ′3) (12). The confirmation of the betalactam CTX-M gene was done using CTX-M-Uni-f (CGCTTTGCGATGTGCAG) and CTX-M-Uni-r (ACCGCGATATCGTTGGT) (16,17). While the confirmation of betalactam SHV gene was done by SHV-f (ATGCGTTATATTCGCCTGTG) and SHV-r (TGCTTTGTTATTCGGGCCAA). The PCR reaction mixture for all protocols was carried out according to the manufacturer's instructions. The master mix reaction was prepared by adding 12.5 µl of 2X Taq Premix (Ge-Net, Bio-Korea), 1 µl of each forward and reverse primers 10 mmol (IDT, USA), 8 µl of PCR grade water and finally 2.5 µl of the DNA template final concentration 2 ng/µl. PCR cycling conditions were done by using a thermocycler (BioRad, T100, BioRad - USA) that included one cycle at 94°C for ten min for initial denaturation, while 35cycle for 30 sec for DNA denaturation. Also 35 cycles for each annealing, extension and final extension at 1 min, 45 sec and 5 min respectively. The PCR products were separated by 1.2% of agarose gel (Promega, USA), which contained Prime Safe Dye (GeNet, Bio, Korea). The electrophoresis condition was set at 80 V, 300 mA ,50 min by using Wide Mini-Sub Cell GT gel electrophoresis systems and basic power supply (Bio-Rad, USA), A 4 µl of 100 bp DNA ladder, (GeNet Bio, Korea) was used as molecular weight standard. After that, the gel was viewed using Gel doc Ez system to detect the specific bands (18,19).

Results

Through bacterial examination of 75 fish gut samples were obtained 26 isolates that belonged to the ESBL resistant *E. coli* with the percentage 34%. All isolates gave metallic sheen phenomenon on EMBA as shown on figure 1, and

isolates were confirmed by biochemical tests, *E. coli* isolates gave positive results for indole and methyl red while gave negative results for each Voges- Proskauer, citrate utilization, H_2S production and urease tests. ESBL producing *E. coli* isolates were confirmed molecularly by PCR by using ECO223-F and ECO445-R genes were give 100% positive results with 232bp band figure 2. The results showed that all ESBL producing *E. coli* isolates gave positive PCR application products 550 bp for universal CTXM-gene figure 3, while all ESBL *E. coli* isolated in this study did not contain the SHV gene.

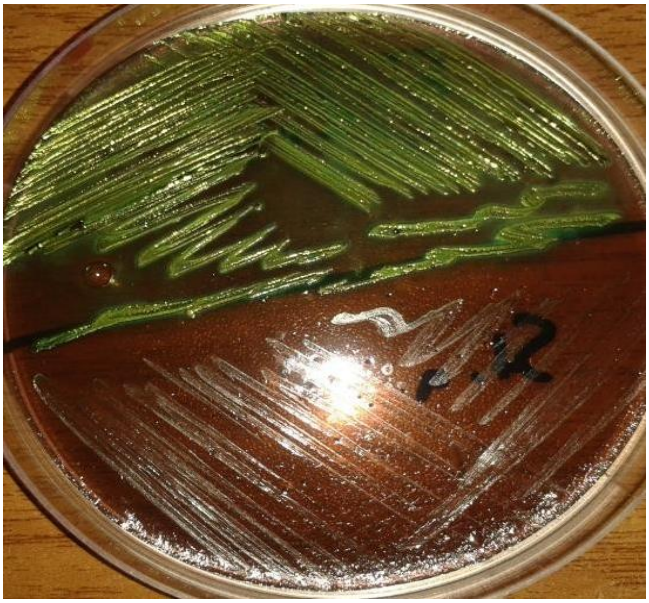

Figure 1: *E. coli* metallic sheen phenomena in eosin methylene blue agar.

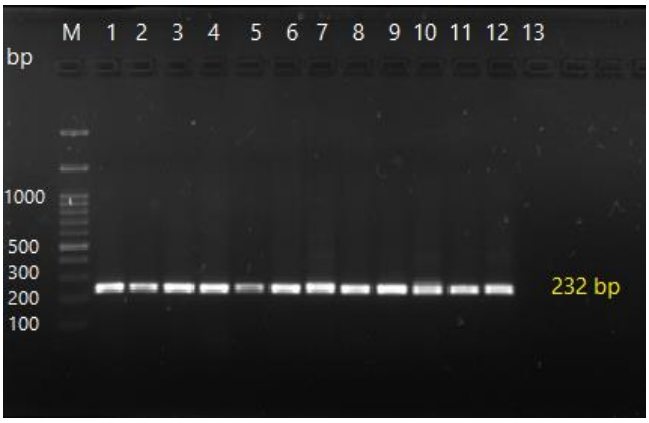

Figure 2: Gel electrophoresis of PCR final products for *E. coli*. Lane M= 100 bp DNA marker. Well 1-12 positive *E. coli* samples giving, 232 bp product size. Well 13 negative controls.

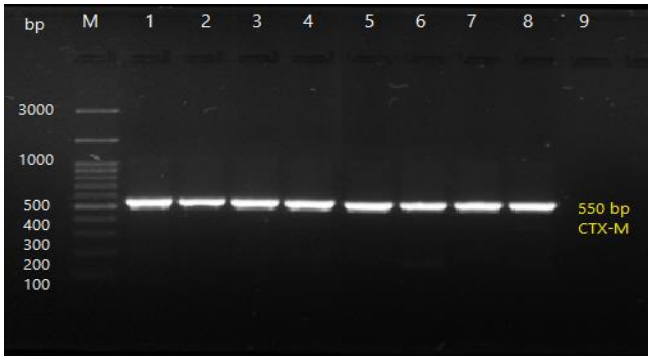

Figure 3: PCR final products of universal CTX-M gene. Lane M: 100 bp DNA marker. Well 1-8 positive fish samples 550 bp product size. well 9 negative controls.

Discussion

Currently, foodborne *E. coli* are becoming a serious threat to public health as new strains are increasingly recorded that possess ESBLs genes. Extended-spectrum beta-lactamase-producing *Escherichia coli* constitutes an emerging global health problem with fish that are considered as potential reservoirs and serve as vehicles of transmission to humans and other fish due to direct contact or through shared environments (4,9).

The results of this study showed that 34% of *E. coli* isolates that resisted to cefotaxime when growing on MacConkey agar supplemented with cefotaxime (MA+), that belonging to ESBLs producing *E. coli*, this result in agreement with Nguyen *et al*. (20) who recorded 29.3%in fish and shrimp, and with Kumar *et al*. (3) who revealed that 38.8% of finfish samples from the fresh fish market that belonging to ESBLs *E. coli*. And disagreement with Brahmi *et al*. (21), Said *et al*. (22) and Ahmad *et al*. (23) who recorded 22, 2, 16, and 9.78% respectively for ESBLs *E. coli* from fish in different countries. The variation in these results may be belonging to the difference in the geographic distribution and/ or weather variation (24).

PCR analysis showed that all isolates under study harbored bla CTX-M genes 100%, While bla SHV gene was not detected. These results were consistence with the many studies that were done in different areas around the world such as studies of Ahmad *et al*. (22), Elhadi and Alsamman (25) that indicated the blaCTX-M gene is a predominate gene of ESBLs Enterobacteriaceae, especially extended spectrum cephalosporin resistant (ESCRs) producing *E. coli* in fish gut contents, more than other β-lactamases as SHV and TEM genes (26,16).

Most fish is affected by resistant *E. coli* in the aquatic environment, these bacteria either inhabit contaminated water or are found in the body of fish that are apparent normally (27). Also, industrial and hospital Sewage is

another environmental contamination source of spreading ESBL *E. coli* to freshwater (28). Therefore, these bacteria represent a highly complex challenge between animals, humans, and environments (28), and due to the horizontal transfer of antibiotic resistance genes between strains of bacteria which facilitated the production of global health problems (29).

Conclusions

ESBLs producing *E. coli* were detected in fish (*Cyprinus carpio*) with a common CTX-M gene, SHV gene was not detected in this study. fish is considered as important source of transmission of ESBLs *E. coli*.

Acknowledgments

The authors would like to thanks the College of Veterinary Medicine, University of Mosul, Mosul, Iraq.

Conflict of interest

The authors declare that he has no conflict of interest.

References

1. Faber TA, Hernot DC, Parsons CM, Swanson KS, Smiley S, Bechtel PJ, Fahey GC. Protein digestibility evaluations of meat and fish substrates using laboratory, avian, and ileal cannulated dog assays. J Amin Sci. 2010;88:1421-1432. DOI: 10.2527/jas.2009-2140
2. Kousara R, Shafia N, Andleeb S, Mazhar AN, Akhtara T, Khalid S. Assessment and incidence of fish associated bacterial pathogens at hatcheries of Azad Kashmir, Pakistan. Braz J Biol. 2020;80(3):1-8. DOI: 10.1590/1519-6984.217435
3. Kumar HS, Parvathi A, Karunasagar I, Karunasagar I. Prevalence and antibiotic resistance of *Escherichia coli* in tropical seafood. World J Microbiol Biotechnol. 2005;21(5):619-623. DOI: 10.1007/s11274-004-3555-8
4. Goltz J. Investigating factors associated with extended-spectrum beta-lactamase-producing enterobacteriaceae colonization and/or infection in humans: A one health approach [master's thesis]. Ontario: The University of Guelph; 2020;1-192 p.
5. Olopade A, Bitrus AA, Momoh-Zekeri AH, Bamaiyi PH. Multi-drug resistant phenotypes of extended-spectrum β-lactamase (ESBL)-producing *E. coli* from layer chickens. Iraqi J Vet Sci. 2022;36(4):945-951.DOI: 10.33899/IJVS.2022.132655.2117
6. Hassen B, Jouini A, Elbour M, Hamrouni S, Maaroufi A. Detection of extended-spectrum β-lactamases (ESBL) producing enterobacteriaceae from fish trapped in the Lagoon area of Bizerte, Tunisia. BioMed Res Int. 2020;7132812:1-9. DOI: 10.1155/2020/7132812
7. Cardozoa MV, Borgesa C, Beraldoa LG, Malutab RP, Pollo A, Borzia MM, Santosc LF, Kariyawasamd S, de Avilaa FA. Shigatoxigenic and atypical enteropathogenic *Escherichia coli* in fish for human consumption. Braz J Microbiol. 2018;4(9):936-941. DOI: 10.1016/j.bjm.2018.02.013
8. Adelowo OO, Caucci S, Banjo OA, Nnanna OC, Awotipe EO, Peters FB. Extended spectrum beta-lactamase (ESBL)-producing bacteria isolated from hospital wastewaters, rivers and aquaculture sources in Nigeria. Environ Sci Pollut Res. 2018;25:2744-2755. DOI: 10.1007/s11356-017-0686-7
9. Baniga Z, Yaovi M, Hounmanou G, Kudirkiene E, Lughano J, Kusiluka M, Robinson H, Dalsgaard A. Genome based analysis of extended spectrum b lactamase producing *Escherichia coli* in the aquatic environment and Nile perch (*Lates niloticus*) of Lake Victoria, Tanzania. Front Microbiol. 2020;11(108):1-11. DOI: 10.3389/fmicb.2020.00108
10. Choudhury A, Nath M, Vandana UK, Kumar D. Screening for extended-spectrum beta-lactamases producing *Escherichia coli* isolates in fish sample and profiling for antibiotics susceptibility. BACT EMP. 2021;4(1):25-29. DOI: 10.36547/be.2021.4.1.25-29
11. Saiful Islam M, Abdus Sobur M, Rahman S, Mohammed Ballah F, Ievy S, Siddique MP, Rahman M, Abdul Kafi M, Rahman M. Detection of blaTEM, blaCTX-M, blaCMY, and blaSHV genes among extended-spectrum beta- lactamase- producing *Escherichia coli* isolated from migratory birds travelling to Bangladesh. Microb Ecol. 2022;83:942-950. DOI: 10.1007/s00248-021-01803
12. Ahmed IM. Detection of CTX-M gene in extended spectrum β-lactamases producing Enterobacteriaceae isolated from bovine milk. Iraqi J Vet Sci. 2021;35(2):397-402. DOI: 10.33899/ijvs.2020.126909.1412
13. Al-Sharook M, Hassan A. Isolation and identification of extended-spectrum beta-lactamase (ESBL)-producing *Escherichia coli* from broiler in Erbil, Iraq. Iraqi J Vet Sci. 2017;31(1):31-8. DOI: 10.33899/ijvs.2017.126717
14. Moremi N, Manda EV, Falgenhauer L, Ghosh H, Imirzalioglu G, Matee M, Chakraborty T, Mshana S. Predominance of CTX-M 15 among ESBL producers from environment and fish gut from the shores of lake Victoriain Mwanza, Tanzania. Front Microbiol. 2016;7:1862. DOI: 10.3389/fmicb.2016.01862
15. Silva V, Nunes J, Gomes N, Capita R, Calleja C, Pereira Jd, Torres C, Igrejas G, Poeta P. Detection of antibiotic resistance in *Escherichia coli* strains: can fish commonly used in raw preparations such as sushi and sashimi constitute a public health problem. J Food Prot. 2019;82(7):1130-1134. DOI: 10.4315/0362-028X.JFP-18-575
16. Ly TH, Hoa TT, Huyen HM. Antibiotic resistance and molecular characteristics of extended-spectrum beta-lactamase-producing *Escherichia coli* isolated from fish pond. Tap Chi Khoa Hoc. 2018;54(8):114-123. DOI: 10.22144/ctu.jen.2018.045
17. Ahmed IM, Al-dabbagh SY, Jwher DM. Molecular characterization of extended spectrum cephalosporin resistant *Escherichia coli* isolated from dogs. Iraqi J Vet Sci. 2021;35(3):473-478. DOI: 10.33899/ijvs.2020.127032.1441
18. Altaee AJ, Al-dabbagh SY. Molecular identification of virulence genes of *Pseudomonas aeruginosa* isolated from fish (*Cyprinus carpio*) in Mosul city. Iraqi J Vet Sci. 2022;36(4): 953-958. DOI: 10.3899/IJVS.2022.132660.2119
19. Al- Dabbagh SY. Molecular characterization of extended spectrum beta-lactamase producing *Klebsiella pneumoniae* isolated from cows in Mosul city, Iraqi J Vet Sci. 2022;36(2):375-380. DOI: 10.33899/ijvs.2021.130341.1803
20. Nguyen DP, Nguyen TA, Le TH, Tran NM, Ngo TP, Dang VC, Kawai T, Kanki M, Kawahara R, Jinnai M, Yonogi S. Dissemination of extended-spectrum β-lactamase-and AmpC β-lactamase-producing *Escherichia coli* within the food distribution system of Ho Chi Minh City, Vietnam. Biomed Res Int. 2016;(1):1-9. DOI: 10.1155/2016/8182096
21. Brahmi S, Touati A, Remy CD, Sotto A, Pantel A, Lavigne JP. High prevalence of extended-spectrum b-lactamase-producing Enterobacteriaceae in wild fish from the Mediterranean Sea in Algeria. Microb Drug Resist. 2018;24(3):290-298. DOI: www.doi.org/10.1089/mdr.2017.0149
22. Ahmad K, Siqaf M, Abid GE, Somayya R, Qasim U. Phenotypic and genotypic analysis of beta-lactamases in *Escherichia coli* isolated from fish. Pakistan J Zool. 2022;54(4):1983-1986. DOI: 10.17582/journal.pjz/2019102319
23. Said LB, Hamdaoui M, Jouini A, Boudabous A, Slama KB, Torres C, Klibi N. First detection of CTX-M-1 in extended spectrum b-lactamase-

التحري عن جراثيم الايشيريكيا القولونية المنتجة للبيتالاكتاميز من الأسماك في مدينة الموصل

ابتهال نوفل محمود و سمية ياسين عبد الله الدباغ

فرع الأحياء المجهرية، كلية الطب البيطري، جامعة الموصل، الموصل، العراق

الخلاصة

تشكل الإيشريكيا القولونية المنتجة لأنزيم البيتالاكتاميز ذات الطيف الواسع مشكلة صحية ناشئة عالميا، إذ تعد الأسماك كمستودعات محتملة لـ هذه الجراثيم وتعمل كوسيلة لنقل جينات المقاومة للبيتالاكتام إلى سلالات أخرى من البكتيريا. تم جمع ٧٥ عينة من اسماك الكارب من أسواق الأسماك المحلية في مدينة الموصل للفترة من (تشرين الأول ٢٠٢١- شباط ٢٠٢٢). تم وضع كل عينة بصورة منفصلة بأكياس بلاستيكية ونقلها مباشرة وتحت ظروف التبريد الى مختبر الأحياء المجهرية، كلية الطب البيطري. تم عزل وتوصيف جراثيم الإيشريكيا القولونية ذات الطيف الواسع باستخدام وسط أكار الماكونكي المضاف له السيفوتاكسيم ٢ مكغم/مل. إذ تم الحصول على ٢٦ عزلة بنسبة ٣٤% من عينات معي الأسماك. تم إجراء فحص البلمرة المتسلسل لأثبات نتائج العزل باستعمال بادئات خاصة لجراثيم الايشريكيا القولونية، الجين الأمامي والعكسي للايشريكيا القولونية-٢٢٣. اجري فحص جينات المقاومة باستخدام بادئات السيفوتاكاميز والسلفاهيدريل. أظهرت جميع العزلا ت امتلاكها لجين السيفوتاكساميز ١٠٠% فيما لم تمتلك أي من العزلات لجين السلفاهيدريل. أوضحت هذه الدراسة أن الأسماك تلعب دورا رئيسا في نقل الإيشريكيا القولونية المقاومة للبيتا لاكتام ذات الطيف الواسع الى الإنسان نتيجة التعامل معها أو تسويقها أو عن طريق استهلاك الأسماك الملوثة أو المصابة.

producing *Escherichia coli* in seafood from Tunisia. J Food Prot. 2017;80:1877-1881. DOI: 10.4315/0362-028X.JFP-16-324

24. Jiang H, Tang D, Liu Y, Zhang X, Zeng Z, Xu L, Hawkey PM. Prevalence and characteristics of b-lactamase and plasmid-mediated quinolone resistance genes in *Escherichia coli* isolated from farmed fish in China. J Antimicrob Chemother. 2012;67:2350-2353. DOI: 10.1093/jac/dks250
25. Elhadi N, Alsamman K. Incidence and antimicrobial susceptibility pattern of extended-spectrum-Î²-lactamase-producing *Escherichia coli* isolated from retail imported mackerel fish. Afr J Biotechnol. 2015;14(23):1954-1960. DOI: 10.5897/AJB2015.14698
26. Hoa TT, Nakayama T, Huyen HM, Harada K, Hinenoya A, Phuong NT, Yamamoto Y. Extended-spectrum beta-lactamase-producing *Escherichia coli* harbouring *Sul* and *Mcr*-1 genes isolates from fish gut contents in the Mekong Delta, Vietnam. Appl Microbiol. 2019;71(1):78-85. DOI: 10.1111/lam.13222
27. Rocha RD, Leite LO, Sousa OV, Vieira RH. Antimicrobial susceptibility of *Escherichia coli* isolated from fresh-marketed Nile Tilapia (*Oreochromis niloticus*). J Pathog. 2014;2014:1-5. DOI: 10.1155/2014/756539
28. Jikoo H, Jowoo G. Characterization of antimicrobial resistance of *Escherichia coli* recovered from foods of animal and fish origin in Korea. J Food Prot. 2012;75(5):966-972. DOI: 10.4315/0362-028X.JFP
29. Altayb HN, Salih EK, Moglad EH. Molecular detection of beta-lactamase blaCTX-M group 1 in *Escherichia coli* isolated from drinking water in Khartoum State. J Water Health. 2020;18(6):1091-1097. DOI: 10.2166/wh.2020.160