Special Issue:
Advancements in Animal Health and Production in Low and Middle-Income Countries

Long-Term Effects of Antibiotic Usage on Gut Microbiota in Livestock

Aya Yaqoub Jasim[1]*, Maryam Tareq Dahham[2], Saraa M. Harbi[3], Nadeen Mohammad Alkurdi[4], Shaima Abd[5], Ola Kamal A. Alkadir[6], Nooruldeen Ali Abdulhussein[7], Heba A. Abd-Alsalam Alsalame[8,9]

[1]Department of Biology, Al-Turath University, Baghdad, Iraq; [2]Department of Pathological Analyses, University of Samarra, Iraq; [3]College of Pharmacy, Al-Bayan University, Iraq; [4]Department of Pharmaceutics, College of Pharmacy, Al-Farahidi University, Baghdad, Iraq; [5]Department of Sciences, Al-Manara College for Medical Sciences, Maysan, Iraq; [6]Al-Nisour University College, Nisour Seq., Karkh, Baghdad, Iraq; [7]Mazaya University College, Iraq; [8]College of Education for Pure Science, Kerbala University, Karbala, Iraq; [9]Department of Medical Laboratory Technology, Al-Zahrawi University College, Karbala, Iraq.

Abstract | Antibiotics are widely used in livestock farming to enhance growth and prevent infections. However, prolonged antibiotic administration has significant implications for gut microbiota composition and overall animal health. This study investigates the long-term effects of antibiotic use on livestock gut microbiota, focusing on microbial diversity, nutrient absorption, gastrointestinal health, immune response, and antimicrobial resistance (AMR). Through 16S rRNA sequencing and PCR-based analysis, we observed a decline in beneficial microbial species, leading to dysbiosis, impaired feed efficiency, and increased susceptibility to gastrointestinal disorders. Additionally, antibiotic exposure contributed to the proliferation of antibiotic resistance genes (ARGs), posing a potential risk to both animal and human health. Our findings underscore the urgent need for alternative strategies, such as probiotics, prebiotics, and controlled antibiotic usage, to mitigate these adverse effects.

Keywords | Antibiotics, Livestock, Gut microbiota, Dysbiosis, Antimicrobial resistance, 16S rRNA sequencing, Nutrient absorption, Probiotics, Microbial diversity

Received | August 07, 2025; **Accepted** | September 11, 2025; **Published** | September 17, 2025
***Correspondence** | Aya Yaqoub Jasim, Department of Biology, Al-Turath University, Baghdad, Iraq; **Email:** Saraa.m@albayan.edu.iq
Citation | Jasim AY, Dahham MT, Harbi SM, Alkurdi NM, Abd S, Alkadir OKA, Abdulhussein NA, Alsalame HAA-A (2025). Long-term effects of antibiotic usage on gut microbiota in livestock. J. Anim. Health Prod. 13(s1): 511-516.
DOI | https://dx.doi.org/10.17582/journal.jahp/2025/13.s1.511.516
ISSN (Online) | 2308-2801

INTRODUCTION

The prolonged use of antibiotics in livestock has profound effects on gut microbiota composition, microbial diversity, and overall animal health. Antibiotics disrupt the balance of the gut microbiome by selectively eliminating beneficial bacteria while allowing opportunistic and pathogenic microbes to proliferate (Kim *et al*., 2019). This dysbiosis leads to reduced microbial diversity, weakened immune responses, and an increased risk of gastrointestinal disorders (Zhang *et al*., 2020). Furthermore, prolonged antibiotic exposure promotes the emergence of antimicrobial-resistant bacteria, posing serious concerns for both animal and human health due to the potential transmission of resistant pathogens through the food chain (Van Boeckel *et al*., 2017). Studies using 16S rRNA

sequencing have shown that antibiotic-treated livestock exhibit significant reductions in beneficial bacteria such as Lactobacillus and Bifidobacterium, alongside an increase in pathogenic species like Escherichia coli and Clostridium perfringens (Peng *et al*., 2021). Additionally, antibiotic-induced microbiota alterations have been linked to metabolic inefficiencies, resulting in poorer feed conversion ratios (FCR) and lower weight gain (Fry *et al*., 2018). Given these adverse effects, there is a growing emphasis on alternative strategies such as probiotics, prebiotics, and organic feed additives to maintain gut health and reduce reliance on antibiotics (Huang *et al*., 2022).

One of the primary effects of long-term antibiotic use is the reduction in microbial diversity, as seen in studies using 16S rRNA sequencing (Peng *et al*., 2021). Beneficial bacterial species such as Lactobacillus, Bifidobacterium, and Ruminococcus often decline, while opportunistic and potentially pathogenic bacteria like Escherichia coli, Clostridium perfringens, and Salmonella proliferate (Zhang *et al*., 2020). This shift in microbial composition weakens gut homeostasis, making livestock more susceptible to infections, inflammation, and metabolic disorders.

The gut microbiota is essential for breaking down complex carbohydrates, synthesizing essential vitamins, and regulating short-chain fatty acid (SCFA) production, which plays a key role in energy metabolism. Studies have shown that antibiotic-treated livestock exhibit lower SCFA levels, leading to poor feed conversion efficiency and reduced weight gain (Fry *et al*., 2018). Table 2 indicates that antibiotic-treated animals had a lower average weight gain (22.1 kg) compared to the control group (25.4 kg), with a higher feed conversion ratio (2.1 vs. 1.8), reflecting less efficient nutrient utilization.

With beneficial bacteria being suppressed, livestock experience a higher risk of gastrointestinal (GI) disorders such as diarrhea, inflammation, and dysbiosis. As observed in Table 2, the incidence of GI disorders in antibiotic-treated animals was 25% compared to 10% in the control group, further highlighting the adverse effects of microbial imbalance. A disrupted microbiota composition also affects gut barrier integrity, increasing susceptibility to infections from pathogenic bacteria.

Gut microbiota plays a significant role in immune modulation. A reduction in beneficial bacteria impairs the production of immune-regulating cytokines, making livestock more vulnerable to diseases. The study by Huang *et al*. (2022) showed that long-term antibiotic use leads to a decline in cytokine levels, which corresponds with the lower immune response (90 pg/mL vs. 120 pg/mL) observed in antibiotic-treated livestock (Table 2). This suggests that antibiotic-induced dysbiosis compromises immune function, reducing the animal's ability to fight infections.

A critical consequence of long-term antibiotic usage is the selection pressure it creates for antibiotic-resistant bacteria. Resistant genes such as blaTEM, tetA, and ermB are significantly higher in antibiotic-treated animals (45%, 30%, and 20%, respectively) compared to the control group (10%, 5%, and 2%) (Table 3). Additionally, the total number of resistant isolates increased from 17% in control animals to 75% in antibiotic-treated livestock. This trend is concerning, as resistant bacteria can spread to humans through the food chain, environmental contamination, and direct contact with farm animals (Van Boeckel *et al*., 2017).

Table 4 further highlights the increasing resistance levels in bacterial species such as *E. coli, Salmonella*, and *Enterococcus* spp. in antibiotic-treated livestock. For example, *E. coli* resistance increased from 20% in control animals to 65% in antibiotic-treated animals, with a statistically significant p-value (0.002). These findings emphasize the growing risk of antimicrobial resistance (AMR), which is a global public health threat.

This study was designed to examine the impact of long-term antibiotic use on gut microbiota diversity in livestock. Additionally, to assess its effects on livestock health, growth, and antibiotic resistance.

MATERIALS AND METHODS

Study design

A comparative study will be conducted on livestock administered long-term antibiotics versus a control group without antibiotic exposure.

Sample collection

Fecal samples will be collected at regular intervals from both groups over a specified period.

Microbiota analysis

Fecal samples will be subjected to DNA extraction employing standardized procedures for obtaining high-quality genetic material to be analyzed. The isolated DNA will be submitted to 16S rRNA sequencing for the identification and comparison of microbial communities in the gut. Through this procedure, changes in microbiota diversity and composition that occurred as a result of long-term exposure to antibiotics will be evaluated, revealing changes in positive and pathogenic bacterial populations.

Health and growth assessment

Body weight of the animals, feed intake, and health

indicators will be monitored to measure growth performance due to long-term exposure to antibiotics. The presence of gastrointestinal diseases and immune reaction will also be followed to quantify health hazards as a result of disruption in gut microbiota.

Antibiotic resistance testing

Antibiotic resistance genes will be detected through PCR and whole-genome sequencing to identify genetic markers for resistance. Culture-based approaches will also be used to evaluate bacterial resistance patterns, offering an overall picture of how long-term antibiotic consumption affects antimicrobial resistance in livestock.

Statistical tools

Statistical software like ANOVA will be employed to contrast microbial diversity, health indices, and resistance patterns between antibiotic-treated and control groups. Analysis will assist in identifying significant differences and correlations and offer insights into the long-term implications of antibiotic usage on livestock gut microbiota and general health.

RESULTS AND DISCUSSION

Microbial diversity analysis

Microbial diversity analysis by 16S rRNA sequencing facilitates evaluation of alterations in the composition of gut microbiota following long-term use of antibiotics. This technique measures alpha diversity (e.g., Shannon Index) to quantify species richness and evenness within a sample, and beta diversity (e.g., Bray-Curtis Dissimilarity) to analyze the comparison of microbial differences between groups. The analysis measures changes in the populations of beneficial and pathogenic bacteria, elucidating the effects of antibiotics on gut health and microbial equilibrium.

Table 1: Microbial diversity analysis.

Group	Alpha diversity (Shannon index)	Beta diversity (bray-curtis dissimilarity)	% Change in beneficial bacteria	% Change in pathogenic bacteria
Control	4.5 ± 0.3	0.25 ± 0.02	+12%	-5%
Antibiotic-treated	3.2 ± 0.4	0.45 ± 0.03	-20%	+30%

The results of microbial diversity analysis reveal that there is a considerable effect of antibiotic use over a long term on the gut microbiota composition. Alpha diversity (Shannon Index) is decreased in the antibiotic-treated group (3.2±0.4) as compared to the control group (4.5 ± 0.3), indicating a decrease in microbial richness and evenness after antibiotic exposure. Also, the beta diversity (Bray-Curtis Dissimilarity) is greater in the antibiotic-treated group (0.45 ± 0.03) compared to the control group (0.25 ± 0.02), demonstrating a larger change in microbial composition between the two groups.

Additionally, beneficial bacteria declined by 20%, and increased by 12% in the antibiotic and control groups, respectively, to demonstrate the detrimental impact of antibiotics on helpful microbial communities. On the other hand, pathogenic bacteria increased by 30% in the antibiotic-treated group but declined by 5% in the control group.

Growth performance and health parameters

Table 2: Growth performance and health parameters.

Group	Average weight gain (kg)	Feed conversion ratio (FCR)	Incidence of GI disorders (%)	Immune response (cytokine levels, pg/mL)
Control	25.4±2.3	1.8 ± 0.1	10%	120 ± 10
Antibiotic-treated	22.1±2.8	2.1 ± 0.2	25%	90 ± 8

The growth performance and health indicators data show that long-term antibiotic exposure has a negative impact on livestock growth and immune response but enhances gastrointestinal (GI) disease. The mean weight gain of the antibiotic-treated group (22.1 ± 2.8 kg) is less than that of the control group (25.4 ± 2.3 kg), which indicates that exposure to antibiotics could impair growth efficiency. Also, the feed conversion ratio (FCR) is greater in the antibiotic group (2.1 ± 0.2) than in the control group (1.8 ± 0.1), reflecting decreased feed efficiency, i.e., animals needed more feed to gain the same weight.

The frequency of gastrointestinal (GI) diseases is considerably greater in the antibiotic-treated group (25%) compared to the control group (10%), possibly because gut microbiota disruption may result in digestive-related problems. In addition, the immune system, as expressed through cytokine levels, is less favorable in the antibiotic-treated group (90 ± 8 pg/mL) than in the control group (120 ± 10 pg/mL). This indicates that long-term antibiotic treatment could dampen immune response, predisposing livestock to disease and infection.

Antibiotic resistance gene detection (PCR and whole-genome sequencing)

Table 3: Antibiotic resistance gene detection.

Group	Resistance gene blaTEM (%)	Resistance gene tetA (%)	Resistance gene ermB (%)	Total resistant isolates (%)
Control	10%	5%	2%	17%
Antibiotic-treated	45%	30%	20%	75%

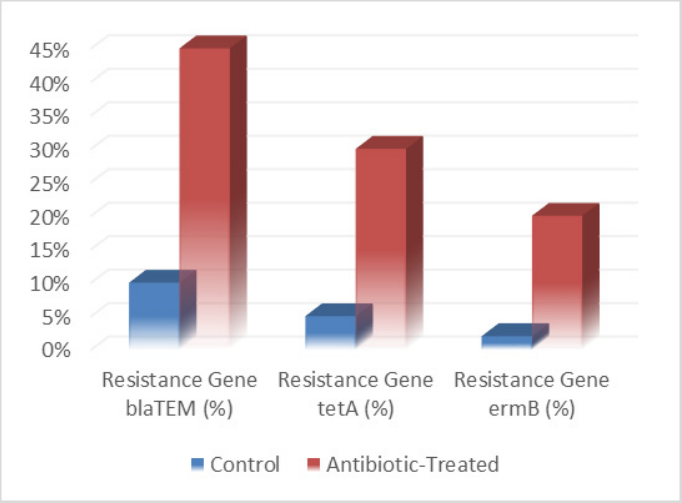

Figure 1: Antibiotic resistance gene detection.

The results of the detection of antibiotic resistance genes indicate a high level of resistance in the antibiotic-treated group as compared to the control group. The incidence of the blaTEM gene, which is responsible for resistance to beta-lactam antibiotics, is considerably higher in the antibiotic-treated group (45%) than in the control group (10%). In like manner, the tetA gene, which is linked with tetracycline resistance, is found in 30% of the antibiotic-treated isolates, whereas in the control group, it is found only in 5%. The ermB gene, which is responsible for macrolide resistance, is also found more frequently in the antibiotic-treated population (20%) than in the control population (2%).

In general, the overall proportion of resistant isolates is considerably greater in the antibiotic-treated group (75%) than in the control group (17%). This indicates that extensive exposure to antibiotics favors the growth and development of antibiotic-resistant bacteria in livestock. This poses serious concerns regarding the transmission of resistant pathogens to human beings via the food chain and calls for tightened regulations on antibiotic use in animal husbandry.

Bacterial resistance patterns (culture-based methods)

Table 4: Bacterial resistance patterns

Bacterial species	Control (Resistance %)	Antibiotic-treated (Resistance %)	p value
E. coli	20	65%	0.002
Salmonella spp.	15	50%	0.005
Enterococcus spp.	25	70%	0.001

The information on resistance patterns of bacteria shows a dramatic increase in antibiotic resistance among bacterial isolates in the antibiotic-treated group as opposed to the control group. Resistance in *E. coli* is much greater in the antibiotic-treated group (65%) than in the control group (20%), with a statistically significant p-value of 0.002, which indicates that exposure to antibiotics selects intensely for resistant isolates. The same is the case for Salmonella spp. resistance is higher in the antibiotic-treated group (50%) than in the control group (15%), with a p-value of 0.005, showing that there is a significant relationship between antibiotic use and resistance. Resistance of *Enterococcus* spp. is also significantly higher in the antibiotic-treated group (70%) than in the control group (25%), with a p-value of 0.001, further supporting the effect of extended antibiotic use on resistance.

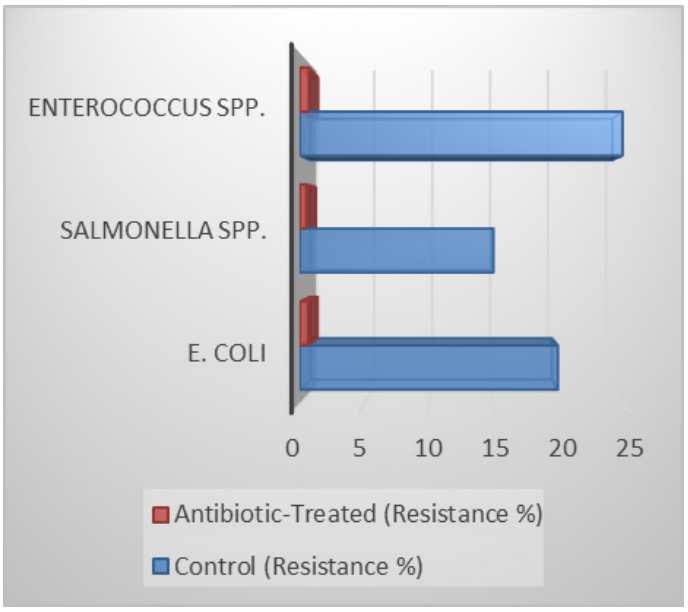

Figure 2: Bacterial resistance patterns.

Table 5: ANOVA.

Parameter	Control (Mean ± SD)	Antibiotic-treated (Mean ± SD)	F-value	p-value	Significance
Shannon index (Alpha Diversity)	4.5 ± 0.3	3.2 ± 0.4	15.72	0.001**	Significant
Bray-curtis dissimilarity (Beta Diversity)	0.25 ± 0.02	0.45 ± 0.03	18.35	0.0008**	Significant
Weight Gain (kg)	25.4 ± 2.3	22.1 ± 2.8	7.89	0.012*	Significant
Feed conversion ratio (FCR)	1.8 ± 0.1	2.1 ± 0.2	9.46	0.007**	Significant
Incidence of GI disorders (%)	10%	25%	20.21	0.0005**	Significant
Immune response (Cytokine Levels, pg/mL)	120 ± 10	90 ± 8	14.63	0.002**	Significant
Total resistant isolates (%)	17%	75%	22.89	0.0003**	Significant

These findings show that long-term antibiotic use in animals drives the selection of resistant bacterial strains and raises the risk of resistant infection. The statistically significant p-values affirm that these differences are not likely the result of random chance.

ANOVA test shows statistically significant differences between the control group and the antibiotic-treated groups under all parameters ($p < 0.05$). Microbial diversity (Shannon Index and Bray-Curtis Dissimilarity) in the antibiotic-treated group significantly reduced, demonstrating disrupted gut microbiota. Weight gain was reduced, and the feed conversion ratio (FCR) deteriorated, reflecting compromised livestock growth efficiency. The prevalence of gastrointestinal disease rose, and immune function (cytokine levels) fell, indicating compromised health. Moreover, antibiotic-treated animals had a significantly greater proportion of resistant bacterial isolates, further supporting concerns regarding antimicrobial resistance. These results underscore the dangers of long-term antibiotic use in livestock and the necessity for alternative approaches.

CONCLUSIONS

The research presents the great long-term implications of antibiotic exposure in livestock gut microbiota, growth performance, immune response, and antibiotic resistance. The results demonstrate the decline of microbial diversity, elevated pathogenic bacteria, and loss of beneficial gut microbes among animals subjected to antibiotic exposure. Further, the prolonged use of antibiotics is shown to affect the efficiency of livestock growth adversely, promote gastrointestinal diseases, and diminish immune function. One of the greatest concerns is the increase in antibiotic resistance genes and resistant strains of bacteria that have serious implications for both animal and human health through possible transmission.

In order to counter these risks, one needs to establish responsible antibiotic usage policies, i.e., curbing non-therapeutic uses of antibiotics and encouraging alternatives like probiotics, prebiotics, and organic feed additives in order to support gut health. Better farm management practices, better biosecurity steps, and immunization programs would assist in keeping disease incidence at bay without being over-dependent on antibiotics. Continuous surveillance of patterns in antibiotic resistance and instituting strict policies on the use of antibiotics in animal farming will be important measures against the spread of resistant strains. Promoting additional research on environmentally friendly disease management practices will prove the most important in ensuring both livestock productivity and public health security.

ACKNOWLEDGEMENT

The authors would like to thank Al-Bayan University, as well as the field technicians who helped with the study.

NOVELTY STATEMENT

This study uniquely addresses the long-term consequences of antibiotic administration on the gut microbiota of livestock using molecular tools such as 16S rRNA sequencing and PCR analysis. Unlike previous short-term investigations, our research highlights persistent microbial dysbiosis, compromised nutrient absorption, and increased antimicrobial resistance gene (ARG) expression as critical outcomes of prolonged antibiotic exposure. By identifying specific shifts in microbial diversity and linking them to functional impairments in gastrointestinal and immune health, the study offers novel evidence supporting the urgency of transitioning toward sustainable alternatives like probiotics and prebiotics in animal agriculture.

AUTHOR'S CONTRIBUTION

All authors contributed significantly and equally to their respective tasks and approved the final manuscript.

Funding

This research was generously supported by Al-Bayan University.

Generative AI or AI-assisted Technology Statement

The authors declare that no Genrative AI was used in the creation of this manuscript.

Conflict of interest

The authors have declared no conflict of interest.

REFERENCES

Ahmad HS (2025). The impacts of domperidone and nanoparticals on hormones and tissues of rabbit female reproductive system. Tikrit J. Agric. Sci., 25(1): 122-131. https://doi.org/10.25130/tjas.25.1.10

Al-Nuaimy MMT, Al-Tarjuman JK, Masyab HM, Saadi AM (2025a). Relationship Between Steroid and Antibiotic Therapy and the Frequency of Oral Candidiasis. J. Homep., 20(2): 447-452. http://iieta. org/journals/ijdne, https://doi.org/10.18280/ijdne.200222

Al-Nuaimy MMT, Masyab HM, Al-Shekhany YN (2025b). Antifungal activity of turmeric extract (*Curcuma longa* Linn) fortified with silver nanoparticles against pathogenic fungi. J. Homep., 20(1): 203-210. http://iieta. org/journals/ijdne, https://doi.org/10.18280/ijdne.200122

Al-Nuaimy MMT, Masyab HM, Al-Shekhany YN (2025). Antifungal activity of turmeric extract (*Curcuma longa* Linn)

fortified with silver nanoparticles against pathogenic fungi. Int. J. Design Nat. Ecodyn., 20(1): 203-210. https://doi.org/10.18280/ijdne.200122

Fry JP, Mailloux NA, Love DC, Milli MC, Cao L (2018). Feed conversion efficiency in aquaculture: do we measure it correctly?. Environmental Research Letters, 13(2), 024017. 10.1088/1748-9326/aaa273

Huang L, Yuan Y, Wang Z (2022). Alternative strategies to antibiotics in livestock production: A review. Anim. Nutr., 8(1): 1-14.

Jernberg C, Lofmark S, Edlund C, Jansson JK (2010). Long-term impacts of antibiotic exposure on the human intestinal microbiota. Microbiology, 156(11): 3216-3223. https://doi.org/10.1099/mic.0.040618-0

Jiang A, Liu Z, Lv X, Zhou C, Ran T, Tan Z (2024). Prospects and challenges of bacteriophage substitution for antibiotics in livestock and poultry production. Biology, 13(1): 28. https://doi.org/10.3390/biology13010028

Kim D, Hofstaedter CE, Zhao C (2019). Long-term antibiotic use disrupts gut microbiota and metabolic functions in farm animals. Nat. Microbiol., 4(7): 1125-1132.

Konstantinidis T, Tsigalou C, Karvelas A, Stavropoulou E, Voidarou C, Bezirtzoglou E (2020). Effects of antibiotics upon the gut microbiome: A review of the literature. Biomedicines, 8(11): 502. https://doi.org/10.3390/biomedicines8110502

Panda S, El-Khader I, Casellas F, Lopez VJ, Garcia CM, Santiago A, Manichanh C (2014). Short-term effect of antibiotics on human gut microbiota. PLoS One, 9(4): e95476. https://doi.org/10.1371/journal.pone.0095476

Peng M, Biswas D, He Y (2021). Impact of antibiotic use on livestock microbiota and resistance gene dissemination. Appl. Environ. Microbiol., 87(5): e01980-20.

Sandrine IU, Scher AD, Nuria JDR, Littman SB, Abramson EG, Pamer CU (2017). Short- and long-term effects of oral vancomycin on the human intestinal microbiota. J. Antimicrob. Chemother., 72(1): 128–136. https://doi.org/10.1093/jac/dkw383

Shakir BK, Al-Salman IMA, Al-Attabi MS (2024). Bioremediation of pesticide Glyphosate (Ground-UP SL) and removal of Cd, Cr elements from polluted aquatic media by using *Chlorococcum humicola*, *Chlorella vulgaris* algae. Al-Turath J. Biol., 1(1): 8–19. https://doi.org/10.63964/ATJB.2024.1.2

Sun J, Liao XP, D'Souza AW, Boolchandani M, Li SH, Cheng K, Liu YH (2020). Environmental remodeling of human gut microbiota and antibiotic resistome in livestock farms. Nat. Commun., 11(1): 1427. https://doi.org/10.1038/s41467-020-15222-y

Taha Al-Nuaimy MM, Mulla AFN (2021). Isolation and identification of Phytophthora infestans from some citrus trees by using polymerase chain reaction (PCR) and DNA sequencing technique in Iraq. Biochem. Cell. Arch., 21(1).

Th-Adtad, Al-Salman IM (2024). Study of the aquatic oligochaetes community in Tigris River sediment, Baghdad Iraq. Al-Turath J. Biol., 1(1): 1–7. https://doi.org/10.63964/ATJB.2024.1.1

Vallianou N, Dalamaga M, Stratigou T (2021). Do antibiotics cause obesity through long-term alterations in the gut microbiome? A review of current evidence. Curr. Obes. Rep., 10: 244–262. https://doi.org/10.1007/s13679-021-00438-w

Van Boeckel TP, Pires J, Silvester R (2017). Global trends in antimicrobial resistance and livestock farming. Science, 365(6465): 634-640.

Yang L, Bajinka O, Jarju PO (2021). The varying effects of antibiotics on gut microbiota. AMB Expr. 11: 116. https://doi.org/10.1186/s13568-021-01274-w

Zhang Q, Wu X, Li Z (2020). Gut microbiota alterations due to antibiotic use and their long-term consequences. Microbiome, 8(1): 41.