



Document heading

Molecular Study of Enteropathogenic *Escherichia Coli* Isolation from Clinical Samples

Lames H Almanseekanaa

Basic Medical Science, College of Dentistry, University of Karbala, Kerbala, Iraq

Corresponding author: - lames.h@uokerbala.edu.iq

ORCID <https://orcid.org/0000-0001-6594-1715>

Article Info.

Article history:

Received 24 February 2022

Revised 25 April 2022

Published 15 June 2022

Keywords:

molecular, characterises, Enteropathogenic, *Escherichia coli* isolation.

How to cite:

Lames H Almanseekanaa.

Molecular study of enteropathogenic *Escherichia coli* isolation from clinical samples.

Aca. Intl. J. Med. Sci., 2022;1(1):06-14:

<https://doi.org/10.59675/M202202>

Copyright:

© 2022 A.I. Publishers.

All rights reserved.

Abstract

Escherichia coli regarded as the most important causes of intestinal illnesses in the world and also a significant public health challenge. Intestinal sicknesses which are caused by *E. coli* infection are often under diagnosed while doing usual microbiological analysis, particularly in resource constrained settings. However, the use of molecular tests could be helpful to identify the distribution of intestinal illnesses of *E. coli* infection and also its clinical importance.

The distribution analysis of virulence factors which exist among various *E. coli* pathotypes which include Shiga toxin-producing *E. coli* (STEC) will offer substantial insights with regard to the mechanisms through which various *E. coli* strains lead to diseases and result in the growth of different *E. coli* types. This present investigation therefore is an attempt to evaluate the incidence of intestinal illnesses of *E. coli* in clinical samples taken from patients.

The samples from 150 patients and 15 controls were collected and then screened for *E. coli*. Compatible colonies taken from 150 individuals (150 patients and 15 controls) were characterized through performing biochemical test, a set of Polymerase Chain Reaction (PCR) for recognition of six virulence factors. The clinical samples from patients were within two months -two year attending. Pyelonephritis were associated by pili (*pap gene*, hemolysin (*hly gene*), aerobactin (*aer gene*), translocon pore-forming subunit *espB* and Shiga toxins (Stx1 and Stx2) respectively. They were related with six intestinal illnesses pathotypes. The incidence of *hlyA* and *papC* (both 40%) was highest, and was followed by *espB*, *aer*, *stx1*, *stx2* (30%, 25%, 20% and 18%) respectively. Although, how and whether these mixtures of genes affect their pathogenicity is an issue that have to be investigated and elucidated. The findings obtained in this study highlight the significance of epidemiological and microbiological surveillance of STECs. It also determines if the development of control measures will diminish the risks which are associated with intestinal illnesses from *E. coli*.

Introduction

Escherichia coli is considered as an ever present microbial kind commensal of individuals and also animals of warm blooded types. However, some strains are recognized to have developed the ability that leads to both intestinal and extra intestinal sicknesses [1]. *E.coli* bacteria are known as Gram-negative facultative anaerobic bacilli. They are not glomerular and have peritrichous flagella for mobility. These bacteria are considered potential causative agents of various diseases such as cholecystitis, pneumonia, pyelonephritis, cystitis, urinary tract infections, neonatal meningitis, septicemia, infections of the central nervous system and respiratory tract [2]. On the other hand, it is worth noting that many *E. coli* strains are commensal flora found in the gut, and the barrier between *E. coli* symbiosis and virulence mainly is dependent on the equilibrium which exists between host immune status and bacterial acquisition capacity viral factor. Infectious diarrheal and gastro has been a main source of disease and death in younger children and is mostly found in developing countries [3]. Enteropathogenic *E. coli* from the Study and Malnutrition and Malnutrition and Malnutrition and Enteric Diseases Encyclopedia identifies the main reasons of moderate to very severe diarrhea in younger children [4]. The infection causes severe watery diarrhea with fever, vomiting and dehydration [5]. A full profile of virulence factors is the code of all genes. The pathogen *E. coli* with an interpersonal disease is a serious cause of diarrheal, often with a high degree of morbidity, which is reported in developing countries [6]. Among the diarrheagenic pathotypes of *E. coli*, and Enteropathogenic *E. coli* are more frequent and frequently occurring in waterborne occurrences worldwide [7].

In particular, in certain geographical markets, for example in some certain developing countries, infections caused by *E. coli* diarrheal have been transferred through human contacts, like enteroaggregative *E. coli* (EAaggEC), endemic [8]. Furthermore, the treatment with human manures is frequently unusable or lacking. Therefore, it principals to a wide spreading of these pathogens in the surrounding environment and then they might have contact with Shiga toxin-producing *E. coli* (STEC). We examined the presence of pathogenic *E. coli*, mainly enteric ones, in specimens isolated from infant clinical specimens [9]. Molecular characterization has manifested high degree of diversity among virulence factors in different strains of atypical EPEC [10]. The pathogenicity of degree of extraintestinal *E. coli* isolates is dependent on the existence of fimbriae adhesions (fimA, sfa/foc, and yfcV) which are documented as very important factors that are responsible for the related adherence [11]. Adherence is considered as an important requirement for initiating and colonization to convinced host cells. Some other actual issues are known as haemolysins (hlyA and hlyF) [12].

The purposes of this study were to detect *E. coli* virulence factors in clinical samples collected from hospital from Karbala, Iraq, and to introduce PCR assays as an approach for detection.

Materials and Methods

Sample collection

This present study aims at evaluating the number of *E. coli* pathotypes which circulate in younger children with diarrheal in Karbala. Accordingly, stool samples were taken from children (2 month-2 years) of age with diarrheal. A total of (n=150) stool samples and 15 samples control, were collected and processed during. from May 2021 until November 2021. All collected samples were then directly transferred to the laboratory and were analysed within 24 hours.

Sample processing

Escherichia coli isolates were obtained by culturing stool samples were inoculated on eosin methylene blue agar and incubated at 37°C for 24h. identified *E. coli* by biochemical tests of indole production, sugar ferment, Voges-Proskauer reactions, and by the hydrogen sulphide Public Health England [13].

DNA Preparation

The collected cultures of bacterial *E. coli* were Incubated at 37 °C on nutrient broth for 18 hours. An estimated concentration of each *E. coli* cultures after the incubation period was approximately 10⁶ CFU ml⁻¹ of vegetative forms. A bacteriological loop was used to carry part of the one colony into 500 µL distilled water, which was then 30 sec vortexed, suspending culture then, boiled for 10 min and finally centrifuged for 7 min at 14,000 rpm, put on ice for 10 min and centrifuged for 10 min at 14,000 and the supernatant was utilized for PCR [14].

Oligonucleotide primers and PCR conditions

The extracted DNA was, the PCR primers were selected for each enterotoxins which partially amplified the (*stx1*, *stx2*, *espB*, *pap*, *sfa*, *hlyA* and *aer* genes each as indicated [15], the positive control (*E.coli* ATCC 25992) showed in (Table 1,2 and 3).

Table 1: Reaction mixture of PCR for detection E. coli toxin genes

Component	Volume (µL)
GoTaq Green Master (25)	25.0
Upstream primer (10 µM)	2.5
Downstream primer (10 µM)	2.5
DNA Template	2.5
Nuclease-free water	17.5
Total	50.0

Table 2: primers used for detection of E. coli toxin genes

Primer Identified Genes	primer sequences (5'-3')	Product Gene size bp
stx1	F GAAGAGTCCGTGGGATTACG R AGCGATGCAGCTATTAATAA	302 bp
Stx2	F GGGTACTGTGTGCCTGTTACTGG R GCTCTGGATGCATCTCTGGT	510 bp
espB	F GGC GTT TTT GAG AGC CA R GAT GCC TCC TCT GCG A	260 bp
papC	F GACGGCTGTACTGCAGGGTGTGGCG R ATATCCTTTCTGCAGGGATGCAATA	328 bp
aer	F TACCGGATTGTTCATATGCAGACCGT R AATATCTTCCTCCAGTCCGGAGAAG	602 bp
hlyA	F AACAAGGATAAGCACTGTTCTGGCT R ACCATATAAGCGGTCATTCCCGTCA	1177 bp

Table 3: Primers used for PCR amplification of detection of *E. coli* toxin genes

Target gene	Amplification (35 cycles)				
	Primary denaturation	Secondary denaturation	Annealing	Extension	Final Extension
<i>stx1</i>	95 °C	95°C	60°C	72°C	72°C
<i>Stx2</i>	95°C	94°C	56°C	72°C	72°C
<i>espB</i>	94°C	95°C	60°C	72°C	72°C
<i>papC</i>	95°C	95°C	54°C	72°C	72°C
<i>aer</i>	94°C	95°C	55°C	72°C	72°C
<i>hlyA</i>	95°C	94°C	56°C	72°C	72°C

Agarose gel Electrophoresis

Extracted genomic DNA Agarose gels (1% w/v) were managed in 1xTAE buffer and 1 µL of each DNA extract was electrophoresed at 95 V minutes following PCR amplification.in 1xTAE buffer and post-stained in 1x TAE buffer while GeneRuler™ 1 kb DNA ladder containing 0.1% The gel was pre-stained with Maestrosafe™ Nucleic Acid were utilized as DNA size marker [16]. At last, all related gels were analyzed and captured by UV trans-illuminator Gel Documentation System (Syngene, UK).

Results and Discussion

All 65 *E. coli* O157:H7 isolates were detected to be appeared as gram negative rod under light microscope, mostly motile indole and methyl red tests and negative reactions on voges – proskaure, oxidase and citrate tests. provided positive reactions on catalase, Triple Sugar Iron agar medium provided yellow colour with capability for gas production. The results obtained in this study were in contrast as reported by [17], As they reported *E. coli* Ten bacterial isolates were obtained from n=25 urine samples collected from medical city hospital in Baghdad. These isolates of *E. coli* were identified by cultural and biochemical tests.

The obtained results demonstrated to determine the prevalence of selected virulence genes among *E.coli*, which showed to positive towards *stx1*, *stx2*, *espB*, *PapC*, *aer*, *hlyA* (6.89%,5.51%,25.51%,31.03%,12.41%&27.58%) would produce amplicons of 302bp,510bp,260 bp,328 bp,602 bp &1177 bp respectively in size (Figure 1-6). The results in our study were in line with [18] Among the adhesions, the Haemolysin A and P fimbriae were the most predominant. The result has been in contrast with A total of 63 clinical samples were collected and screened for the presence of *E. coli* O157:H7.

Another study by [19] recognised 239 Shiga toxin-producing *E. coli* strains isolated was observed in 199 separate (83.26%). Haemolytic uremic syndrome patients were ehxA-positive53 (26.63%) and 99 (49.75%) faces (diarrheal circulation). The results obtained in this study were in contrast as reported by [20] *stx1* profile was the furthestmost public and was obtainable in 20.4% (202/990) of the isolates, followed by *stx2* (4.54%; 45/990) and *stx1* and *stx2* profile (2.92%; 29/990). The findings were compatible with what mentioned showing that such characteristics usually are coming in accordance with those which belong to *E. coli* [21].

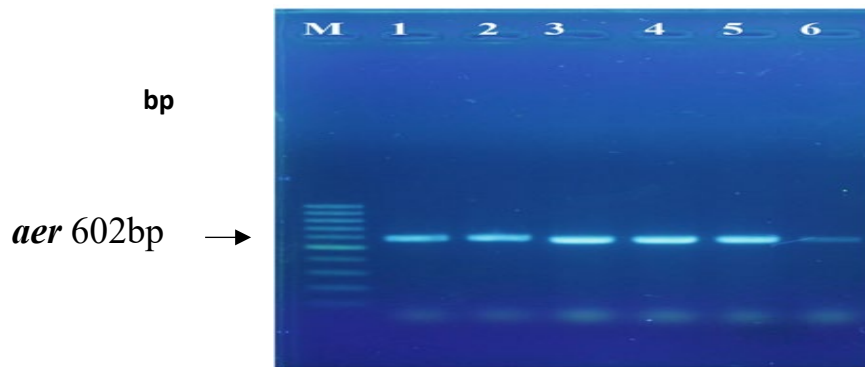


Figure 1: Agarose gel electrophoresis of PCR product of *aer* gene (602 bp) amplified from some of *E. coli* isolates, electrophoresed on 1% (w/v) agarose gel. Lane M: 100 bp DNA ladder; lanes: 2-6 *E. coli* isolates. Lane 1: (*E. coli* ATCC 25992) positive control.

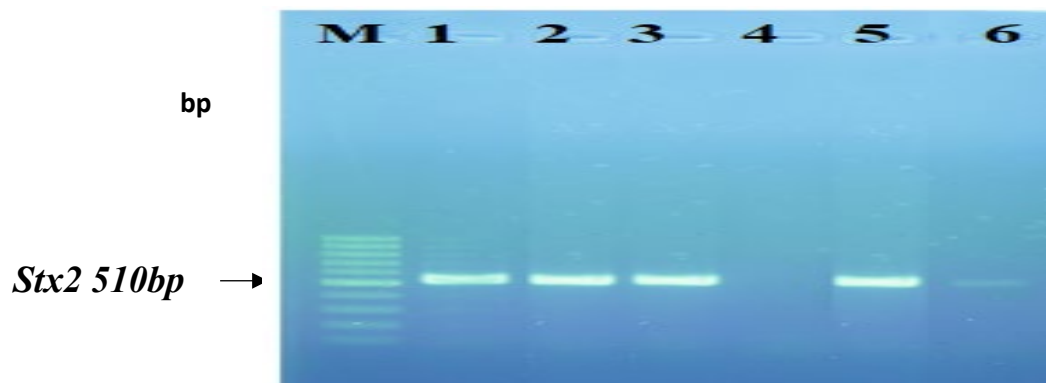


Figure 2: Agarose gel electrophoresis of PCR product of *Stx2* gene (510 bp) amplified from some of *E. coli* isolates, electrophoresed on 1% (w/v) agarose gel. Lane M: 100 bp DNA ladder; lanes: 2-6 *E. coli* isolates. Lane 1: (*E. coli* ATCC 25992) positive control.

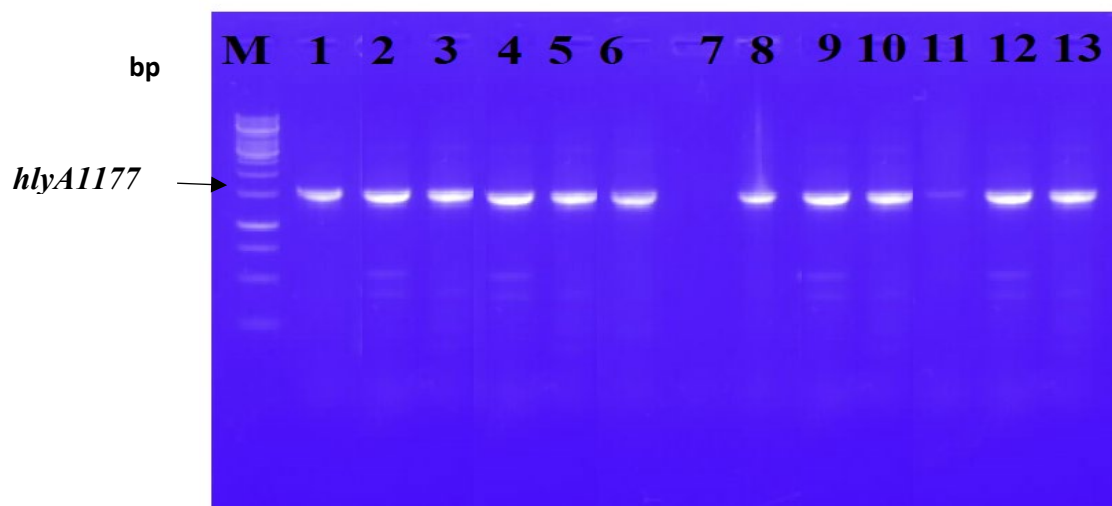


Figure 3: Agarose gel electrophoresis of PCR product of *hlyA* gene (1177 bp) amplified from some of *E. coli* isolates, electrophoresed on 1% (w/v) agarose gel. Lane M: 10000 bp DNA ladder; lanes: 2-13 *E. coli* isolates. Lane 1: (*E. coli* ATCC 25992) positive control.

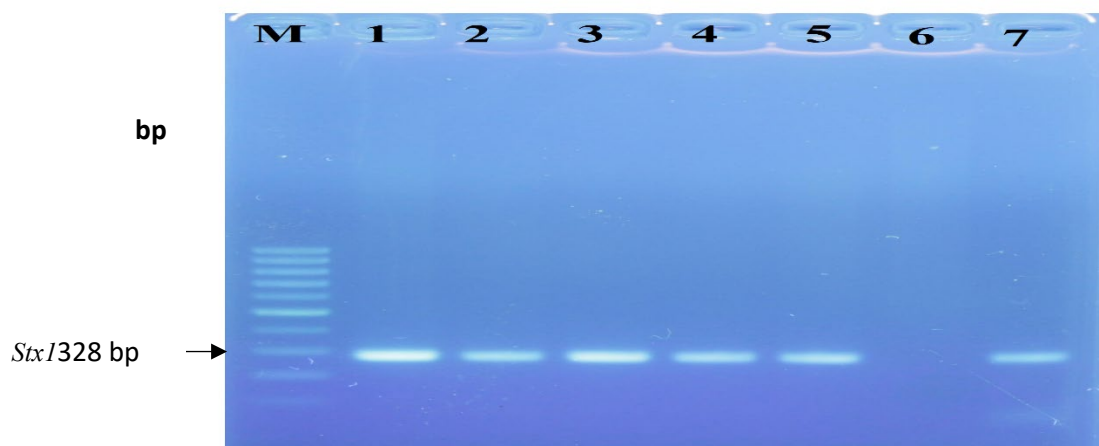


Figure 4: Agarose gel electrophoresis of PCR product of *Stx1* gene (328 bp) amplified from some of *E. coli* isolates, electrophoresed on 1% (w/v) agarose gel. Lane M: 100 bp DNA ladder; lanes: 2-7 *E. coli* isolates. Lane 1: (*E. coli* ATCC 25992) positive control.

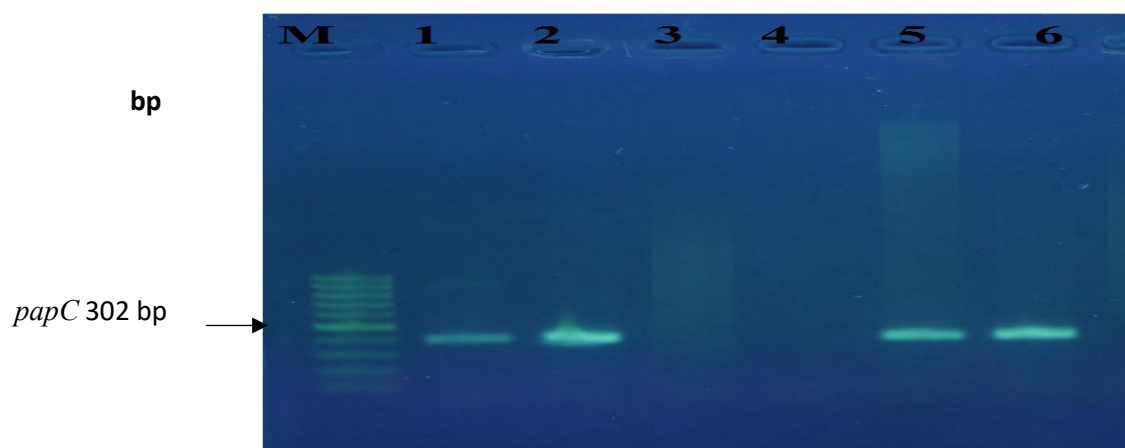


Figure 5: Agarose gel electrophoresis of PCR product of *papC* gene (302 bp) amplified from some of *E. coli* isolates, electrophoresed on 1% (w/v) agarose gel. Lane M: 100 bp DNA ladder; lanes: 2-6 *E. coli* isolates. Lane 1: (*E. coli* ATCC 25992) positive control.

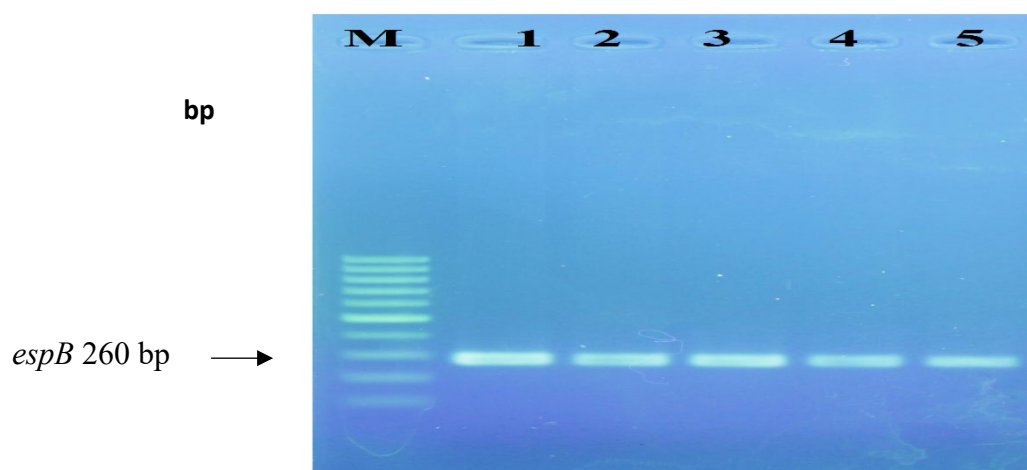


Figure 6: Agarose gel electrophoresis of PCR product of *espB* gene (260 bp) amplified from some of *E. coli* isolates, electrophoresed on 1% (w/v) agarose gel. Lane M: 100 bp DNA ladder; lanes: 2-5 *E. coli* isolates. Lane 1: (*E. coli* ATCC 25992) positive control.

Conclusion

There is a high incidence of *E. coli* in human and their environment. The statistics explained in the current study specifies a very great expression of the diarrheal toxin genes of *E. coli* isolated in medical samples demonstrating that the pathogenic strains of the microorganism are highly adapted for toxins.

Acknowledgments

The authors wish to thank University of Warith Al-Anbiyaa college medicine for their support. We are also grateful to the Microbiology Laboratory of the Hospital in Karbala city.

References

1. Scheutz F, Nielsen EM, Frimodt-Møller J, Boisen N, Morabito S, Tozzoli R, et al. Characteristics of the enteroaggregative Shiga toxin/verotoxin-producing *Escherichia coli* O104:H4 strain causing the outbreak of haemolytic uraemic syndrome in Germany, May to June 2011. *Eurosurveillance*. 2011;16(24):19889.
2. Makvana S, Krilov LR. *Escherichia coli* infections. *Pediatrics in Review*. 2015;36(4):167-71.
3. Heng J, Zhao Y, Liu M, Liu Y, Fan J, Wang X, et al. Substrate-bound structure of the *E. coli* multidrug resistance transporter MdfA. *Cell Research*. 2015;25(9):1060-73.
4. Troeger C, Forouzanfar M, Rao PC, Khalil I, Brown A, Reiner Jr RC, et al. Estimates of global, regional, and national morbidity, mortality, and aetiologies of diarrhoeal diseases: a systematic analysis for the Global Burden of Disease Study 2015. *Lancet Infect Dis*. 2017;17(9):909-48.
5. Platts-Mills JA, Liu J, Rogawski ET, Kabir F, Lertsethtakarn P, Sigua M, et al. Use of quantitative molecular diagnostic methods to assess the aetiology, burden, and clinical characteristics of diarrhoea in children in low-resource settings: a reanalysis of the MAL-ED cohort study. *Lancet Glob Health*. 2018;6(12):e1309-18.
6. Umaru GA, Adamu Z, Ishaya D, Abubakar YU, Hussaini A, Umar M, et al. Prevalence and antimicrobial resistance pattern of *Escherichia coli* in drinking waters in Jalingo Metropolis, Taraba State, North-Eastern Nigeria. *Microbiol Res Int*. 2015;3(1):8-13.
7. van den Beld MJ, de Boer RF, Reubsat FA, Rossen JW, Zhou K, Kuiling S, et al. Evaluation of a culture-dependent algorithm and a molecular algorithm for identification of *Shigella* spp., *Escherichia coli*, and enteroinvasive *E. coli*. *J Clin Microbiol*. 2018;56(10):e00510-18.
8. Deku JG, Duedu KO, Kinanyok S, Kpene GE, Feglo PK. Phylogeneticity and virulence profiles of clinical *Escherichia coli* isolates in the Ho Teaching Hospital of Ghana. *Biomed Res Int*. 2022.
9. Hebbelstrup Jensen B, Olsen KE, Struve C, Krogfelt KA, Petersen AM. Epidemiology and clinical manifestations of enteroaggregative *Escherichia coli*. *Clin Microbiol Rev*. 2014;27(3):614-30.
10. Bryan A, Youngster I, McAdam AJ. Shiga toxin producing *Escherichia coli*. *Clin Lab Med*. 2015;35(2):247-72.
11. Bugarel M, Martin A, Fach P, Beutin L. Virulence gene profiling of enterohemorrhagic (EHEC) and enteropathogenic (EPEC) *Escherichia coli* strains: a basis for molecular risk assessment of typical and atypical EPEC strains. *BMC Microbiol*. 2011;11(1):1-10.
12. Awad WS, El-Sayed AA, Mohammed FF, Bakry NM, Abdou NEM, Kamel MS. Molecular characterization of pathogenic *Escherichia coli* isolated from diarrheic and in-contact cattle and buffalo calves. *Trop Anim Health Prod*. 2020;52(6):3173-85.
13. Zhang R, Wilson VL, Hou A, Meng G. Source of lead pollution, its influence on public health and the countermeasures. *Int J Health Anim Sci Food Saf*. 2015;2(1).
14. Espinosa I, Báez M, Percedo MI, Martínez S. Evaluation of simplified DNA extraction methods for *Streptococcus suis* typing. *Rev Salud Anim*. 2013;35(1):59-63.

15. Sarowska J, Futoma-Koloch B, Jama-Kmiecik A, Frej-Madrzak M, Ksiazczyk M, Bugla-Ploskonska G, et al. Virulence factors, prevalence and potential transmission of extraintestinal pathogenic *Escherichia coli* isolated from different sources: recent reports. *Gut Pathog.* 2019;11(1):1-16.
16. Kadhim NJ, AL-Karawi NJ, Almanseekanaa LH, Ogaili RH. Molecular characterization and antibiotic pattern of the *Klebsiella pneumoniae*. *Vet Pract.* 2021;22(2).
17. Al-Baer AS, Hussein AA. Optimum conditions for cytosine deaminase production by local isolate of *Escherichia coli* E9. *World J Pharm Res.* 2017;6(16):88-96.
18. Amani J, Ahmadpour A, Fooladi AA, Nazarian S. Detection of *E. coli* O157:H7 and *Shigella dysenteriae* toxins in clinical samples by PCR-ELISA. *Braz J Infect Dis.* 2015;19:278-84.
19. Liu H, Hua X, Zhang YN, Zhang T, Qu J, Nolte TM, et al. Electrocatalytic inactivation of antibiotic-resistant bacteria and control of antibiotic resistance dissemination risk. *Environ Pollut.* 2021;291:118189.
20. Gomes JF, Lopes A, Gonçalves D, Luxo C, Gmurek M, Costa R, et al. Biofiltration using *C. fluminea* for *E. coli* removal from water: comparison with ozonation and photocatalytic oxidation. *Chemosphere.* 2018;208:674-81.
21. Rotariu O, Ogden ID, MacRitchie L, Forbes KJ, Williams AP, Cross P, et al. Combining risk assessment and epidemiological risk factors to elucidate the sources of human *E. coli* O157 infection. *Epidemiol Infect.* 2012;140(8):1414-29.