

The Prevalence of Multidrug-Resistant Bacteria Detected in Poultry Products in Mandya, India

Jeet Bahadur Moktan¹, Rajesh Venkataraman¹, Yogendra Shrestha^{1*}

¹Department of Pharmacy Practice, Sri Adichunchanagiri College of Pharmacy, Adichunchanagiri University, BG Nagara, Karnataka, India.

Abstract

Antimicrobials blended at a subtherapeutic level to improve feed efficiency and growth are becoming more popular. Antibiotic use in food-producing animals results in the deposition of drug residues in meat and eggs. Evidence of infection by environmental and agricultural organisms is emerging. As Southern India makes a significant contribution to the poultry product of India, we intend to identify possible organisms in poultry products, as well as their antibiotic resistance patterns, in Mandya, Karnataka, India. The sample from the freshly sacrificed chicken was taken from each of the seven taluks in the Mandya district. Gram staining followed by a biochemical test was performed to isolate the organisms. The antibiotic susceptibility test (AST) was performed by a modified Kirby Bauer disk diffusion method and AST was interpreted using the Clinical Laboratory Standard Guideline (CLSG) 2020. A total of 105 samples were collected from seven taluks in the Mandya district. Of these, 94.29% (99/105) of samples were cultured positive for bacterial growth. At least 71.43% of each poultry product was culture positive for bacteria. The predominance of nine different organisms was observed. Almost all isolated organisms were resistant to lincomycin (95.96%), bacitracin (88.89%), and erythromycin (82.83%). Organisms developed minimal resistance to gentamicin (3.03%) and ciprofloxacin (16.16%). The study concluded that the poultry products were contaminated with *Staphylococcus aureus*, *Klebsiella oxytoca*, *Proteus mirabilis*, *Escherichia coli*, *Citrobacter freundii*, *Citrobacter koseri*, *Salmonella spp.*, *Morganella morgani*, and NFGNB. All of these organisms have developed multi-drug resistance.

Keywords: Microorganism, Antimicrobial resistance, Multidrug-resistant organisms, Poultry products

INTRODUCTION

Antimicrobial resistance (AMR) has been associated with higher mortality, lengthy hospital stays, delayed recovery, and long-term disability [1]. Every year, it kills at least 70 million people [2]. At least half of hospital prescriptions involve inappropriate antimicrobial use [1]. Shrestha *et al.* reported antibiotic overuse, with isolated bacteria resistant to half of the antibiotics [3]. Misuse, self-medication, and irrational antimicrobial use are thought to be the root causes of drug-resistant pathogens. To address this issue, policymakers are focusing on reducing antibiotic use by spreading infection control knowledge and adopting and implementing hospital infection control practices, as well as forming active hospital infection control teams in each hospital to monitor and contain the spread of infections [1]. However, COVID-19's desperate situation had affected the implementation of guidelines and had led to the overuse of antibiotics [3, 4]. On the other hand, evidence of infection by commensal environmental and agricultural organisms is emerging [5].

Antimicrobials blended at a subtherapeutic level to improve feed efficiency and growth are becoming more popular [6, 7]. The poultry and fish industries consume the maximum feed. It is reported that 46 different antibiotic residuals were found in food from animals' origins. Some of them were commonly

prescribed antibiotics in humans, such as amikacin, amoxicillin, cephalixin, chloramphenicol, ciprofloxacin, clarithromycin, doxycycline, gentamycin, neomycin, norfloxacin, sulfamethoxazole, tetracycline, tobramycin, and trimethoprim were detected [8]. Antibiotic use in food-producing animals results in the deposition of drug residues in milk, meat, and eggs [9-11]. The residues then cause the development of antimicrobial-resistant bacteria in animals, which are then released into the environment and on various animal-derived food items [12, 13]. Nelson *et al.* reported *non-typhoidal Salmonella serovars* in 50% of the poultry samples, with 97% of them resistant to tetracycline [14]. Neogi *et al.* observed *Campylobacter spp.* in 32% of poultry

Address for correspondence: Yogendra Shrestha, Department of Pharmacy Practice, Sri Adichunchanagiri College of Pharmacy, Adichunchanagiri University, BG Nagara, Karnataka, India. dryogendra@ gmail.com

This is an open-access article distributed under the terms of the Creative Commons Attribution-Non Commercial-Share Alike 4.0 License, which allows others to remix, tweak, and build upon the work non commercially, as long as the author is credited and the new creations are licensed under the identical terms.

How to cite this article: Moktan JB, Venkataraman R, Shrestha Y. The Prevalence of Multidrug-Resistant Bacteria Detected in Poultry Products in Mandya, India. Arch Pharm Pract. 2023;14(1):35-9. <https://doi.org/10.51847/UWgyw11wYg>

samples with Multi-Drug Resistant (MDR), i.e., tetracycline, amoxicillin, streptomycin, fluoroquinolones, and macrolides [15, 16].

India is the world's third-largest producer of eggs and the seventh-largest producer of chicken meat. Southern India makes a significant contribution to the poultry product of India [17]. It is important to know the possible organism present in poultry products and their resistance to the antibiotic, which could help in the design of an effective AMR prevention program. In this study, we intend to identify possible organisms in poultry products, as well as their antibiotic resistance patterns, in Mandya, Karnataka, India.

MATERIALS AND METHODS

Sampling Site

The study was conducted in the Mandya district of Karnataka, in southern India, between April and August 2022. The sample was taken from each of the seven taluks in the Mandya district: Krishnarajpet, Nagamangala, Maddur, Malavalli, Mandya, Pandavapura, and Shirangapattana. The ethical approval was obtained from the Adichunchanagiri Institute of Medical Sciences institutional ethical committee (*Clearance No. AIMS/IEC/1875A/2020*). The samples were collected with the permission of the district health officer (Supplementary 1).

Sample Collection

After obtaining oral consent from the shop owner, the sample was collected from the chicken shop. 20 g of fresh litter from a bird cage, 20 g of the intestine, 50 g of liver, 50 g of meat, and 50 g of gizzard from a freshly sacrificed chicken. Every sample was taken in a sterile container. All sample containers were placed in ice-filled thermocol boxes and transported to the laboratory within 6 hours. GPS tracker smartphone applications were used to record the location. To reduce sampling errors, samples were taken from three different locations in each taluk.

Microbiological Procedure

The samples were pre-enriched in 225ml of 1% buffered peptone water (HI MEDIA®) in a sterile stomacher bag, shaken, and incubated (OPTECH®) at 35 °C for 24 hours. Samples were carefully enriched by transferring 100 µl of pre-enriched content into 9.9 ml of Rappaport Vassiliadis Soyabean Meal Borth (RVSM) and incubating them at 37 °C for 24 hours. The loopful of RVSM content was then subcultured on Xylose Lysine Deoxycholate agar (XLD Agar) for 24 hours at 37°C. On the inoculated XLD agar plates, the Gram stain was performed. To isolate the organism, biochemical tests where indole, citrate utilization, triple sugar iron, peptone water, and fermentation tests were performed. After that, the antibiotic susceptibility test (AST) was performed by modified Kirby Bauer disk diffusion method for erythromycin 15 mcg (E), gentamicin 10 mcg (GEN), ciprofloxacin 5 mcg (CIP), nalidixic acid 30 mcg (NA), chloramphenicol 30 mcg (C), cefalexin 30 mcg (CN), amoxicillin 10 mcg (AMX), lincomycin 2mcg (L), sulphadiazine 100 mcg (SZ), cefixime 5 mcg (CFM), doxycycline 30 mcg (DO), and bacitracin 10 IU (B). The AST was interpreted using the Clinical Laboratory Standard Guideline (CLSG) 2020.

RESULTS AND DISCUSSION

Prevalence of Bacteria in the Poultry Products

A total of 105 samples were collected from seven taluks in the Mandya district (*fresh litter, intestine, liver, meat, and gizzard; each with 21 samples*). Of these, 94.29% (99/105) of samples were cultured positive for bacterial growth. The predominance of nine different organisms was observed. *Staphylococcus aureus* was isolated from 57.57% (57/99) of the total growth, followed by *Klebsiella oxytoca* 9.09% (9/99), *Proteus mirabilis* 9.09% (9/99), *Escherichia coli* 6.06% (6/99), *Citrobacter freundii* 6.06% (6/99), *Citrobacter koseri* 3.03% (3/99), *Salmonella spp.* 3.03% (3/99), *Morganella morgani* 3.03% (3/99), and non-fermenting gram-negative bacilli (NFGNB) 3.03% (3/99) in that order. At least 71.43% of each poultry product was culture positive for bacteria (**Table 1**).

Table 1. Isolation of organisms from different poultry samples

Organisms	N	Liver	Intestine	Gizzard	Meat	Litter
<i>Citrobacter freundii</i>	6	0	6 (6.06)	0	0	0
<i>Staphylococcus aureus</i>	57	15.79% (9/57)	10.53% (6/57)	21.05% (12/57)	21.05% (12/57)	31.58% (18/57)
<i>Citrobacter koseri</i>	3	0	100% (3/3)	0	0	0
<i>Proteus mirabilis</i>	9	33.33% (3/9)	33.33% (3/9)	33.33% (3/9)	0	0
<i>Salmonella spp.</i>	3	0	0	0	0	100% (3/3)
<i>Klebsiella oxytoca</i>	9	0	0	33.33% (3/9)	66.67% (6/9)	0
<i>Escherichia coli</i>	6	0	100% (6/6)	0	0	0
<i>Morganella morgani</i>	3	100% (3/3)	0	0	0	0
NFGNB	3	0	0	0	100% (3/3)	0
Total	99	15.15% (15/99)	24.24% (24/99)	18.18% (18/99)	21.21% (21/99)	21.21% (21/99)

Antibiotic Resistance Profile

The antimicrobial susceptibility test results for 99 pathogens isolated from poultry samples are shown in **Table 2**. Almost all isolated organisms were resistant to lincomycin (95.96%), bacitracin (88.89%), and erythromycin (82.83%). Isolated organisms developed at least 50% of resistance to cefalexin

(50.5%) and sulphadiazine (69.69%). Whereas isolated organisms developed resistance between 20% to 47% to amoxicillin (38.38%), cefixime (28.28%), chloramphenicol (20.20%), nalidixic acid (46.46%), and doxycycline (39.39%). Organisms developed minimal resistance to gentamicin (3.03%) and ciprofloxacin (16.16%) (**Table 2**).

Table 2. Antibiotic resistance in the isolated organisms

Organisms	N	Erythromycin	Gentamicin	Ciprofloxacin	Nalidixic acid	Chloramphenicol	Cefalexin	Amoxicillin	Lincomycin	Sulphadiazine	Cefixime	Doxycycline	Bacitracin
<i>Citrobacter freundii</i>	6	6 (100%)	0	1 (16.67%)	3 (50%)	3 (50%)	0	6 (100%)	6 (100%)	6 (100%)	2 (33.33%)	3 (50%)	6 (100%)
<i>Citrobacter koseri</i>	3	0	0	1 (33.33%)	3 (100%)	1 (33.33%)	0	1 (33.33%)	3 (100%)	3 (100%)	1 (33.33%)	3 (100%)	3 (100%)
<i>Proteus mirabilis</i>	9	7 (77.78%)	3 (33.33%)	3 (33.33%)	6 (66.67%)	4 (44.44%)	8 (88.89%)	6 (66.67%)	4 (44.44%)	9 (100%)	2 (22.22%)	6 (66.67%)	7 (77.78%)
<i>Salmonella spp.</i>	3	1 (33.33%)	0	1 (33.33%)	3 (100%)	1 (33.33%)	0	1 (33.33%)	3 (100%)	3 (100%)	0	3 (100%)	3 (100%)
<i>Klebsiella oxytoca</i>	9	8 (88.89%)	0	2 (22.22%)	5 (55.56%)	0	4 (44.44%)	7 (77.78%)	9 (100%)	8 (88.89%)	2 (22.22%)	3 (33.33%)	9 (100%)
<i>Escherichia coli</i>	6	6 (100%)	0	1 (16.67%)	2 (33.33%)	1 (16.67%)	4 (66.67%)	3 (50%)	6 (100%)	4 (66.67%)	0	0	6 (100%)
<i>Morganella morgani</i>	3	3 (100%)	0	1 (33.33%)	1 (33.33%)	1 (33.33%)	2 (66.67%)	2 (66.67%)	3 (100%)	3 (100%)	0	0	3 (100%)
<i>Staphylococcus aureus</i>	57	48 (84.21%)	0	6 (10.53%)	21 (36.84%)	9 (15.79%)	30 (52.63%)	9 (15.79%)	57 (100%)	30 (52.63%)	21 (36.84%)	21 (36.84%)	48 (84.21%)
NFGNB	3	3 (100%)	0	0	2 (66.67%)	0	2 (66.67%)	3 (100%)	3 (100%)	3 (100%)	0	0	3 (100%)

Total	99	82 (82.83%)	3 (3.03%)	16 (16.16%)	46 (46.46%)	20 (20.20%)	50 (50.5%)	38 (38.38%)	95 (95.96%)	69 (69.69%)	28 (28.28%)	39 (39.39%)	88 (88.89%)
-------	----	-------------	-----------	-------------	-------------	-------------	------------	-------------	-------------	-------------	-------------	-------------	-------------

This is the first study, to our knowledge, that has collected a range of poultry product samples and used multiple antibiotics in southern India intending to identify the possible organisms and assess AMR in them. The study revealed the prevalence of *Staphylococcus aureus*, *Klebsiella oxytoca*, *Proteus mirabilis*, *Escherichia coli*, *Citrobacter freundii*, *Citrobacter koseri*, *Salmonella spp.*, *Morganella morgani*, and NFGNB. We observed higher levels of *Staphylococcus aureus* contamination in the poultry products. Intestines were contaminated with the most different organisms (5/9), followed by meat, liver, and gizzard (each with 3/9), and litter (2/9). A high prevalence of *Staphylococcus aureus* was detected in the litter, *Klebsiella oxytoca* in meat, *Proteus mirabilis* equally in meat, liver, and intestine, *Escherichia coli*, *Citrobacter koseri*, and *Citrobacter freundii* in the intestine, *Salmonella spp.* in the litter, *Morganella morgani* in the liver, and NFGNB in the meat (**Table 1**). The study revealed a high number of different organisms contamination in poultry products than previously observed in poultry products [18-20]. There is a high possibility that harmful organisms will spread from poultry flocks, which have been shown to be common reservoirs, to humans through the food chain [21]. Inspections by regulatory agencies must be done promptly to prevent the spread of contaminated organisms from poultry products to humans.

The complete resistance of isolated organisms to at least one antibiotic has been observed. Almost all isolated organisms developed complete resistance to lincomycin (8/9), bacitracin (7/9), and sulphadiazine (6/9). Five different antibiotics were completely resistant to *Citrobacter freundii*, *Citrobacter koseri*, *Salmonella spp.*, and NFGNB. *Morganella morgani* had complete resistance to four antibiotics, *Escherichia coli* had complete resistance to three antibiotics, and *Proteus mirabilis* and *Staphylococcus aureus* had complete resistance to two antibiotics each. *Citrobacter freundii* was resistant to erythromycin, lincomycin, amoxicillin, and sulfadiazine. *Citrobacter koseri* had developed complete resistance to nalidixic acid, lincomycin, sulphadiazine, doxycycline, and bacitracin. *Proteus mirabilis* was completely resistant to sulphadiazine. Resistance to nalidixic acid, lincomycin, bacitracin, doxycycline, and sulphadiazine has completely evolved in *Salmonella spp.* Against *Klebsiella oxytoca*, bacitracin and lincomycin lost all of their effectiveness. *Escherichia coli* displayed total resistance to erythromycin, lincomycin, and bacitracin. *Morganella morgani* for the drugs erythromycin, lincomycin, sulphadiazine, and bacitracin, total resistance was seen. *Staphylococcus aureus* became completely resistant to lincomycin. It was revealed that NFGNB was completely resistant to erythromycin, amoxicillin, lincomycin, cefixime, and bacitracin (**Table 2**). The rise of multidrug-resistant pathogens could be the result

of improper maintenance of the use of antibiotics among food animals [14, 22]. Studies from various countries have revealed the existence of antibiotic residues in poultry products [8-11]. As the antibiotic used in food animals for prevention, and treatment of infection as well as growth promoters. However, antibiotic non-therapeutic use has been particularly widespread in poultry production [18]. The use of low doses of antibiotics to increase production contributes to the emergence of resistant poultry-contaminated organisms. The consumption of antibiotic residues contained in poultry products contributes to the emergence of resistant bacteria in humans. The strict enforcement of laws governing the use of antibiotics on animals used for human consumption is crucial to preventing antibiotic resistance.

The study's limitation is the lack of molecular testing of the genes that indicate antimicrobial resistance.

CONCLUSION

The study concluded that the poultry products were contaminated with *Staphylococcus aureus*, *Klebsiella oxytoca*, *Proteus mirabilis*, *Escherichia coli*, *Citrobacter freundii*, *Citrobacter koseri*, *Salmonella spp.*, *Morganella morgani*, and NFGNB. All of these organisms have developed multi-drug resistance. A large sample-size study with periodic follow-ups is needed to corroborate the findings.

ACKNOWLEDGMENTS: We thank Dr. B Ramesh (Dean & Principal, Sri Adichunchanagiri College of Pharmacy) and Dr. M. G. Shivaramu (Principal, Adichunchanagiri Institute of Medical Sciences) for providing a favourable environment to conduct the study.

CONFLICT OF INTEREST: None

FINANCIAL SUPPORT: This study was partially supported by the Sri Adichunchanagiri College of Pharmacy, BG Nagara.

ETHICS STATEMENT: None

REFERENCES

- Murray CJL, Ikuta KS, Sharara F, Swetschinski L, Aguilar GR, Gray A, et al. Articles Global burden of bacterial antimicrobial resistance in 2019: a systematic analysis. *Lancet*. 2022;399(10325):629-55.
- O'Neill J. Tackling drug-resistant infections globally: final report and recommendations. 2016 [cited 2022 Sept 30]. Available from: https://amr-review.org/sites/default/files/160525_Final_paper_with_cover.pdf
- Shrestha Y, Shivalingegowda RK, Avinash MJ, Kenchegowda SB, Moktan JB, Doddasamiah SM, et al. The rise in antimicrobial resistance: An obscure issue in COVID-19 treatment. *PLOS Glob Public Health*. 2022;2(7):e0000641. doi:10.1371/journal.pgph.0000641
- Shrestha Y, Venkataraman R, Moktan JB, Mallikarjuna S, Narayan SS, Madappa MH, et al. The Association of Medication Complexity with

- COVID-19 Severity and its Impact on Pharmacotherapy Evaluation. *J Young Pharm.* 2022;14(3):323-6.
5. Jadhav SS, Kumar JG, Tripathi P, Matani A, Ganesan SK, Panchal AC, et al. Febrile neutropenia: An unusual cause of Pantoea agglomerans bacteremia in acute myeloid leukemia. *J Precis Oncol.* 2022;2(1):49.
 6. Sarmah AK, Meyer MT, Boxall AB. A global perspective on the use, sales, exposure pathways, occurrence, fate, and effects of veterinary antibiotics (VAs) in the environment. *Chemosphere.* 2006;65(5):725-59.
 7. Marshall BM, Levy SB. Food animals and antimicrobials: impacts on human health. *Clin Microbiol Rev.* 2011;24(4):718-33.
 8. Treiber FM, Beranek-Knauer H. Antimicrobial Residues in Food from Animal Origin-A Review of the Literature Focusing on Products Collected in Stores and Markets Worldwide. *Antibiotics (Basel).* 2021;10(5):534. doi:10.3390/antibiotics10050534
 9. Chowdhury S, Hassan MM, Alam M, Sattar S, Bari MS, Saifuddin AK, et al. Antibiotic residues in milk and eggs of commercial and local farms at Chittagong, Bangladesh. *Vet World.* 2015;8(4):467-71.
 10. Sattar S, Hassan MM, Islam SK, Alam M, Al Faruk MS, Chowdhury S, et al. Antibiotic residues in broiler and layer meat in Chittagong district of Bangladesh. *Vet World.* 2014;7(9):738-43.
 11. Hassan MM, El Zowalaty ME, Lundkvist Å, Järhult JD, Khan Nayem MR, Tanzin AZ. Residual antimicrobial agents in food originating from animals. *Trends Food Sci Technol.* 2021;111:141-50.
 12. Landers TF, Cohen B, Wittum TE, Larson EL. A review of antibiotic use in food animals: perspective, policy, and potential. *Publ Health Rep.* 2012;127(1):4-22.
 13. Rahman MS, Hassan MM, Chowdhury S. Determination of antibiotic residues in milk and assessment of human health risk in Bangladesh. *Heliyon.* 2021;7(8):07739. doi:10.1016/j.heliyon.2021.e07739
 14. Nelson A, Manandhar S, Ruzante J, Gywali A, Dhakal B, Dulal S, et al. Antimicrobial drug-resistant non-typhoidal *Salmonella enterica* in the commercial poultry value chain in Chitwan, Nepal. *One Health Outlook.* 2020;2(1):1-18. doi:10.1186/s42522-020-00025-4
 15. Neogi SB, Islam MM, Islam SKS, Akhter AHMT, Sikder MMH, Yamasaki S, et al. Risk of multi-drug resistant *Campylobacter* spp. and residual antimicrobials at poultry farms and live bird markets in Bangladesh. *BMC Infect Dis.* 2020;20(1):278. doi:10.1186/s12879-020-05006-6
 16. Salih SM, Jafar NA, Noomi BS. Prevalence of mycoplasma infection in poultry (*Gallus gallus domesticus*) and evaluation of some diagnostic techniques. *J Adv Pharm Edu Res.* 2020;10(1):191-5.
 17. Government of India. Department of Animal Husbandry, Dairy, and Fisheries. National Action Plan for Egg & Poultry-2022 for Doubling Farmers' Income by 2022. 2022 [cited 2022 September 30]. Available from: <https://www.dahd.nic.in/sites/default/files/Seeking%20Comments%20on%20National%20Action%20Plan-%20Poultry-2022%20by%2012-12-2017.pdf>
 18. Bhushan C, Khurana A, Sinha R, Nagaraju M. Antibiotic resistance in poultry environment: Spread of resistance from poultry farm to agricultural field. Centre for Science and Environment, New Delhi. 2017. [cited on 25 November 2022]. Available from: <http://www.cseindia.org/userfiles/Antibioticspercent20npercent20Chickenpercent2030percent20july.pdf>
 19. Schwaiger K, Schmied EM, Bauer J. Comparative analysis on antibiotic resistance characteristics of *Listeria* spp. and *Enterococcus* spp. isolated from laying hens and eggs in conventional and organic keeping systems in Bavaria, Germany. *Zoonoses Public Health.* 2010;57(3):171-80. doi:10.1111/j.1863-2378.2008.01229.x
 20. Costa M, Cardo M, Cara d'Anjo M, Leite A. Assessing antimicrobial resistance occurrence in the Portuguese food system: Poultry, pigs and derived food, 2014-2018. *Zoonoses Public Health.* 2022;69(4):312-24. doi:10.1111/zph.12920
 21. Saravanan S, Purushothaman V, Murthy TR, Sukumar K, Srinivasan P, Gowthaman V, et al. Molecular Epidemiology of Nontyphoidal *Salmonella* in Poultry and Poultry Products in India: Implications for Human Health. *Indian J Microbiol.* 2015;55(3):319-26. doi:10.1007/s12088-015-0530-z
 22. Food safety and standard authority of India. Food safety and standard (contaminants, toxins, and residues) regulations. 2011. [Internet]. 2011 [cited 25 November 2022]. Available from: https://www.fssai.gov.in/upload/uploadfiles/files/Compendium_Contaminants_Regulations_20_08_2020.pdf