SINDH UNIVERSITY RESEARCH JOURNAL (SCIENCE SERIES)
ISSN (Print) 1813-1743, ISSN (online) 2791-0547
Vol. 55:01 (2023).
Doi: https://doi.org/10.26692/surj-ss.v55i01.4769

Prevalence and Antimicrobial Resistance of E.coli and Salmonella isolated from Poultry litter in District Shaheed Benazir Abad

PARVEEN LAGHARI[1], DILDAR HUSSAIN KALHORO[1*], HASINA BALOCH[1], RAZA NIZAMANI[2], ABDUL AHAD SOOMRO[2]

[1]Faculty of Animal Husbandry and Veterinary Sciences, Sindh Agriculture University, Tandojam-70050, Pakistan
[2]Central Veterinary Diagnostic Laboratory, Tandojam, Directorate of Veterinary Research and Diagnosis, Government of Sindh, Pakistan

Cite this:
Parveen L., DH Kalhoro, H. Baloch, R. Nizamani, AA Soomro (2023). Prevalence and antimicrobial resistance of E.Coli and Salmonella isolated from Poultry litter in District Shaheed Benazir Abad. Sindh Uni. Res.J. (SS)

Corresponding author
drdildarkalhoro@gmail.com

Abstract

Poultry industry provides rich and cheap resources of protein requirements to the people in the form of chicken meat and eggs production. A total of 100 poultry litter samples were collected from different poultry farms of district Shaheed Benazir Abad. Collected samples were transported to Central Veterinary Diagnostic Laboratory Tandojam for isolation and identification of microorganisms from different areas including, Sakrand, Nawab shah, Qazi Ahmed and Dour of District Shaheed Benazir Abad. Overall results showed that (84%) samples were found positive for *E. coli* and *Salmonella* species. Higher prevalence of *E.coli* 17(68%) was observed in Sakrand, while lower 12(48%) was observed in Dour . High prevalence of *Salmonella* 9(36%) was observed in Nawab shah, while lower 5(20%) was observed in dour. The results of antimicrobial sensitivity indicated that *E.coli* species were resistant to Tetracycline, Ciprofloxacin, Sulphafurazole and Oxacillin. However, *E. coli* species were sensitive to Erythromycin, Amikacin and Gentamycin. The isolated *Salmonella* species have shown resistance to Tetracycline, Ciprofloxacin and Sulphafurazole. While, *Salmonella* species were highly sensitive against the Amoxicillin, Erythromycin and Gentamycin. In conclusion, higher prevalence of *E. coli* was observed in Sakrand, while higher prevalence of *Salmonella* was observed in Nawab shah and lower prevalence of *E.coli* and *Salmonella* was observed in Dour. Whereas, the Tetracycline, Ciprofloxacin, Sulphafurazole were highly resistant to *E.coli* and *Salmonella*.

Keywords: E.Coli, Antimicrobial resistance, Poultry litter, Salmonella.

INTRODUCTION

Poultry plays a vital role in producing animal derived protein for human food from the meat and egg sources. Poultry production markets differ around the world based on geographical regions, demand, supply, and consumption, which is increasing all over the world with the population, especially in Asian countries as compared to the rest of the world (Belova *et al*. 2012; Mujahid *et al*., 2019). China, the United States, Brazil, Russia, Mexico, India, and Pakistan have recently become the top producers of poultry (Putri *et al*., 2018). Many diseases affect poultry birds, including bacterial infections (Colibacillosis and Pullorum disease), viral infections (New Castle disease and Avian influenza), and parasitic infections (Coccidiosis and Heterakios Gallinarum), which results in significant losses to the poultry industry (Schiavone *et al*., 2020; Umar *et al*., 2019).

The disease outbreak, mostly occurs due to poor management, the floor of poultry farm is the one an important disease outbreak source that is always covered with bedding material called poultry litter that include rice husk or wood straw. poultry litter may contain a large waste material include poultry feathers, urine, feces and spoiled poultry feed (Jayathilakan *et al*., 2012). There are many pathogenic organisms in the poultry litter, but the most common pathogen present in poultry farms are *E. coli* and *Salmonella* causing severe losses due to high morbidity and mortality (Alali *et al*., 2010).

Antimicrobial drugs are widely used to inhibit the growth of microorganisms in intensive farming management systems for therapeutic or prophylactic uses (Jajere *et al.*, 2019). Antibiotic resistance is very common in poultry birds and common antibiotics are ineffective against previously sensitive bacteria (Capita and Alonso-Calleja *et al.*, 2013). Monitoring of bacterial resistance to antimicrobial drugs is essential in poultry for human consumption and the spread of antimicrobial resistance bacteria present in food-producing animals (Collignon *et al.*, 2016). The World Organization for Animal Health has declared a list of antibiotics resistant such as Quinolones, Cephalosporin and Macrolides groups that were previously effective against bacterial pathogens especially *E. coli* and *Salmonella* spp. (Davies and Wales, 2019; Sellah and Drissi, *et al*., 2015). World health organization recommends reduced use of antimicrobial drugs in food-producing animal to decrease the adaptation of antibiotics resistance by bacteria (Belanger *et al*., 2011). Therefore, the present study was designed to know Prevalence and antimicrobial resistance of E.*coli* and *salmonella* isolated from poultry litter in district shaheed Benazir Abad.

MATERIALS AND METHODS

The study was conducted at Central veterinary diagnostic Laboratory (CVDL) Tandojam to know the prevalence and antimicrobial resistance of E. coli and Salmonella from poultry litter.

Sampling

A total of one hundred litter samples were collected from poultry farms four talukas i.e., Taluka Sakrand, Qazi Ahmed, Nawab Shah, and Dour of District Shaheed Benazir Abad. The 25 samples of litter from different farms of each taluka were taken in sterile polythene bags using standard methods. The litter samples were brought to laboratory for further examination.

Bacteriological identification tests

To isolate and identify the pathogens on different media a number of biochemical tests were performed. Isolation and identification of salmonella was done by the standard method described in ISO 6579 (2002) and 6579-1 (2017). The isolation and identification of E. coli was done by the method described by Lie et al., (2008).

Antimicrobial sensitivity and resistance Test

The antimicrobial resistance to *E. coli* and *Salmonella* was determined by an agar disk diffusion assay. Guidelines from the clinical and Laboratory Standards Institute (CLSI) for disk diffusion methods that was used to determine antimicrobial resistance of antibiotics (Humphries, *et al*., 2018). 3.8 gram of Muller Hinton agar measured and 100 ml double distilled water was prepared and autoclaved at 121^0C temperature for 15 minutes at 15 lb pressure. After cooling the media was poured in sterile Petri dish to solidify. The Bacteria were inoculated on each of the plates with the sterile cotton swab. The Antibiotic discs of 14 antibiotics i.e., Oxacillin, Streptomycin, Polymyxin-B, Amoxicillin, Nalidixic acid, Amikacin, Ciprofloxacin, Sulphafurazole, Doxycycline, Sulfamethoxazole, Gentamycin, Tetracycline, Erythromycin, and Cefixime were placed with the help of a disc dispenser on the surface of agar plate and slightly pressed with a sterile forceps to make it fixed on the surface of medium. The Petri dishes were incubated at 37^0C temperature for 24 hours. After overnight incubation, the size of inhibition zone was recorded.

RESULTS

A total of 100 poultry litter samples were collected from different poultry farms of district Shaheed Benazir Abad. Among 100 litter samples, (84%) samples were positive for *E.coli* and *Salmonella* from four Talukas such as Sakrand, Nawab shah, Qazi Ahmed and Dour in district Shaheed Benazir Abad.

Table 1. The taluka wise distribution, number and % of E.coli and salmonella contaminated samples in District Shaheed Benazir Abad.

Taluka	N	*E. coli*	*Salmonell*
Sakrand	25	17 (68%)	06 (24%)
Nawab shah	25	13 (52%)	09 (36%)
Qazi Ahmed	25	14 (56%)	08 (32%)
Dour	25	12 (48%)	5 (20%)
Total	**100**	**56 (56%)**	**28 (28%)**
N= Total number of samples			

Table 1 shows that maximum samples were contaminated with E. coli as compared to Salmonella. This suggests that the E. coli is dominant pathogenic bacteria prevailing in the environment of poultry farms at all four talukas. The Sakrand Taluka was found with highest number of farms contaminated with E. coli (68%) followed by Qazi Ahmed taluka (56%). The

Salmonella, on other hand was common in the farms of Taluka Nawab Shah (36%) followed b Qazi Ahmed (32%). If considered both pathogens, the farms of Qazi Ahmed Taluka were found with high contamination and low sanitation. While the farms of Taluka Dour showed very low contamination with both E. coli and Salmonella in its farms. Prevalence of these high-risk pathogens in the local poultry farms of District Shaheed Benazir Abad suggest that the meet quality of these farms can be of low standards and farmers have to take advanced measures to decontaminate the environment of their farms.

Antimicrobial susceptibility test of E. coli species isolated from the poultry litter samples

E. coli isolated from 100 samples were observed for the antimicrobial resistant to the fourteen antibiotics i.e., Oxacillin, Streptomycin, Polymyxin-B, Amoxicillin, Nalidixic acid, Amikacin, Ciprofloxacin, Sulphafurazole, Doxycycline, Sulfamethoxazole, Gentamycin, Tetracycline, Erythromycin, and Cefixime. The results of resistance indicated that *E. coli* were highly resistant to most of antibiotics including oxacillin, Streptomycin, Nalidixic acid, Tetracycline, Ciprofloxacin, Sulphafurazole, Doxycycline, Sulfamethoxazole, and Cefixime (Table 2). The *E. coli* species has shown intermediate sensitivity to the Amoxicillin with an inhibition zone of 14 mm. However, *E. coli* species were sensitive to Erythromycin, Amikacin and Gentamycin showing an inhibition zone of 23%, 22%, and 20% respectively, (Table: 2).

Table 2. The response of E. coli spp. isolated from various farms of four talukas against various well-known antibiotics.

Antibiotics	Zone of inhibition (mm)**	Resistant Level
Oxacillin	02	Resistant
Streptomycin	04	Resistant
Polymyxin-B	02	Resistant
Amoxicillin	15	Intermediate
Nalidixic acid	02	Resistant
Amikacin	22	**Sensitive**
Ciprofloxacin	03	Resistant
Sulfafurazole	04	Resistant
Doxycycline	03	Resistant
Sulfamethoxazole	2.5	Resistant
Gentamycin	20	**Sensitive**
Tetracycline	2.6	Resistant
Erythromycin	23	**Sensitive**
Cefixime	1.4	Resistant

**According to CLSI Standards, antibiotics sensitivity is shown in millimeter (mm) Sensitive >20, Intermediate 15-19, Resistant <14.

Antimicrobial susceptibility test of salmonella species isolated from poultry litter samples

S*almonella* isolate from 100 samples were tested for the antimicrobial resistance to the 14 well-known antibiotics. The isolated *salmonella* species have shown high resistance to majority of antibiotics including Polymyxin-B, Nalidixic acid, Tetracycline, Ciprofloxacin, doxycycline. Sulfamethoxazole, and Sulfafurazole. *Salmonella* spp. were intermediately sensitive to Amikacin, Oxacillin, Streptomycin and Cefixime. While, the most of *Salmonella spp.* have shown sensitive response against the Amoxicillin, Erythromycin and Gentamycin, as shown in the Table: 3.

Table 3. The response of Salmonella spp. isolated from various farms of four talukas against various well-known antibiotics.

Antibiotics	Zone of inhibition (mm)**	Resistant Level
Oxacillin	14	Intermediate
Streptomycin	16	Intermediate
Polymyxin-B	1.25	Resistant
Amoxicillin	24	Sensitive
Nalidixic acid	1.3	Resistant
Amikacin	17	Intermediate
Ciprofloxacin	2.6	Resistant
Sulphafurazole	4.5	Resistant
Doxycycline	6.0	Resistant
Sulfamethoxazole	5.5	Resistant
Gentamycin	20	Sensitive
Tetracycline	2.1	Resistant
Erythromycin	23	Sensitive
Cefixime	16	Intermediate

**According to CLSI Standards, antibiotics sensitivity is shown in millimeter (mm) Sensitive >20, Intermediate 15-19, Resistant <14.

DISCUSSION

The study was carried out to observe the prevalence and antimicrobial resistance of *E.coli*and *Salmonella* from poultry litter. In this study the highest prevalence of *E. coli* 56 (56%) and *Salmonella* 28 (28%) were observed from poultry litter. The results obtained by Ghanbarpour*et al*. (2011) revealed similar the prevalence of *E. coli* 63.64% in poultry litter. However, all comparative results were attributed to the differences in environmental conditions, dietary habits, age of the sampling, and mixed feed contamination with other

microbes. Findings of Islam *et al*., (2014) reported that poultry litter may contain different type of microorganisms, the presence of *Salmonella* in both feed and litter poses an alarming situation for the public health. In their study, the prevalence of 3% pathogenic *E.coli* was recorded in internal environment from the poultry farms. A similar kind of result was supported by Jeffrey *et al*., (1998) that the overall prevalence of *E.coli* and *Salmonella* spp. from four poultry farms were 62.50 % and 49.91 % respectively. Whereas, *E. coli* and *Salmonella* prevalence was recorded as 87.50% and 66.66% respectively from litter samples, these results suggested that poultry litter is always the major source of both *E. coli* and *Salmonella* spp. form the birds reared in litter system.

Similarly, a high prevalence rate of *E. coli* and *Salmonella* spp. 58% and 28% by Ahmed *et al*., (2007), and Biswas *et al*. (2004) was observed, they suggested the higher prevalence of *Salmonella* spp. in the same environmental sources and conditions as in India and Bangladesh. The isolated *E. coli* and *Salmonella* from poultry litter samples were tested in-vitro for antibiotic susceptibility by an antibiotic disk diffusion method. According to the findings of our study results, *E. coli* species were highly resistant to Tetracycline, Ciprofloxacin and Sulfafurazole. While *E. coli* species have shown intermediate sensitivity to Amoxicillin. However, *E. coli* spp. were highly sensitivity to Erythromycin, Amikacin and Gentamycin as 23%, 22%, and 20% respectively antibiotics. Similar results were reported by Dhanarani, *et al*., (2009) who reported that *E. coli* (12.5%) isolates were susceptible to Ampicillin 57%, Erythromycin 25%, Tetracycline 4% to Chloramphenicol 40%, Kanamycin 75%, and Streptomycin.

However, it was observed that the *E.coli*species were found resistant to Ciprofloxacin and Polymyxin-B antibiotics, which have been also reported previously by Ljubojević, *et al*., (2016), Khan *et al.* (2005), and Islam *et al.,* (2008) who isolated antibiotic-resistant *E. coli* from poultry litter to multiple antibiotics. Khan *et al.,* (2005) isolated nineteen Quinolone group drug-resistant *E. coli* isolates during 2002-03 from 200 poultry litter samples from Arkansas. They found that all isolates were resistant to multiple antibiotics such as Gentamicin, Kanamycin, Chloramphenicol, and Streptomycin. Similar results were observed by Islam *et al.,* (2008) from Bangladesh. They reported that all 30 positive *E. coli* spp. From poultry litter samples were resistance to Tetracycline (96.6%), Ciprofloxacin (30%), Penicillin (100%), Erythromycin (66.6%), Gentamycin (50%) and Chloramphenicol (100%). Adelowo *et al.,* (2009) study results showed multiple-resistances to antimicrobial drugs (Ampicillin, Tetracycline, Cotrimoxazole, Colistin, Gentamicin, Streptomycin, and Nalidixic acid).

Furtula *et al.* (2010) reported antibiotic resistance in *E. coli*. to seven antibiotics such as β-lactam antibiotics, Amoxicillin, Ceftiofur, Tetracyclines, and Sulphonamides. Adelowo *et al.,* (2014)) reported similar results on antibiotic resistance in 36 *E. coli* isolated from litter of poultry farms in Southwestern Nigeria. Results showed that resistance to Tetracycline was 81%, Sulphamethoxazole 67%, Streptomycin 56%, Trimethoprim 47%, Ciprofloxacin 42%, Ampicillin 36%, Chloramphenicol 22%, Neomycin 14%, and Gentamicin 8%. Krnjaić *et al.,* (2005) observed the prevalence of antibiotics-resistant of *E. coli* strains isolated from chicken to Tetracycline (100%), Ampicillin (95%), Streptomycin (95%), Trimethoprim (60%), Neomycin (60%), Nalidixic acid (60%), Amoxicillin-clavulanate (45%), Cephalexin (20%), Ciprofloxacin/Enrofloxacin (15%), and Gentamycin (5%).

S*almonella* isolates were observed for the antimicrobial resistant to the fourteen antibiotics. The isolated *salmonella* species has shown highly resistant While, most of *Salmonella spp*. have shown resistance against the Tetracycline, Ciprofloxacin and sulfafurazole *Salmonella* spp. were intermediate sensitive to Amikacin, Oxacillin, Streptomycin and Cefixime. The isolated *salmonella* species has shown sensitivity to Amoxicillin, Erythromycin and Gentamycin as24%, 23%, 20% respectively. *Salmonella* spp. While, most of *Salmonella spp.* Have similar results were observed by Gutierrez, (2020) who observed antimicrobial-resistant profiles of *Salmonella* in poultry litter from Florida. Isolated *Salmonella* isolates (n = 290) were resistance to Tetracycline (29.8%), Sulfisoxazole (23.4%), and Streptomycin (14.9%).

Alam, *et al*., (2020) studied the antibiotic-resistance in *Salmonella* species isolated from broiler farms in Bangladesh. Overall prevalence of *Salmonella* was observed 35%, with the highest multi drug resistant observed was found to Tetracycline (97.14%), Chloramphenicol (94.28%), Ampicillin (82.85%), and Streptomycin (77.14%).

CONCLUSIONS

Higher prevalence of *E.coli* was observed in poultry litter than *Salmonella* species. Taluka wise higher prevalence of *E.coli and Salmonella* was observed in Sakrand and Nawab shah, whereas, lower prevalence was observed in Dour. *E. coli* and *Salmonella were* shown highly resistant to Tetracycline, Ciprofloxacin and Sulphafurazole.

CONFLICT OF INTEREST: The authors declare no conflict of interest in this publication.

FUNDING: No funding has been obtained for this research.

REFERENCES

Adelowo, O.O., Fagade, O.E. and Agersø, Y. (2014) A ntibiotic resistance and resistance genes in *Escherichia coli* from poultry farms, southwest Nigeria. *The Journal of Infection in Developing Countries* 8: 1103–1112.

Ahmed, A.O., Raji, M.A., Mamman, P.H., Kwanashie, C.N., Raufu, I.A., Aremu, A., and Akorede, G.J., (2019). Salmonellosis: Serotypes, prevalence and multi-drug resistant profiles of *Salmonella enterica* in selected poultry farms, Kwara State, North Central Nigeria. *Onderstepoort Journal of Veterinary Research*, 86(1), 1-8.

Alali, W.Q., S. Thakur, R.D. Berghaus, M.P. Martin, and W.A. Gebreyes, (2010).Prevalence and distribution of *Salmonella* in organic and conventional broiler poultry farms. *Foodborne Pathogens and Disease*, 7(11), 1363-1371.

Alam, S. B., Mahmud, M., Akter, R., Hasan, M., Sobur, A., Nazir, K. H. M., ...&Rahman, M. (2020). Molecular detection of multidrug resistant*Salmonella* species isolated from broiler farm inBangladesh. *Pathogens*, *9*(3), 201.

Al-Zenki, S., Al-Nasser Al-Safar, A., Alomirah, H., Al-Haddad, A., Hendriksen, R.S., and Aarestrup F.M, (2007). Prevalence and antibiotic resistance of *Salmonella*isolated from a poultry farm and pr0Cessing plant environment in the State of Kuwait. *Foodborne Pathogens and Disease*, 4(3), 367-373.

Ao, T.T., Feasey, N.A., Gordon, M.A., Keddy, K.H., Angulo, F.J., and. Crump J.A., (2015).Global burden of invasive nontyphoidal*Salmonella* disease, 2010. *Emerging Infectious Diseases*, 21(6), 941.

Bélanger, L, (2011). "*E.coli* from animal reservoirs as a potential source of human extraintestinal pathogenic *E.coli*. *FEMS Immunology and Medical Microbiology* 62.1: 1-10.

Belova, A., L. Smutka., E. Ros0Chatecka and A. Bazina, (2012). AGRIS on-line Papers in Economics and Informatics, Czech University of Life Sciences Prague, *Faculty of Economics and Management* 4: 1-15.

Blaak, Hetty, (2015). Distribution, numbers, and diversity of ESBL-producing *E. coli* in the poultry farm environment. *PloS one* 10.8: 0135402.

Borda-Molina D, J. Seifertand, A. Camarinha-Silva, (2018). Current perspectives of the chicken gastrointestinal tract and its microbiome. *Computational and structural biotechnology journal*, 16.131-139.

Caffrey, N., Nekouei, O., Gow, S., Agunos, A., and Checkley, S., (2017). Risk factors ass0Ciated with the A2C resistance pattern among *E. coli* isolates from broiler fl0Cks in Canada. *Preventive Veterinary Medicine*, 148, 115-120.

Capita, R. and Alonso-Calleja C., (2013). Antibiotic-resistant bacteria: a challenge for the food industry. *Critical Reviews in Food Science and Nutrition*, 53(1),11-48.

Clavijo, V. and M.J.V. Flórez, (2018). The gastrointestinal microbiome and its ass0Ciation with the control of pathogens in broiler chicken production: a review. *Poultry science*, 97(3), 1006-1021.

Collignon, P.C., Conly, J.M., Andremont, A., McEwen S.A., Aidara-Kane A., (2016). World Health Organization Advisory Group, Bogotá Meeting on Integrated Surveillance of Antimicrobial Resistance, World Health Organization ranking of antimicrobials according to their importance in human medicine: a critical step for developing risk management strategies to control antimicrobial resistance from food animal production. *Clinical Infectious Diseases*, 63(8), 1087-1093.

Dahshan, H, A.M.M., Abd-Elall, A.M. Megahed, M.A. Abd-El-Kader.and E.E. Nabawy, (2015). Veterinary antibiotic resistance, residues, and ecological risks in environmental samples obtained from poultry farms, Egypt. *Environmental monitoring and assessment*, 187(2) .2.

Davies, R. and A. Wales, (2019). Antimicrobial resistance on farms: a review including biosecurity and the potential role of disinfectants in resistance selection. *Comprehensive Reviews in Food Science and Food Safety*, 18(3), .753-774.

Dhanarani, T. S., Shankar, C., Park, J., Dexilin, M., Kumar, R. R., &Thamaraiselvi, K. (2009). Study on acquisition of bacterial antibiotic resistance determinants in poultry litter. *Poultry science*, *88*(7), 1381-1387.

Djeffal, S, B. Mamache, R., Elgroud, S. Hireche, and O. Bouaziz, (2018). Prevalence and risk factors for *Salmonella* spp. contamination in broiler chicken farms and slaughterhouses in the northeast of Algeria. *Veterinary world*, 11(8), 1102.

Founou, L.L., Founou R.C., and Essack, S.Y, (2016).Antibiotic resistance in the food chain: A developing country-perspective. *Frontiers in Microbiology*, 7, 1881.

Friend, A.L., Roberts, S.D., Schoenholtz, S.H., Mobley J.A., and Gerard P.D., (2006). Poultry litter application to Loblolly pine forests: Growth and nutrient containment. *Journal of Environmental Quality*, 35, 837-848.

Furtula, V., Farrell, E. G., Diarrassouba, F., Rempel, H., Pritchard, J., &Diarra, M. S. (2010). Veterinary pharmaceuticals and antibiotic resistance of *Escherichia coli* isolates in poultry litter from commercial farms and controlled feeding trials. *Poultry science*, *89*(1), 180-188.

Gast, R.K. and Jr. R. E. Porter, (2020). *Salmonella* infections. *Diseases of poultry*.717-753.

Gast, R.K. and Porter, Jr. R. E., (2020). *Salmonella* infections. Diseases of Poultry, 717-753.

Gutierrez, A., De, J., & Schneider, K. R. (2020). Prevalence, Concentration, and Antimicrobial Resistance Profiles of *Salmonella* Isolated from Florida Poultry Litter. *Journal of Food Protection*, *83*(12), 2179-2186.

Gutierrez, A., De, J., & Schneider, K. R. (2020). Prevalence, Concentration, and Antimicrobial Resistance Profiles of *Salmonella* Isolated from Florida Poultry Litter. *Journal of Food Protection*, *83*(12), 2179-2186.

Heredia, N. and S. García, (2018). Animals as sources of food-borne pathogens: A review. *Animal nutrition*, 4(3).250-255.

Humphries, R.M., Kircher, S., Ferrell, A., Krause, K.M., Malherbe, Hsiung R. A., and Burnham, C.A.D., (2018). The continued value of disk diffusion for assessing antimicrobial susceptibility in clinical laboratories: report from the Clinical and Laboratory Standards Institute Methods Development and Standardization Working Group. *Journal of Clinical Microbiology*, 56(8).

Ibrahim, W.A., S.A., Marouf, A.M. Erfan, S.A. Nasef, and J.K. El Jakee, (2019). The 0Ccurrence of disinfectant and antibiotic-resistant genes in *E.coli* isolated from chickens in Egypt. *Veterinary world*, 12(1).141.

International Standards Organization, *ISO 6579 (2002). Microbiology of Food and Animal Feeding Stuffs–Horizontal Method for the Detection of Salmonella*, International Standards Organization, Geneva, Switzerland.

International Standards Organization, *ISO 6579-1 (2017) (en) Microbiology of the Food Chain—Horizontal Method for the Detection, Enumeration and Serotyping of Salmonella—Part 1: Detection of Salmonella spp*, International Standards Organization, Geneva, Switzerland.

Islam, M. M., Islam, M. N., Sharifuzzaman, F. M., Rahman, M. A., Sharifuzzaman, J.U., Sarker, E.H., and Sharifuzzaman, M.M., (2014). Isolation and identification of *E.coli* and *Salmonella* from poultry litter and feed. *International Journal of Natural and S0Cial Sciences*, 1(1), 1-7.

Islam, M.J., Sultana, S., Das, K.K., Sharmin, N. and Ha san, M.N. (2008) Isolation ofplasmid-mediated multidrug resistant *Escherichia coli* frompoultry. *International Journal of Sustainable Crop Production* 3: 46–50.

Jajere, S. M, (2019).A review of *Salmonella enterica* with particular f0Cus on the pathogenicity and virulence factors, host specificity and antimicrobial resistance including multidrug resistance. *Veterinary world*, 12.4: 504.

Jayathilakan, K., K., Sultana, k. Radhakrishna, and A.S., Bawa, (2012). Utilization of byproducts and waste materials from meat, poultry and fish pr0Cessing industries: a review. *Journal of food science and technology*, 49(3), 278-293.

Jeffrey, JS., Kirk, JH., Atwill, E.R and Cullor, J.S., (1998). Prevalence of selected microbial pathogens in pr0Cessed poultry waste used as dairy cattle feed. *Poultry Science*, 77,808–811.

Khan, A.A., Nawaz, M.S., West, C.S., Khan, S.A. and Lin, J. (2005) Isolation andmolecular characterisation of fluoroquinolone-resistant *Escherichiacoli* from poultry litter. *Poultry Science* 84: 61–66.

Kilonzo-Nthenge, A., Nahasho, S.N., Chen, F., and Adefope, N., (2008).Prevalence and antimicrobial resistance of pathogenic bacteria in chicken and guinea fowl. *Poultry Science*, 87(9), 1841-1848.

Lee, M. D., K. L. Nolan, D. Zavala et al., (2008). "Editorial board for the American association of avian pathologists," *A Laboratory Manual for the Isolation and Identification of Avian Pathogen Louis*, Blackwell Publishing, Hoboken, NJ, USA, 5th edition, 2008.

Ljubojević, D., Puvača, N., Pelić, M., Todorović, D., Pajić, M., Milanov, D., &Velhner, M. (2016).Epidemiological significance of poultry litter for spreading the antibiotic-resistant strains of *Escherichia coli*. *World's Poultry Science Journal*, *72*(3), 485-494.

Lynch, D., Henihan, A. M., Bowen, B., Lynch, D., McDonnell, K., Wapinski, W. K., and Leahy, J. J., (2013). Utilisation of poultry litter as an

energyfeedst0Ck.*Biomass and Bioenergy*, 49, 197-204.

Magsi, H., A. A. Randhawa., and A.H. Shah, (2020). Halal meat production in Pakistan: status and prospects. *Journal of Islamic Marketing*.

Mezal, E.H., A. Sabol, M.A. Khan, N. Ali, R. Stefanova, A and A.A. Khan, (2014)Isolation and molecular characterization of *Salmonella entericas*erovarEnteritidis from poultry house and clinical samples during 2010. *Food Microbiology*. 38: 67-74.

Mujahid H., A.S. Hashmi. M.Z. Khan., M. Tayyab., and W. Shezad, (2019).Protective effect of yeast sludge and whey powder against 0Chratoxicosis in broiler chicks.*Pakistan Veterinary.Journal.,* 39:588-592.Pakistan Economic Survey, (2020).*Poultry Industry in Pakistan*.

Putri, D.D., E. Handharyani., R. D Soejoedono., A. Setiyono., and N.I. Mayasari, (2018). Genotype characterization of Newcastle disease virus isolated from commercial chicken fl0Cks in West Java, Indonesia. *Pakistan. Veterinary.Journal*.l 38: 184-188.

Schiavone, A., Pugliese, N., Circella, E., and Camarda, A., (2020). Ass0Ciation between the poultry red mite Dermanyssusgallinae and potential avian pathogenic *E.coli*. *Veterinary Parasitology*, 284, 109198.

Sellah, M. and M. Drissi, (2015). Resistance to 3rd generation cephalosporin of *E.coli* isolated from the feces of healthy broilers chickens in Algeria. *Journal of Veterinary Medicine and Animal Health*, 7(8), 290-295.

Sohaib, M. and F. Jamil, (2017). An insight of meat industry in Pakistan with special reference to halal meat: a comprehensive review. *Korean journal for food science of animal resources*, 37(3) .329.

Soria, M.A., and Bueno, D.J., (2016). Culture based methods to detect Salmonella From different poultry products. Food Microbiology: Fundamentals, Challenges and Health Implications, E. Perkins, ed., Nova Science Publishers, *New York*, 57-86.

Tabo, D.A., C.D. Diguuimbaye, S.A. Granier, F. Moury, A. Brisabois, R. Elgroud, and Y. Millemann, (2013). Prevalence and antimicrobial resistance of non-typhoidal*Salmonella* serotypes isolated from laying hens and broiler chickenfarms in N'Djamena, Chad. *Veterinary microbiology* 166: 293-298.

Umar, S., Teillaud, A., Aslam, H.B., Guerin, J.L., and Ducatez, M.F., (2019).Molecular epidemiology of respiratory viruses in commercial chicken fl0Cks in Pakistan from 2014 through to 2016. *Bio Medical Center Veterinary Research*, 15(1) ,351.

Velasquez, C.G., K. S. Macklin, S. Kumar, M. Bailey, P.E. Ebner, H.F. Olive, F.S. Martin-Gonzalez, and M. Singh, (2018). Prevalence and antimicrobial resistance patterns of *Salmonella* isolated from poultry farms in southeastern United States. *Poultry science*, 97(6) .2144-2152.