Eurasia Specialized Veterinary Publication

International Journal of Veterinary Research and Allied Sciences

ISSN:3062-357X

2023, Volume 3, Issue 2, Page No: 7-13

Available online at: www.esvpub.com/

Understanding the Biological Characteristics and Diagnostic Approaches for *Escherichia coli* and Colibacillosis

Amyleigh Watts[1], Paul Wigley[2*]

[1]Institute of Infection, Veterinary and Ecological Sciences, University of Liverpool, Leahurst Campus, Neston CH64 7TE, UK.
[2]Bristol Veterinary School, University of Bristol, Bristol BS40 5DU, UK.

***E-mail** ✉ paul.wigley@bristol.ac.uk

ABSTRACT

Escherichia coli is a gram-negative, rod-shaped, polymorphic bacterium, with non-pathogenic strains commonly found in the intestinal flora. It demonstrates stability in the environment, being able to survive in water, soil, and feces for long periods, sometimes many months. *E. coli* produces various enzymes that can break down polyhydric alcohols and carbohydrates such as glucose, galactose, and maltose, leading to the formation of pyruvates, which are then converted into acids such as acetic and formic acid. Despite extensive research on its biochemical properties, no direct relationship has been found between its enzymatic activity and pathogenic potential. Colibacillosis is an acute intestinal infection caused by specific serovars of *E. coli* and is usually transmitted by the fecal-oral route. This article reviews the morphological, staining, cultural, and biochemical characteristics of *E. coli* and its antigenic properties. Additionally, it covers the symptoms and diagnostic methods for colibacillosis caused by different strains of *E. coli*.

Keywords: Colibacillosis, *Escherichia coli*, Enteropathogenic, Enteroinvasive, Enterohemorrhagic, Enterotoxigenic

Received: 10 May 2023
Revised: 29 August 2023
Accepted: 06 September 2023

How to Cite This Article: Watts A, Wigley P. Understanding the Biological Characteristics and Diagnostic Approaches for *Escherichia coli* and Colibacillosis. Int J Vet Res Allied Sci. 2023;3(2):7-13.
https://doi.org/10.51847/HlXsZoecLI

Introduction

Colibacillosis is an acute infection primarily affecting the intestines, caused by certain serovars of *Escherichia coli* [1]. The infection typically manifests as enteritis or enterocolitis, but in some cases, it can spread beyond the intestines, leading to systemic symptoms [2]. *E. coli* is transmitted via the fecal-oral route, often through the consumption of contaminated dairy or meat products. In some cases, household contact can also facilitate the transmission of the infection. Diagnosis is confirmed through the detection of *E. coli* in feces and vomit or in cases of systemic infection, in blood [3].

Colibacillosis encompasses a variety of infections caused by *E. coli*, leading to damage in different body systems, including the gastrointestinal, urinary, and respiratory tracts, as well as the meninges and bloodstream. These infections are most common in small children. Gastrointestinal infections, particularly those causing diarrhea, are the most frequent, and *E. coli* is the leading cause of diarrhea in both adults and infants [4]. Some *E. coli* strains, along with their associated toxins, can cause severe and life-threatening damage to internal organs [5].

This article explores the morphological, staining, cultural, and biochemical characteristics of *E. coli* and its antigenic properties. Additionally, it covers the symptoms and diagnostic methods for colibacillosis caused by various *E. coli* strains.

Results and Discussion

E. coli is a polymorphic, gram-negative, rod-shaped bacterium, with non-pathogenic strains present in the normal intestinal flora [6]. Colibacillosis is typically associated with diarrheal serovars **(Table 1)**.

Table 1. Categories of diarrheal serovars

Pathogen groups	Characteristics of colibacillosis
Enteropathogenic	These strains primarily affect young children, especially during their first year. Transmission typically occurs through contact and household spread.
Enterotoxigenic	Associated with cholera-like infections, these strains are common in hot climates with poor hygiene practices. The infection spreads through contaminated food and water.
Enteroinvasive	These strains cause enterocolitis similar to dysentery. Infection is transmitted via contaminated food and water, with a seasonal pattern observed in the summer and fall. They are mostly found in developing nations.
Enterohemorrhagic	Epidemiological data for this group is limited. The spread of colibacillosis is largely influenced by both general and individual hygiene practices.
Enteroadhesive	These bacteria do not produce cytotoxins, do not penetrate epithelial cells, and lack plasmid adhesion factors. They are characterized by their ability to rapidly adhere to cell surfaces.

E. coli are resilient organisms, capable of surviving in any environment, including water, soil, and feces, for extended periods. In food products, particularly milk, they thrive and form large colonies, with a remarkable ability to endure drying. However, boiling and the use of disinfectants effectively eliminate *E. coli* [7-9].

The main sources of *E. coli* infection are individuals who are either symptomatic or healthy carriers. Those infected with enteropathogenic and enteroinvasive strains pose a greater risk of spreading the pathogen. Other *E. coli* groups are comparatively less hazardous. Individuals infected with enterotoxigenic and enterohemorrhagic strains are only contagious during the early stages of illness, while those with enteroinvasive or enteropathogenic infections can spread the bacteria for 1-2 weeks, sometimes even longer, particularly in children [8-12].

E. coli is transmitted via the fecal-oral route, with enterotoxigenic and enteroinvasive strains typically spread through contaminated food, and enteropathogenic strains often transmitted through household contact. Waterborne transmission is also possible. In environments where hygiene practices are neglected, such as in children's groups, the bacteria can spread through toys, contaminated hands, and objects. Infection with enterohemorrhagic strains is commonly linked to consuming undercooked meat or raw, unpasteurized milk [10-12].

Clinically, colibacillosis is classified into several forms: gastroenteric, enterocolitic, gastroenterocolitic, and generalized. The generalized form can manifest as coli-sepsis or affect various organs and systems, such as the meninges (meningitis) and kidneys (pyelonephritis). The severity of the disease can range from mild to severe [9-12].

This article provides an in-depth examination of the morphological, staining, cultural, and biochemical characteristics of *E. coli*, as well as its antigenic structure. Additionally, the symptoms and diagnostic approaches for colibacillosis caused by various *E. coli* strains are discussed [12-14].

Morphological, cultural, and biochemical characteristics of E. coli

E. coli typically appears as a short, rod-shaped bacterium with rounded ends, measuring between 2-3 µm in length and 0.6-1.0 µm in thickness **(Figure 1)**. These bacteria are generally found as single cells, though occasionally they may appear in pairs in some environments, they can assume a coccus-like shape [15]. When stained using the Gram method, *E. coli* displays a negative result and takes up conventional aniline dyes, often showing a bipolar appearance, particularly in exudates and tissues [16]. Under certain environmental conditions, *E. coli* may also transition into L-forms, gaining the ability to pass in bacterial filters.

E. coli is adaptable to a variety of nutrient media and can thrive at temperatures ranging from 15 to 55 °C, with an optimal growth temperature around 37-38 °C. It is classified as either an aerobe or facultative anaerobe, and it grows well in a pH range of 7.0-7.4. Common media such as MPA (meat peptone agar), MPB (meat peptone broth), Endo, and Levin’s media support its growth [17]. On MPA, after 24 hours, *E. coli* produces smooth,

translucent colonies with a grayish-blue hue, which may merge easily. These colonies may either have smooth, shiny edges (S-shape) or appear flat and dry with slightly wavy edges and a rough surface (R-shape). In broth culture (BCH), *E. coli* causes a strong growth with significant turbidity and the formation of a grayish precipitate that can be disrupted by shaking, and sometimes a film or a parietal ring forms at the surface. When cultured in gelatin, the medium remains solid with no liquefaction. Upon inoculation, a grayish-white growth appears. Additionally, *E. coli* causes milk to coagulate, and litmus milk quickly turns pink due to acid formation, followed by coagulation.

On Levin's diagnostic medium, *E. coli* forms dark purple or black colonies. On Endo's differential medium, lactose-fermenting strains present as raspberry-red colonies, which may or may not have a metallic sheen. On McConkey's medium, the colonies are typically pink or red, although some *E. coli* strains may fail to ferment lactose and thus appear colorless. Colonies from other serogroups tend to be crimson red [18]. Strains that produce hemolysin are characterized by a zone of hemolysis on blood agar. The bacterial culture often emits a fecal odor [19].

E. coli is capable of producing various enzymes that break down a wide range of carbohydrates and polyhydric alcohols, including glucose, galactose, levulose, lactose, maltose, lures, rhamnose, sucrose, dulcite, raffinose, salicin, sorbitol, and glycerin. The breakdown products include pyruvates, which are then converted into acids such as milk, formic acid, and acetic [20]. Interestingly, both pathogenic and non-pathogenic strains of *E. coli* exhibit similar morphological, cultural, and enzymatic characteristics, making it challenging to differentiate pathogenic strains responsible for infectious diarrhea [21].

E. coli antigenic structure

The classification of *E. coli*, in a serological way as developed by F. Kaufman, is based on the analysis of three primary antigens: O, K, and H. In the early stages of research, 146 variants of O-antigen and 88 variants of K-antigen were identified. By the late 1980s, the number of recognized O-antigen serotypes had grown to 171, with over 100 variants of K-antigen and 60 varieties of H-antigen identified in *E. coli*. Further studies revealed 173 distinct O-serogroups, 56 K-antigen types, and 80 H-antigen types [22]. However, it is important to note that not all *E. coli* variants are capable of causing intestinal infections in both humans and animals.

The cell structure of *E. coli* contains three distinct antigen types: O (somatic), K (capsular), and H (flagellar) antigens **(Figure 1)**. The O-antigen is stable at high temperatures, surviving heat treatment at 100 °C for up to two and a half hours. It is a complex composed of lipopolysaccharides and proteins, and it is highly variable, which is why *E. coli* is categorized into more than 160 different O-groups based on this antigen [22].

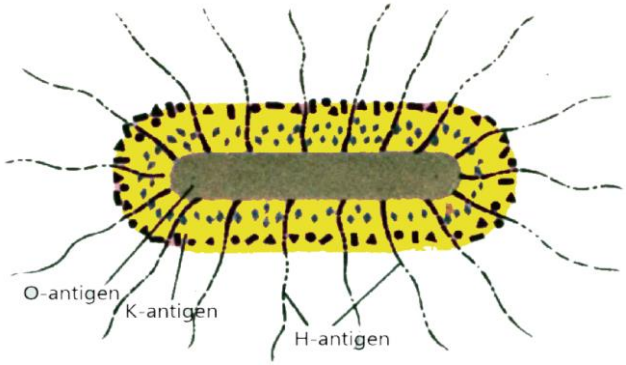

Figure 1. The structure of *E. coli*.

K-antigens, which form a protective surface layer, consist mainly of acidic polysaccharides, and occasionally proteins. These antigens are classified into three categories based on their properties. The L-antigen is heat-sensitive, losing its activity when exposed to 100 °C for one hour. Similarly, the B-antigen loses its agglutination properties under the same conditions. In contrast, the A-antigen is thermostable and remains unaffected at 100 °C but can be inactivated at 120 °C for 2.5 hours. This antigen is found in the mucous membranes of capsule-forming *E. coli* strains, particularly in O-groups such as 08, 09, 020, and 0101 [23].

The H-antigen, located in the flagella of *E. coli*, is composed of a thermolabile protein and can be inactivated at 60 °C for one hour. Due to its location in the fimbriae, it is increasingly referred to as the fimbrial antigen. This

antigen plays a crucial role in the bacteria's ability to adhere to the intestinal epithelial cells, contributing to the pathogenesis of *E. coli*.

The K-antigens envelop the O-antigen and prevent *E. coli* cells from agglutinating in homologous O-sera. They also enhance the bacteria's adhesive properties, allowing it to attach to the intestinal cells. The mixture of O-, K-, and H-antigens characterizes the specific serovar of *E. coli* [24].

In terms of environmental resistance, *E. coli* can survive under harsh conditions. It can persist in soil for six to eleven months, in water for up to 300 days, and in manure for as long as eleven months. When stored in semi-liquid agar under a layer of vaseline oil, *E. coli* retains its pathogenic properties for over three years. However, *E. coli* is sensitive to heat; exposure to 60 °C for just 10 minutes is sufficient to kill the bacteria, and it is immediately killed at 100 °C. Several disinfectants, such as a 2.5% formaldehyde solution, 2% chlorine and sodium hydroxide solutions, and a 3% iodine monochloride solution, effectively eliminate *E. coli* [25].

Enteropathogenic colibacillosis primarily affects young children, with the incubation period typically lasting several days. The condition presents with symptoms such as vomiting, diarrhea, significant intoxication, and dehydration. In severe cases, a septic form may develop. Adults can also contract enteropathogenic colibacillosis, though it often mirrors the clinical presentation of salmonellosis [26].

Enteroinvasive colibacillosis follows a course that is the same as dysentery or shigellosis. The incubation period ranges from 1 to 3 days, with the onset being sudden. Common symptoms include moderate intoxication (such as headache and fatigue), a fever that can range from mild to high, and chills. Abdominal pain, especially around the navel, and diarrhea (sometimes accompanied by blood or mucus) are also observed. Palpation of the abdomen may reveal tenderness along the colon. This form of colibacillosis often manifests in a mild or atypical manner, with a moderate course being less common. The duration of the illness usually does not exceed a few days [27].

Enterotoxigenic colibacillosis typically presents with clinical features similar to those of salmonellosis, food poisoning, or a mild form of cholera. The incubation period is usually 1 to 2 days. Symptoms are characterized by mild intoxication, a lack of significant fever, recurring vomiting, profuse diarrhea, and gradual onset of dehydration, which may lead to oliguria. Cramping pain is usually localized to the epigastric region [28]. This infection is often termed “traveler's diarrhea” because it commonly affects individuals visiting tropical regions for work or leisure, where the climate exacerbates the symptoms, including severe fever with chills, significant intoxication, and pronounced dehydration.

Enterohemorrhagic colibacillosis most frequently affects children and presents with moderate intoxication and subfebrile temperature. Nausea, vomiting, and watery diarrhea are common early signs. In severe cases, by the third or fourth day, cramping abdominal pain intensifies, diarrhea worsens, and the stool may contain blood and pus, losing its typical fecal consistency [29].

Although many cases resolve spontaneously within a week, more severe instances—especially in young children—can lead to complications. Between days 7 and 10, after the diarrhea subsides, hemolytic-uremic syndrome (characterized by hemolytic anemia, thrombocytopenia, and acute renal failure) may develop. Additional neurological symptoms, including limb spasms, muscle rigidity, and altered consciousness (ranging from confusion to coma), are frequent. The mortality rate for patients experiencing these severe complications can reach up to 5% [30].

Complications

Colibacillosis typically does not result in complications. However, infections caused by Enterohemorrhagic *E. coli* may lead to complications affecting hemolytic anemia, urinary system, and neurological disorders [31].

Diagnostics

To diagnose colibacillosis, the pathogen is isolated from vomit or feces. In cases of systemic infection, it may also be detected in urine, blood, bile, or cerebrospinal fluid. After pathogen isolation, bacteriological analysis is performed using nutrient media. Due to the antigenic similarity between colibacillosis-causing agents and bacteria that are part of the normal intestinal flora, serological testing has limited diagnostic value.

In some instances, identifying bacterial toxins in fecal samples may aid in diagnosis. Additionally, patients may show signs of hemolytic anemia, and elevated levels of urea and creatinine in the blood, and urinalysis may reveal proteinuria, leukocyturia, and hematuria [32].

Prevention

Colibacillosis is closely linked to poor hygiene practices. To prevent these infections, it is essential to maintain proper hygiene, particularly when interacting with children, and regularly wash toys, hands, food, and household items. Broad prevention efforts focus on ensuring adherence to sanitary and hygienic standards in childcare centers, food processing facilities, and healthcare settings. Additionally, monitoring the treatment of sewage and the quality of water sources is vital [33].

Patients who recover from colibacillosis are discharged from the hospital once they show clinical improvement and pass three consecutive bacteriological tests. Kids who have been in contact with infected individuals may return to their institutions only after a negative bacteriological test confirms they are not carrying the pathogen. Those excreting pathogenic *E. coli* must be isolated for the entire contagious period. Food industry workers undergo regular testing for pathogen presence, and if a positive result is found, they are temporarily removed from their duties [34-38].

Conclusion

Colibacillosis manifests with varying symptoms and severity depending on the specific group of *E. coli* responsible for the infection. In young children, enteropathogenic colibacillosis typically has an incubation period of several days, presenting symptoms such as vomiting, diarrhea, severe intoxication, and dehydration. Enteroinvasive colibacillosis mirrors the symptoms of dysentery or shigellosis, with an incubation period of 1 to 3 days, acute onset, moderate intoxication, fever (ranging from low-grade to high), chills, abdominal pain (especially around the navel), and diarrhea, which may include blood and mucus.

Enterotoxigenic colibacillosis exhibits symptoms similar to salmonellosis, food poisoning, or a mild form of cholera. The incubation period is 1-2 days, with mild intoxication, little or no fever, repeated vomiting, profuse diarrhea, and progressively worsening dehydration. In severe cases, oliguria may develop. Enterohemorrhagic colibacillosis, most common in children, starts with moderate intoxication and subfebrile temperature, accompanied by nausea, vomiting, and watery diarrhea. In more severe cases, 3 to 4 days into the illness, intense abdominal cramping occurs, diarrhea worsens, and stools may contain blood and pus, indicating the severity of the infection.

Acknowledgments: None

Conflict of Interest: None

Financial Support: None

Ethics Statement: None

References

1. Da Silva GJ, Mendonça N. Association between antimicrobial resistance and virulence in *Escherichia coli*. Virulence. 2012;3(1):18-28. doi:10.4161/viru.3.1.18382
2. Jang J, Hur HG, Sadowsky MJ, Byappanahalli MN, Yan T, Ishii S. Environmental *Escherichia coli*: ecology and public health implications-a review. J Appl Microbiol. 2017;123(3):570-81. doi:10.1111/jam.13468
3. Leimbach A, Hacker J, Dobrindt U. *E. coli* as an all-rounder: the thin line between commensalism and pathogenicity. Curr Top Microbiol Immunol. 2013;358:3-32. doi:10.1007/82_2012_303
4. De Biase D, Lund PA. The *Escherichia coli* acid stress response and its significance for pathogenesis. Adv Appl Microbiol. 2015;92:49-88. doi:10.1016/bs.aambs.2015.03.002
5. Jayamani E, Mylonakis E. Effector triggered manipulation of host immune response elicited by different pathotypes of *Escherichia coli*. Virulence. 2014;5(7):733-9. doi:10.4161/viru.29948
6. Luneva AV, Lysenko YA, Gneush AN, Shantyz AY, Simonov AN, Verevkina MN, et al. Assessment of the biosafety of microorganisms and their joint composition. Pharmacophore. 2021;12(3):42-8. doi:10.51847/M60cnxYHzx
7. Schuldiner S. The *Escherichia coli* effluxome. Res Microbiol. 2018;169(7-8):357-62. doi:10.1016/j.resmic.2018.02.006

8. Mueller M, Tainter CR. *Escherichia coli*. 2022 Oct 17. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2022.
9. Mirhoseini A, Amani J, Nazarian S. Review on pathogenicity mechanism of enterotoxigenic *Escherichia coli* and vaccines against it. Microb Pathog. 2018;117:162-9. doi:10.1016/j.micpath.2018.02.032
10. Gelalcha BD, Brown SM, Crocker HE, Agga GE, Kerro Dego O. Regulation mechanisms of virulence genes in Enterohemorrhagic *Escherichia coli*. Foodborne Pathog Dis. 2022;19(9):598-612. doi:10.1089/fpd.2021.0103
11. Sizonenko MN, Timchenko LD, Rzhepakovskiy IV, DA SP AV, Nagdalian AA, Simonov AN, et al. The new efficiency of the «Srmp»–listerias growth-promoting factor during factory cultivation. Pharmacophore. 2019;10(2):85-8.
12. Farfán-García AE, Ariza-Rojas SC, Vargas-Cárdenas FA, Vargas-Remolina LV. Virulence mechanisms of enteropathogenic *Escherichia coli*. Rev Chilena Infectol. 2016;33(4):438-50. [In Spanish]. doi:10.4067/S0716-10182016000400009
13. Mariotti A, Ezzraimi AE, Camoin-Jau L. Effect of antiplatelet agents on *Escherichia coli* sepsis mechanisms: a review. Front Microbiol. 2022;13:1043334. doi:10.3389/fmicb.2022.1043334
14. Vila J, Sáez-López E, Johnson JR, Römling U, Dobrindt U, Cantón R, et al. Escherichia coli: an old friend with new tidings. FEMS Microbiol Rev. 2016;40(4):437-63. doi:10.1093/femsre/fuw005
15. Gneush AN, Luneva AV, Machneva NL, Lysenko YA, Aniskina MV, Verevkina MN, et al. Biotechnology of Microorganisms growing–fundamentals for the development of a litter biodestructor. J Pharm Res Int. 2021;33(36B):1-1. doi:10.9734/jpri/2021/v33i36B31946
16. Newell DG, La Ragione RM. Enterohaemorrhagic and other Shiga toxin-producing *Escherichia coli* (STEC): where are we now regarding diagnostics and control strategies? Transbound Emerg Dis. 2018;65(Suppl 1):49-71. doi:10.1111/tbed.12789
17. Juarez GE, Galván EM. Role of nutrient limitation in the competition between uropathogenic strains of *Klebsiella pneumoniae* and *Escherichia coli* in mixed biofilms. Biofouling. 2018;34(3):287-98. doi:10.1080/08927014.2018.1434876
18. Mizuochi S, Nelson M, Baylis C, Green B, Jewell K, Monadjemi F, et al. Matrix extension study: validation of the compact dry EC method for enumeration of *Escherichia coli* and non-*E. Coli* coliform bacteria in selected foods. J AOAC Int. 2016;99(2):451-60. doi:10.5740/jaoacint.15-0268
19. Devane M, Dupont PY, Robson B, Lin S, Scholes P, Wood D, et al. Mobilization of *Escherichia coli* and fecal source markers from decomposing cowpats. Sci Total Environ. 2022;853(2):158509. doi:10.1016/j.scitotenv.2022.158509
20. Falcicchio P, Levisson M, Kengen SWM, Koutsopoulos S, van der Oost J. (Hyper)thermophilic enzymes: production and purification. Methods Mol Biol. 2021;2178:469-78. doi:10.1007/978-1-0716-0775-6_29
21. Köhler CD, Dobrindt U. What defines extraintestinal pathogenic *Escherichia coli*? Int J Med Microbiol. 2011;301(8):642-7. doi:10.1016/j.ijmm.2011.09.006
22. Yura T. Regulation of the heat shock response in *Escherichia coli*: history and perspectives. Genes Genet Syst. 2019;94(3):103-8. doi:10.1266/ggs.19-00005
23. Yang S, Xi D, Jing F, Kong D, Wu J, Feng L, et al. Genetic diversity of K-antigen gene clusters of *Escherichia coli* and their molecular typing using a suspension array. Can J Microbiol. 2018;64(4):231-41. doi:10.1139/cjm-2017-0620
24. Nakae K, Ooka T, Murakami K, Hara-Kudo Y, Imuta N, Gotoh Y, et al. Diversification of *Escherichia albertii* H-Antigens and development of H-Genotyping PCR. Front Microbiol. 2021;12:737979. doi:10.3389/fmicb.2021.737979
25. Elmakaoui A, Bourais I, Oubihi A, Nassif A, Bezhinar T, Shariati MA, et al. Chemical composition and antibacterial activity of essential oil of *Lavandula multifida*. J Microbiol Biotechnol Food Sci. 2022;11(6):e7559. doi:10.55251/jmbfs.7559
26. Fukuda S, Toh H, Hase K, Oshima K, Nakanishi Y, Yoshimura K, et al. Bifidobacteria can protect from enteropathogenic infection through production of acetate. Nature. 2011;469(7331):543-7. doi:10.1038/nature09646
27. Aribam SD, Hirota J, Kusumoto M, Harada T, Shiraiwa K, Ogawa Y, et al. A rapid differentiation method for enteroinvasive *Escherichia coli*. J Microbiol Methods. 2014;98(1):64-6. doi:10.1016/j.mimet.2013.11.012

28. Zhang Y, Tan P, Zhao Y, Ma X. Enterotoxigenic *Escherichia coli*: intestinal pathogenesis mechanisms and colonization resistance by gut microbiota. Gut Microbes. 2022;14(1):2055943. doi:10.1080/19490976.2022.2055943
29. Goto T, Shirano M. Enterohemorrhagic *E. coli* (EHEC). Nihon Rinsho. 2012;70(8):1343-7. [In Japanese].
30. Nguyen Y, Sperandio V. Enterohemorrhagic *E. coli* (EHEC) pathogenesis. Front Cell Infect Microbiol. 2012;2:90. doi:10.3389/fcimb.2012.00090
31. Fründt T, Leuffert J, Groth S, Rösch T, Steurer S, Lohse AW, et al. Low incidence of colonic complications after severe Shiga toxin-producing *E. coli* O104:H4 infection. Z Gastroenterol. 2022;60(7):1104-10. [In English]. doi:10.1055/a-1545-5322
32. Lutful Kabir SM. Avian colibacillosis and salmonellosis: a closer look at epidemiology, pathogenesis, diagnosis, control, and public health concerns. Int J Environ Res Public Health. 2010;7(1):89-114. doi:10.3390/ijerph7010089
33. Bachinina KN, Povetkin SN, Simonov AN, Pushkin SV, Blinova AA, Sukhanova ED, et al. Effects of selenium preparation on morphological and biochemical parameters of quail meat. Int Trans J Eng, Manag Appl Sci Technol. 2021;12(13):1213. Available from: http://TUENGR.COM/V12/12A13K.pdf doi:10.14456/ITJEMAST.2021.263
34. Delsignore M, Siddiqui SA. Chapter 8. From waste to food: legislative insights. In waste to food: returning nutrients to the food chain 2022 Feb 15 (p. 1010). Wageningen Academic Publishers. doi:10.3920/978-90-8686-929-9_8
35. Hassoun A, Siddiqui SA, Smaoui S, Ucak İ, Arshad RN, Garcia-Oliveira P, et al. Seafood processing, preservation, and analytical techniques in the age of industry 4.0. Appl Sci. 2022;12(3):1703. doi:10.3390/app12031703
36. Akshita C, Vijay BV, Praveen D. Evaluation of phytochemical screening and antimicrobial efficacy of Mesua ferrea and piper cubeba fruit extracts against multidrug-resistant bacteria. Pharmacophore. 2020;11(2):15-20.
37. Mirza AS, Baig MT, Huma A, Ibrahim S, Shahid U, Jabeen A, et al. Antibacterial activity of methanol extract of capparis decidua edgew (Forssk.) against staphylococcus aureus, bacillus cereus, salmonella typhi, and *Escherichia coli*. Pharmacophore. 2020;11(4):46-50.
38. Tati S, Nurul Fatimah N, Yandri Y, Rahmat Kurniawan R, Syaiful B, Sutopo H. The anticancer, antimalarial, and antibacterial activities of Moracalkon isolated from Artocarpus kemando Miq. J Adv Pharm Educ Res. 2021;11(4):105-10.