Int. j. adv. multidisc. res. stud. 2025; 5(6):1897-1901

Received: 07-11-2025
Accepted: 17-12-2025

International Journal of Advanced Multidisciplinary Research and Studies

ISSN: 2583-049X

Broiler Poultry Farms in Negros Occidental as Potential Reservoirs of Multidrug- and Colistin-Resistant Coliforms

[1] Esther Grace Villagomez, [2] Jonathan Dennis Zamora, [3] Martha Sofia Barredo, [4] Maria Angela Baterna, [5] Beau Fel Marie Talesio, [6] Ariane Ruth Tejada, [7] Kobe Andre Uy, [8] Chypreyllyn Valencia, [9] Karlo Antonio A Palabrica, [10] Clark Martin P Araneta, [11] Alain C Juayang

[1, 2, 3, 4, 5, 6, 7, 8, 9, 11] Medical Technology Program, Colegio San Agustin, Bacolod City, Philippines

[10] College of Veterinary Medicine, Aklan State University, Banga, Aklan, Philippines

[11] Clinical Laboratory, Corazon Locsin Montelibano Memorial Regional Hospital, Bacolod City, Philippines

Corresponding Author: **Alain C Juayang**

Abstract

MDR coliforms have become an increasing and consistent problem in human and animal health nowadays. The overuse of antibiotics, whether in a clinical setting or in animal farming, has been a contributing factor to the emergence of MDR organisms capable of producing ESBL, ampC, and even resisting colistin. The current study was made to detect and isolate MDR coliforms, including those that produce ESBL, ampC, and those that can resist the antibiotic colistin from chicken droppings in two poultry farms in Negros Occidental, Philippines. A total of 120 fecal swabs were analyzed using conventional microbiologic methods, Kirby-Bauer disc diffusion method for antimicrobial susceptibility, and disc broth elution for colistin resistance. ESBL and AmpC were detected using double disk synergy test. Multiple Antibiotic Resistance Index (MARI) was calculated as the ratio between the number of antibiotics that an isolate is resistant to and the total number of antibiotics it was tested for. Out of 120 samples, 62 (51.67%) were identified as *E. coli*, 18 (15%) as *K. pneumoniae*, and 2 (1.67%) as *E. cloacae*. Most of the isolates were resistant to chloramphenicol (85.37%), amoxicillin/clavulanic acid (74.39%) and colistin (69.51%). Moreover, ESBL production was noted in 23 isolates (28.05%), AmpC in 2 isolates (2.44%) with a mean MARI of 0.39, indicating that these organisms originated from high-risk environments. The findings of this study reveal the emerging threats of MDR coliforms in poultry production. It is therefore important for public health authorities to minimize, if not prevent, the potential risk of spreading these MDR coliforms from poultry farms.

Keywords: Poultry Farm, MDR, Colistin Resistance, Coliforms

1. Introduction

Colistin is a polymyxin antibiotic, a cationic antimicrobial peptide used as a last line of defense to treat multi-drug gram-negative infections [1, 2], such as those caused by Enterobacterales*, Acinetobacter baumannii,* and *Pseudomonas aeruginosa*. It was reinstated after being banned in the 1970s due to its adverse effects and safety issues, including nephrotoxicity, skin irritation, respiratory difficulties, and other concerns [3]. Though, colistin was recently reclassified in the category of very high importance for human medicine by the World Health Organization (WHO) and other health agencies.

Colistin is also used in veterinary medicine but is prohibited in many developing countries for its incorporation in animal feeds. Yet, these countries continue to use it to prevent infections and promote growth in animals for human consumption, such as poultry. Due to these practices, the overuse of antimicrobials in livestock production induces the evolution of bacterial resistance, especially against colistin. Existing studies indicate that common bacteria resistant to the action of colistin are widespread, with pigs and chickens in agriculture serving as important reservoirs [4, 5, 6].

With the reports of colistin resistance that have surfaced over the decades, not just in the Philippines but all throughout Asia, certain sectors or regions also need to establish an AMR surveillance system to determine its local prevalence for other public health and epidemiologic purposes. Hence, this study was designed to determine colistin resistance coliforms among certain broiler poultry farms in Negros Occidental as a basis to enable the path in preventing and controlling antimicrobial resistance to promote health and safety not just for the poultry workers but for the whole community as well.

2. Materials and Methods

This descriptive cross-sectional study was conducted at Colegio San Agustin-Bacolod from April 13, 2024 to May 31, 2024. Specimens were taken from two (2) broiler-poultry farms in Negros Occidental. Ethical clearance was obtained from the Research Ethics Committee of Colegio San Agustin-Bacolod with reference number 2024-01-STU-Zamora-RPA 3-Colistin Resistant Coliforms from Poultry Chickens dated April 12, 2024. Poultry owners were informed about the nature and purpose of the study. Poultry farm owners provided written informed consent for inclusion before they participated in the study.

2.1 Isolation and Identification of Isolates

A total of 120 fecal samples from two (2) poultry farms in Negros Occidental from February 2024 to May 2024 were collected. Each sample was subjected onto a modified Landman technique, wherein a 5-mL Tryptic Soy Broth (TSB) with 10-µg colistin, and incubated at 35–37 °C for 18–24 hrs. After 18–24 hours, all inoculated TSB were subcultured on MacConkey Agar and again incubated at 35–37 °C for another 18–24 hrs. After incubation, coliforms were observed on MacConkey Agar with a characteristic pink color. These coliforms were then purified onto a new MacConkey Agar and incubated at 35–37 °C for 18–24 hours.

Purified colonies of coliforms were then subjected to a series of conventional biochemical tests (gram stain reaction, oxidase, sugar fermentation, H_2S production, gas production, ability to decarboxylae or deaminate lysine, motility, indole production, citrate utilization, urease production and growth on Eosin Methylene Blue). Identification of bacterial isolates was made by their colonial morphologies on MacConkey and EMB and their reactions to the different biochemical tests to the standardized guidelines of Bergey's manual [7] for determinative bacteriology.

2.2 Antimicrobial Susceptibility Testing, Phenotypic Resistance Testing and Multiple Antibiotic Resistance Index

In vitro susceptibility testing of the isolated coliforms was done by using a disk diffusion test on Mueller-Hinton agar as previously described [8], against eight antibiotics, and zones of inhibition were interpreted following the guidelines of the Clinical Laboratory Standards Institute [9]. Antibiotics that were used include Amikacin (AN) 30 µg, Aztreonam (ATM), Amoxicillin/Clavulanic Acid (AMC) 20/10 µg, Cefepime (FEP) 30 µg, Cefotaxime (CTX) 30 µg, Imipenem (IMI) 10 µg, Cefoxitin (FOX) 30 µg, and Chloramphenicol (C) 30 µg. Susceptibility to colistin was determined using broth disc elution as described in CLSI, 2024 [9]. Colistin broth disk elution method offers a simple, cost-effective, and reliable alternative for colistin susceptibility testing comparable to broth microdilution and considered a viable screening method [10, 11].

Screening for Extended Spectrum Beta-Lactamase (ESBL) and AmpC is made as described in the EUCAST Guidelines for Detection of Resistance Mechanisms and Specific Resistance of Clinical and/or Epidemiologic Importance [12]. ESBL was detected using a double disk synergy test (DDST) using Aztreonam (ATM), Amoxicillin/Clavulanic Acid (AMC) 20/10 µg, and Cefepime (FEP) 30 µg with a distance of 20 mm from each other. AmpC was detected using resistance to Cefoxitin (FOX) 30 µg and Cefotaxime (CTX) 30 µg.

Reference or standard strains were used to authenticate and validate expected bacteriological assays. This include the use of ATCC 25922 *(E. coli*), ATCC 27853 (*Pseudomonas aeruginosa)* and ATCC 700603 *(K. pneumoniae).* All three ATCC strains were used as reference strains for biochemical reactions, ATCC 25922 and 27853 for *in vitro* susceptibility testing and ATCC 700603 for ESBL determination assay.

The Multiple Antibiotic Resistance Index (MARI), which is an effective, valid, and cost-effective method for tracking antibiotic resistance organisms, was also determined. This was calculated as the ratio between the number of antibiotics that an isolate is resistant to and the total number of antibiotics it was tested for [13]. A MARI greater than 0.2 indicates a high-risk source of contamination or that antibiotics are frequently used.

3. Results

A total of 82 (68.33%) coliforms (37 isolates from farm A and 45 isolates from farm B) were isolated from the collected samples after the modified landman technique. This comprised of 62 (51.67%) *Escherichia coli*, 18 (15%) *Klebsiella pneumoniae,* and 2 (1.67%) *Enterobacter cloacae* that were identified using colonial morphology on MacConkey and EMB and classical biochemical reactions. These isolates were noted to be highly resistant to chloramphenicol (85.37%), amoxicillin/clavulanic acid (74.39%), and colistin (69.51%). Conversely, these isolates were observed to have a low resistance rate to imipenem (17.07%) and amikacin (2.44%). A summary of antibiotic resistance is shown in Figure 1.

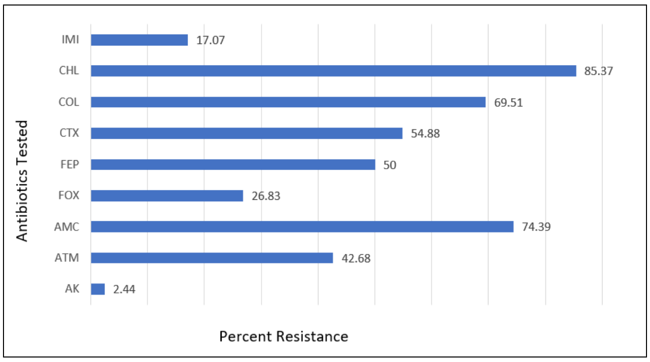

Fig 2: Antibiotic resistance of the isolated coliforms against selected antibiotics

In terms of colistin resistance (MIC90), *E. coli* showed the most frequent coliforms, with 62 (66.13%) out of 82 coliforms, while *K. pneumoniae* is found to be higher compared to *E. coli* with 77.78%.The distribution of colistin resistant coliforms is shown in Table 1.

Table 1: Distribution of Colistin-Resistant Coliforms

Bacterial Isolates	Total	Farm A		Farm B		Total	%
		Freq	%	Freq	%		
E. coli	62	12	21.05	29	50.88	41	71.93
K. pneumoniae	18	10	17.54	4	7.02	14	24.56
E. cloacae	2	0	0	2	3.51	2	3.51
Total	82	22	38.6	35	61.40	57	100

Also, 23 out of 82 (28.05%) isolates were identified to be ESBL producing coliforms and 2 out of 82 (2.44%) to be AmpC producing. The distribution of ESBL and AmpC among coliform isolates is shown in Table 2.

Table 2: Distribution of ESBL and AmpC among Isolated Coliforms

Bacterial Isolates	Frequency		
	Total	ESBL	AmpC
E. coli	62	21 (33.87%)	0 (0%)
K. pneumoniae	18	2 (11.11%)	1 (5.56%)
E. cloacae	2	0 (0%)	1 (50%)
Total	82	23 (28.05%)	2 (2.44%)

To measure the extent of antimicrobial resistance in the group isolates studied, MARI was determined, as it also serves as an important epidemiological tool that measures drug resistance. In this study, 51 (62.20%) isolates were noted to have a MARI of more than 0.2. A summary of isolates with individual MARI is presented in Table 3 below.

Table 3: Multi-Antibiotic Resistance Index of the Isolated Coliforms

MARI	*E. coli*	*K. pneumoniae*	*E. cloacae*	Total
1.0	0	0	0	**0**
0.9	2	0	0	**2**
0.8	3	2	0	**5**
0.7	3	1	0	**4**
0.6	15	2	0	**17**
0.5	0	0	0	**0**
0.4	7	3	1	**11**
0.3	9	2	1	**12**
0.2	13	7	0	**20**
0.1	8	1	0	**9**
0	2	0	0	**2**
Total	**62**	**18**	**2**	**82**

4. Discussions

This study provides data on the presence of not just multidrug-resistant coliforms but also of colistin-resistant coliforms and the presence of ESBL and AmpC-producing coliforms in poultry farms. In this study, *E. coli* predominates among the bacterial isolates present in the fecal samples collected. Though *E. coli* is considered to be a normal inhabitant of the gastrointestinal tract of mammals, poultry and other vertebrates; certain strains can also cause colibacillosis, which is an invasive infection among birds[14]. Therefore, *E. coli* can be isolated from both healthy and infected populations.

Colistin, also known as polymyxin E, is considered the last-resort drug to manage multi-drug resistant gram-negative bacteria. Its target is the lipopolysaccharide found in gram negative bacteria's outer cell membrane. There are also bacteria that are intrinsically resistant to colistin, like *Proteus mirabilis* and *Serratia marcescens,* while others acquire resistance through alteration of LPS, overproduction of efflux pumps, or capsule formation. Colistin is considered one of the clinically important antibiotics; however, it poses a significant global risk to public health owing to the excessive use of antibiotics together with bacterial evolution, as it has many mechanisms being employed to develop resistance as well as other stimulants in the environment [15].

Colistin resistance among poultry samples is not new, as there are previous studies that indicate the presence of colistin-resistant coliforms in poultry production, such as in China in 2015 [16], Malaysia in 2016 [17], Cambodia, and nearby countries in 2021 [18]. Findings in Lebanon have also shown an increased prevalence of AmpC and ESBL-producing bacteria in poultry farms [19]. As far as the Philippines is concerned, there are already a number of studies about colistin resistance on broilers or poultry farms. As noted in the papers, similar results of low to no colistin resistance were noted, such as in the study of Sarmiento *et al* [20] and Gundran *et al* [21]. The paper of Casamina *et al* [4] also describes the prevalence (less than 20%) of colistin-resistance in swine. In contrast to previous studies, this recent study showed colistin resistance as high as 69.51%.

Typically, when antibiotics are applied on a poultry farm, they tend to kill the susceptible isolates. However, isolates that possess special traits of resistance against antibiotics remain and survive. Thus, this leads to the transfer of resistance to other bacteria horizontally by plasmids. The high prevalence of multidrug resistant coliforms may be due to the use of antibiotics in compound feeds, as they have been an integral part of poultry production, not just to prevent infectious disease but to promote growth among livestock [14]. For example, some feeds may contain 2 to 10 grams per ton of colistin for broilers and poultry. This has then led to continuous exposure of the gut microbiome to low dosages, thus resulting in the acquisition of drug resistance. This condition is kind of worrisome as these resistant bacteria might be transferred to humans by chain, as poultry meat is largely consumed by humans or by workers via direct contact.

Overuse of antibiotics or exposure to low doses of antibiotics, especially from food sources or dietary supplements, could result in antibiotic residue that could lead to changes in the gut microbiota. In addition, the effects of these antibiotics can be cumulative and can be associated with health problems like obesity, carcinogenicity, reproductive effects, and teratogenicity [22].

MARI indicated that 51 (62.2%) out of 82 isolates were resistant to at least 3 classes of antibiotics and that they exceeded the 0.2 cut-off. This current study also showed a mean MARI of 0.39, indicating that it originated from a high-risk source of contamination where antibiotics are often used either to a great degree or in large amounts. Most isolates were resistant to chloramphenicol, amoxicillin/clavulanic acid, and colistin. The antibiotic resistance in this paper, however, is somewhat higher compared to other published studies mentioned in the paper by Nhung *et al* [23], Dawadi *et al* [14], and Islam *et al* [24]. On the contrary, these data are lower when compared to the study by Afunwa *et al* [25], in which MARI was noted to be higher than 0.5.

Poultry also generates large volumes of excretion, comprising solid waste and waste water. In many cases, manure can be used to provide a nutrient-rich source of fertilizer or livestock feed supplement. The application of manure may also result in the contamination of the environment with antimicrobial residues, antimicrobial-resistant bacteria, and resistant genes. This is because disposal of animal waste, such as manure, is considered another source of transmission of resistant pathogens from animals to the environment. As resistant bacteria and antibiotic residues accumulate in the soil, it also promotes the proliferation of antibiotic resistant commensal bacteria inhabiting the soil.

There are already initiatives to control antibiotic resistance, such as banning the use of colistin as a feed additive in poultry and broiler production. In 2021, certain countries have agreed to ban or have implemented a ban on colistin to be incorporated in the feeds of broilers and poultry. These include Argentina, Brazil, China, India, Japan, Malaysia, and Thailand [17] and several Latin American countries [24]. Nonetheless, there are countries that still use colistin as a feed additive, especially low- and middle-income countries.

Poultry in the Philippines is still considered one of the major sources of food for human consumption and contributes significantly to the country's economy. It continues to provide income and employment opportunities and meet domestic demands. Unfortunately, it also shows that poultry can be a source of MDR pathogens, as waste products can shed AMR bacteria into the environment. To gain a wider perspective regarding this issue, it would be appropriate to have a more comprehensive study or large-scale studies focusing on more resistance patterns to better map its epidemiology. In order to prevent this possibility, strict laws should be internationally and globally implemented to mitigate widespread resistance to antibiotics on the WHO list of Critically Important Antimicrobials for Human Medicine. Additionally, the frequency and status of antibacterial drug consumption must be assessed periodically in the study area to generate data to support evidence-based policies and interventions for AMR.

5. Conclusion

This study shows that fecal material from poultry farms carries multidrug resistant coliforms, in which E. coli is the most frequent. Drug resistant coliforms in poultry farms have been identified, and because of their resistance to clinically significant antibiotics, they are considered a matter of public health importance. This paper provides evidence of the high incidence of multi-drug resistant coliforms sampled in poultry farms, presenting a zoonotic risk to humans, especially via direct contact with broilers and consumption of these poultry-derived products. The presence of ESBL, ampC, and colistin resistance may also aggravate the situation, as these phenotypes make the pathogen more difficult to treat using the usual antibiotics.

6. Acknowledgment

The team would like to thank the help of Mr. Jericho Carmona, Ms Desiree Diel, Mr Paolo Hilado and Ms Gemma delos Reyes for making this study possible.

7. Authors' Contribution

Conceptualization and planning were made by ACJ, KAAP, EGV, CMA and JDZ. The specimen collection was made by MSB, MAB, BFMT, and ART. Experimentations that include bacterial isolation, identification, and susceptibility testing were performed by EGV, JDZ, MSB, MAB, BFMT. ART, KAU, and CV. ACJ and KAAP supervised the execution of the experiments. Data analysis was accomplished by EGV, JDZ, KAU, and ACJ. BFMT and ART. KAU, CV took care of the preparation of documents for Ethics and Biosafety Clearance. The first draft was prepared by ACJ, CMA and KAAP. All authors have read and agreed to the published version of the manuscript.

8. Declarations and Final Statement

The authors declare no conflict of interest or grant from any funding agency for this study. The datasets generated during and/or analyzed during the current study are available from the corresponding author upon reasonable request. No artificial intelligence (AI) tools or technologies were used in the conception of the study, writing, analysis, or editing of this paper. All work presented herein is the result of original human effort, and any external sources have been appropriately cited.

9. References

1. Ahmed F, Mahmud T, Naher S, Rana J, Ara R, Saif-Ur-Rahman KM. Effectiveness of Colistin in carbapenem resistant Acinetobacter baumannii - A systematic review. Health Science Review. 2023; 8.
2. Andrade F, Silva D, Rodrigues A, Pina-Vaz C. Colistin Update on Its Mechanism of Action and Resistance, Present and Future Challenges. Microorganisms. 2020; 8:11.
3. Kumar H, Chen BH, Kuca K, Nepovimova E, Kaushal A, Nagraik R, *et al*. Understanding of Colistin Usage in Food Animals and Available Detection Techniques: A Review. Animals. 2020; 10:10.
4. Casamina NV, Victoriano-Belvis AF. Detection of Colistin Resistant Enterobacteriaceae among Backyard Farm Swine and Swine Raisers in Tanay, Rizal Swine Farm. Philippine Journal of Science. 2023; 153:911-914.
5. Barroga TR, Morales R, Benigno C, Castro SJ, Caniban M, Cabullo MF, *et al*. Antimicrobials Used in Backyard and Commercial Poultry and Swine Farms in the Philippines: A Qualitative Pilot Study. Frontiers in Veterinary Science. 2020; 7:329.
6. Badr H, Samir A, El-Tokhi EI, Shahein M, Rady F, Hakim A, *et al*. Phenotypic and Genotypic Screening of Colistin Resistance Associated with Emerging Pathogenic Escherichia coli Isolated from Poultry. Veterinary Sciences. 2022; 9:6.
7. World Health Organization. Manual for the Laboratory Identification and Antimicrobial Susceptibility Testing of Bacterial Pathogens of Public Health Importance in the Developing World. Switzerland: WHO, 2003. Retrieved July 02, 2024, from: https://www.who.int/publications/i/item/WHO-CDS-CSR-RMD-2003.6.
8. Bergey DH. Bergey's Manual of Determinative Bacteriology. Lippincott Williams and Wilkins, 1994.
9. CLSI. CLSI M100 Performance Standards for Antimicrobial Susceptibility Testing (34th ed.). Wayne, PA, USA: CLSI, 2024.
10. Chauhan S, Kaur N, Saini A, Chauhan J, Kumar H. Assessment of Colistine Resistance in Gram Negative Bacteria from Clinical Samples in Resource-Limited Settings. Asian Pacific Journal of Tropical Medicine. 2022; 15(8):367-373.
11. Sanda ZS, Sanda S, Ulker KH. Comparison of Modified

Colistin Broth Disk Elution, Automated System, and Broth Microdilution Results in Determining Colistin Susceptibility in Multidrug-Resistant Gram Negative Bacteria. Acta Microbiologica Bulgaria. 2025; 41(3):334-340.
12. EUCAST. Guidelines for Detection of Resistance Mechanisms and Specific Resistance of Clinical and/or Epidemiologic Importance, 2017.
13. Ayandele AA, Oladipo EK, Oyebisi O, Kaka MO. Prevalence of Multi-Antibiotic Resistant *Escherichia coli* and *Klebsiella species* obtained from a Tertiary Medical Institution in Oyo State, Nigeria Qatar Med J. 2020; 1:9.
14. Dawadi P, Bista S, Bista S. Prevalence of Colistin-Resistant Escherichia coli from Poultry in South Asian Developing Countries. Veterinary Medicine International, 2021.
15. Mondal AF, Khare K, Saxena P, Debnath P, Mukhopadhyay K, Yadav D. A Review on Colistin Resistance: An Antibiotic of Last Resort. Microorganisms. 2024; 12:4.
16. Danaei B, Sarmastzadeh T, Khalili F. The battle against colistin-resistant E. coli and the need for a one health approach. New Microbes New Infection. 2023; 54.
17. Yu CY, Ang GY, Chin PS, Ngeow YF, Yin WF, Chan KG. Emergence of mcr-1-mediated colistin resistance in Escherichia coli in Malaysia. International Journal of Antimicrbial Agents. 2016; 47:504-505.
18. Pungpian C, Lee S, Trongjit S, Sinwat N, Angkititrakul S, Prathan R, *et al*. Colistin resistance and plasmid-mediated mcr genes in *Escherichia coli* and Salmonella isolated from pigs, pig carcass and pork in Thailand, Lao PDR and Cambodia border provinces. Journal of Veterinary Science. 2021; 22:5.
19. Olaitan AO, Dandachi I, Baron SA, Daoud Z, Morand S, Rolain JM. Banning colistin in feed additives: A small step in the right direction. The Lancet Infectious Diseases. 2021; 21:29-30.
20. Sarmiento AN, Micu R, Dayrit G. Presence of Extended Spectrum Beta-lactamase-producing Escherichia coli from Meat Handler and Poultry Cecal Samples. Philippine Journal of Science. 2023; 153:323-331.
21. Gundran R, Cardenio P, Villanueva M, Sison F, Benigno C, Kreausukon K, *et al*. Prevalence and distribution of blaCTX-M, blaSHV, blaTEM genes in extended- spectrum β- lactamase- producing E. coli isolates from broiler farms in the Philippines. BMC Veterinary Research. 2019; 15:227.
22. Bacanli MG. The two faces of antibiotics: An overview of the effects of antibiotic residues in foodstuffs. Archives of Toxicology. 2024; 98:1717-1725.
23. Nhung NT, Chansiripornchai N, CarriqueMas J. Antimicrobial Resistance in Bacterial Poultry Pathogens: A Review. Frontiers of Veterinary Science. 2017; 4:126.
24. Islam S, Urmi UL, Rana M, Sultana F, Jahan N, Hossain B, *et al*. High abundance of the colistin resistance gene mcr-1 in chicken gut-bacteria in Bangladesh. Scientific Reports. 2020; 10.
25. Afunwa RA, Ezeanyinka J, Afunwa EC, Udeh AS, Oli AN, Unachukwu M. Multiple Antibiotic Resistant Index of Gram-Negative Bacteria from Bird Droppings in Two Commercial Poultries in Enugu, Nigeria. Open Journal of Medical Microbiology. 2020; 10:4.
26. Umair MU, Hassan B, Farzana R, Ali Q, Sands K, Mathias J. International manufacturing and trade in colistin, its implications in colistin resistance and One Health global policies: A microbiological, economic, and anthropological study. Lancet Microbe. 2023; 4:4.