Research Article

Multi-Drug Resistance Pattern of Bacterial Flora Obtained from Necropsy Samples of Poultry

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Abstract | For determining the multi- drug resistance pattern of bacterial flora of poultry origin, six caecal, two air sacs and one tracheal sample from necropsy of poultry birds (broiler chicken) including one feed sample were collected from poultry farms of Jaipur division. Four different bacterial species name as viz. *Escherichia coli* (38.88%), *Pseudomonas aeruginosa* (27.77%), *Staphylococcus aureus* (22.22%) and *Streptococcus* spp. (11.11%) were obtained from processed samples. *Escherichia coli* were hundred percent resistant to ampicillin, gentamicin, tetracycline, doxycycline hydrochloride. The only antibiotic to which *E. coli* isolates were susceptible (57.14%) was ampicillin sulbactam. *Pseudomonas aeruginosa* showed 100% resistance to ceftriaxone, meropenem, ciprofloxacin, erythromycin and colistin while 60% were sensitive to ampicillin sulbactam, ceftazidime, cefoperazone and rifampicin. The isolates of *Staphylococcus aureus* and *Streptococcus* spp. were 100% resistant to most of screened antibiotics and retained sensitivity to only imipenem and chloramphenicol. The detected resistance pattern indicated the gross severity of problem and underline that for preventing the spread of bacterial resistance, it is critically important to have regulated antibiotic usage policies and surveillance system for implementing effective control of multidrug resistant organisms. Further the study suggested molecular screening of these isolates in regard to antibiotic resistance genes.

Keywords | Bacterial flora, Multidrug, Resistance, Poultry, Necropsy

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INTRODUCTION

Poultry has emerged as a major source of meat and is one of the fastest growing agricultural sectors in India enabling it to export broilers and meet the increasing demand. The current poultry production is growing at the rate of 8-10% per annum with an annual turnover of 30,000 crore units. Bacterial infections continue to pose a grave threat to quality and economics of poultry production (Kearney, 2010; Sams, 2001). Various gram positive and gram negative microorganisms are considered to be normal residents in intestines and respiratory tract of birds. A large variety of both facultative and strict anaerobes colonize the caecum. Approximately twenty bacteria have been reported from poultry including common pathogens such as *Escherichia coli*, *Pseudomonas* spp. *Staphylococcus aureus* and *Streptococcus* spp. These foodborne pathogens are not only significant contributor in human infections but also have important role in poultry health (Mead, 2004).

Avian pathogenic *E. coli* is responsible for causing colisepticemia, coligranuloma and air sacculitis. *Staphylococcus aureus* causes arthritis, septicemia, bumble foot and omphalitis, *Pseudomonas aeruginosa* causes respiratory infection,

Table 1: Prevalence detail of various isolated microorganisms in studied samples.

S. No.	Name of Sample	No. of Sample	*Escherichia coli*	*Pseudomonas aeruginosa*	*Staphylococcus aureus*	*Streptococcus* spp.
1	Ceca	6	3	3	4	0
2	Trachea	1	1	1	0	1
3	Air sac	2	2	0	0	1
4	Feed sample	1	1	1	0	0
Total No. Bacteria			7 (38.88%)	5 (27.77%)	4 (22.22%)	2 (11.11%)

Table 2: Multidrug resistant pattern of *Escherichia coli* isolates obtained from collected samples.

S. No.	Antibiotics	Isolate ID							Percentage (%)		
		E1	E2	E3	E4	E5	E6	E7	Sensitive	Intermediate	Resistant
1	Ampicillin (10 µg)	R	R	R	R	R	R	R	-	-	100.0
2	Ampicillin/ Sulbactam (10/10 µg)	I	S	S	I	S	R	S	57.14	28.57	14.28
3	Amoxicillin Clavulanic Acid (20/10 µg)	R	R	R	R	R	R	I	-	14.28	85.71
4	Cefipime (30 µg)	S	S	R	I	S	R	R	42.85	14.28	42.85
5	Cefixime (5 µg)	R	S	R	R	S	R	R	28.57	-	71.42
6	Ceftazidime/Clavulanic Acid (30/10 µg)	R	I	R	R	S	R	R	14.28	14.28	71.42
7	Ceftriaxone (30µg)	I	S	R	I	S	R	R	28.57	28.57	42.85
8	Cefoperazone (75 µg)	I	S	S	S	R	S	R			
9	Imipenem (10 µg)	I	I	S	I	S	I	R	28.57	57.14	14.28
10	Meropenem (10 µg)	I	R	R	R	S	R	R	14.28	14.28	71.42
11	Faropenem	S	R	S	R	R	S	R	42.85	-	57.15
12	Ciprofloxacin (5 µg)	I	R	R	I	R	R	R	-	28.57	71.42
13	Norfloxacin (10 µg)	S	I	I	S	R	R	R	28.57	28.57	42.85
14	Gentamicin (10 µg)	R	R	R	R	R	R	R	-	-	100
15	Tobramycin (10 µg)	R	R	I	R	R	I	R	-	28.57	71.42
16	Doxycycline (30 µg)	R	R	R	R	R	R	R	-	-	100
17	Tetracycline (30 µg)	R	R	R	R	R	R	R	-	-	100
18	Azithromycin (15 µg)	S	R	R	R	R	R	R	14.28	-	85.71
19	Erythromycin (15 µg)	S	R	R	R	R	R	R	14.28	-	85.71
20	Chloramphenicol (30 µg)	R	R	S	S	R	S	R	42.85	-	57.15
21	Nitrofurantoin (300 µg)	S	I	R	R	I	I	R	14.28	42.85	42.85
22	Trimethoprim (5 µg)	S	I	R	R	S	R	R	28.57	14.28	57.14
23	Rifampicin	R	R	S	S	R	R	R	28.57	-	71.43
24	Polymyxin-B (300 units)	S	I	R	S	R	R	R	28.57	14.28	57.14
25	Colistin (10 µg)	S	R	R	R	R	R	R	14.28	-	85.72

R- Resistant, I- Intermediate, S- Sensitive

sinusitis, keratitis/keratoconjuctivitis and septicemia and *Streptococcus* spp. are known to cause pyogenic infections, septicemia, endocarditis and lameness along with many diverse diseases (Sams, 2001). Therefore, hygienic practices and safe produce with no contamination of pathogens are required so that healthy and whole some product may be produced from poultry sector.

In past years, uses of antibiotics have increased due to huge demand of infection free poultry. In addition to being used as therapeutic agents, the antibiotics are also being employed as growth promoters in poultry industries. Such

Table 3: Multidrug resistant pattern of *Pseudomonas aeruginosa* isolates obtained from collected sample

S. No.	Antibiotics	Isolate ID					Percentage (%)		
		P1	P2	P3	P4	P5	Sensitive	Intermediate	Resistance
1	Ampicillin (10 µg)	R	I	R	R	R	-	20.0	80.0
2	Ampicillin Sulbactam (10/10 µg)	S	S	S	R	R	60.0	-	40.0
3	Amoxicillin Clavulanic Acid (20/10 µg)	R	S	S	R	R	40.0	-	60.0
4	Cefipime (30 µg)	R	I	I	I	R	-	60.0	40.0
5	Cefixime (5 µg)	I	R	R	R	R	-	20.0	80.0
6	Ceftazidime (30 µg)	S	I	S	S	I	60.0	40.0	-
7	Ceftriaxone(30µg)	R	R	R	R	R	-	-	100.0
8	Cefoperazone (75 µg)	I	S	S	S	R	60.0	20.0	20.0
9	Imipenem (10 µg)	R	I	I	I	I	-	80.0.	20.0
10	Meropenem (10 µg)	R	R	R	R	R	-	-	100.0
11	Faropenem	S	R	S	R	R	40.0	-	60.0
12	Ciprofloxacin (5 µg)	R	R	R	R	R	-	-	100.0
13	Norfloxacin (10 µg)	S	R	S	R	R	40.0	-	60.0
14	Gentamicin (10 µg)	R	S	S	R	I	40.0	20.0	40.0
15	Tobramycin (10 µg)	R	R	S	S	R	40.0	-	60.0
16	Doxycycline (30 µg)	R	I	I	R	R	-	40.0	60.0
17	Tetracycline (30 µg)	R	I	S	R	R	20.0	20.0	60.0
18	Azithromycin (15 µg)	R	R	R	S	R	20.0	-	80.0
19	Erythromycin	R	R	R	R	R	-	-	100.0
20	Chloramphenicol (30 µg)	S	R	R	R	R	20.0	-	80.0
21	Nitrofurantoin (300 µg)	I	S	S	R	R	40.0	20.0	40.0
22	Trimethoprim (5 µg)	R	S	R	R	R	20.0	-	80.0
23	Rifampicin	R	S	S	S	R	60.0	-	40.0
24	Polymyxin-B (300 units)	S	R	R	S	R	40.0	-	60.0
25	Colistin (10 µg)	R	R	R	R	R	-	-	100.0

R- Resistant, I- Intermediate, S- Sensitive

extensive use of antibiotic promotes selection pressure mechanism of antibiotics resistance, which facilitate the acquaintance of multidrug resistance of residing bacterial population as well as common pathogens. The misuse of antibiotics in poultry industry is a serious problem and has put both humans and animal at the risk of acquiring multidrug resistance as well as limiting the therapeutic choices for treatment of bacterial diseases (Aarestrup, 2000; Tilak, 2011).

At present, about 70% of pathogenic microorganisms leading to nosocomial infections are resistant to at least one antimicrobial drug that were previously effective (Fair and Tor, 2014). Infections caused by multidrug resistant *Pseudomonas aeruginosa* have been associated with significant increase in poultry morbidity and mortality. Contaminated poultry products may disseminate the resistant pathogenic microorganisms to humans via food or coming in direct contact with the animal, causing illness, mortality and high treatment costs (Pitout et al., 2005). The development of multi drug resistance in bacteria has garnered attention regarding the sensible use of antimicrobial agents in veterinary medicine, nutrition and agriculture (Caprioli et al., 2000). Keeping these concerns of multidrug resistance in consideration, this study is undertaken to identify the prevalent pathogens and their multidrug resistance pattern in poultry for common antibiotics being used clinically. The outcomes can unravel the potential effects of the shifts in antibiotic resistance and the potential risk of transmission of antibiotic resistance.

MATERIALS AND METHODS

Collection of Samples

A total of 54 sick poultry birds of different ages from farms of Jaipur division were necropsied and after post-mortem, ten samples comprising of six caeca, two air sacs and one trachea swab sample with gross lesions were collected. One representative sample of poultry feed from same farm was also collected.

Table 4: Multidrug resistant pattern of *Staphylococcus aureus* isolates obtained from collected samples.

S. No.	Antibiotics	Isolate ID				Percentage (%)		
		S1	S2	S3	S4	Sensitive	Intermediate	Resistance
1	Ampicillin (10 µg)	R	R	R	R	-	-	100.0
2	Ampicillin Sulbactam (10/10 µg)	R	R	R	R	-	-	100.0
3	Amoxicillin Clavulanic Acid (20/10 µg)	R	R	R	R	-	-	100.0
4	Cefipime (30 µg)	R	R	R	R	-	-	100.0
5	Cefixime (5 µg)	R	R	R	R	-	-	100.0
6	Ceftazidime/ Clavulanic Acid (30/10 µg)	R	I	R	I	-	50.0	50.0
7	Ceftriaxone (30µg)	R	R	R	R	-	-	100.0
8	Cefoperazone (75 µg)	R	R	R	R	-	-	100.0
9	Imipenem (10 µg)	S	S	S	S	100.0	-	-
10	Meropenem (10 µg)	S	R	I	I	25.0	50.0	25.0
11	Faropenem	R	R	R	R	-	-	100.0
12	Ciprofloxacin (5 µg)	R	R	R	R	-	-	100.0
13	Norfloxacin (10 µg)	I	R	S	S	50.0	25.0	25.0
14	Gentamicin (10 µg)	R	R	R	R	-	-	100.0
15	Tobramycin (10 µg)	R	R	R	R	-	-	100.0
16	Doxycycline (30 µg)	R	R	R	R	-	-	100.0
17	Tetracycline (30 µg)	R	R	R	R	-	-	100.0
18	Azithromycin (15 µg)	R	S	R	S	50.0	-	50.0
19	Erythromycin	R	R	R	R	-	-	100.0
20	Chloramphenicol (30 µg)	S	R	R	R	25.0	-	75.0
21	Nitrofurantoin (300 µg)	R	I	I	I	-	75.0	25.0
22	Trimethoprim (5 µg)	R	R	R	R	-	-	100.0
23	Rifampicin	R	R	R	R	-	-	100.0
24	Polymyxin-B (300 units)	R	R	R	R	-	-	100.0
25	Colistin (10 µg)	R	R	R	R	-	-	100.0

R- Resistant, I- Intermediate, S- Sensitive

Isolation and Identification of Bacteria

Samples were primarily inoculated on nutrient broth at 37°C for 18- 24 hrs and further were taken on nutrient agar plates for obtaining pure culture. All obtained single pure colonies were characterized with primary and secondary biochemical characteristics such as gram staining, catalase and oxides test to identify the bacterial isolates. The organism were further identified on Mac Conkey agar plates, Eosine Methylene Blue agar plates, Citramide agar plates, Mannitol salt agar plates and Edward agar plates. The organism were isolate and characterized as per standards described by Carter et al. (1990) and Quinn et al. (1994).

Detection of Antibiotic Sensitivity/ Multidrug Resistance Pattern

Antibiotic sensitivity pattern were determined with total 25 antibiotics, as per method of agar disk diffusion on Mueller Hinton agar as per technique of Bauer-Kirby (Bauer et al., 1966, Sharma et al., 2017) using commercially available antibiotic impregnated disks (HiMedia Laboratories, Mumbai). After inhibition zone measurement, result as sensitive, intermediate or resistant and interpretations were made according to guidelines recommended by Clinical and Laboratory Standards Institute (CLSI, 2016).

RESULTS

In the present study, a total of 18 bacterial isolates comprising twelve gram negatives (66.67%) and six gram positives (33.33%) were obtained. Of these 18 isolates detected, 38.88% were *Escherichia coli*, 27.77% were of *Pseudomonas aeruginosa,* 22.22% were *Staphylococcus aureus* and 11.11% prevalence was detected for *Streptococcus* spp. All tested samples were positive with mix bacterial infection of at least any two bacterial organisms (Table 1).

In this study, sensitivity pattern of total 25 antibiotics for each studied genus were screened and majority of the isolates detected were found multidrug resistant (MDR).

Among the *Escherichia coli* isolates, 100% individuals were resistant to ampicillin, gentamicin, tetracycline, doxycycline hydrochloride, and more than 85.0% resistance observed against colistin, azithromycin, erythromycin and amoxicillin clavulanic acid. While 57.14% and 42.85% isolates were sensitive to ampicillin sulbactam and cefepime, respectively. More intermediates were also observed against beta-lactams and quinolone antibiotics (Table 2).

The *Pseudomonas aeruginosa* isolates were found 100% resistant towards ceftriaxone, meropenem, ciprofloxacin, erythromycin and colistin while 60.0% sensitivity was observed against ampicillin sulbactam, ceftazidime, cefoperazone and rifampicin. Isolates showed variable multidrug resistance patterns for other antibiotics as well (Table 3). Imipenem was most effective for *Staphylococcus aureus* isolates while most of isolates were resistant for 18 antibiotics out of total 25 studied antibiotics as mentioned in Table 4. Similarly higher resistance was observed among *Streptococcus* isolates against all studied 25 antibiotics and only two antibiotics (imipenem and chloramphenicol) were found effective against streptococcus. Overall higher group of cephalosporins and penem group of antibiotics were found more effective in present study in compare to routinely using antibiotics in veterinary practices.

DISCUSSIONS

In the present study, samples were obtained from routinely dead poultry birds to ascertain involvement of common microorganisms and their antibiotic resistance pattern. In agreement to present study, higher prevalence of gram negative is also reported by Kolar et al. (2002) from poultry samples in Czech Republic. They reported 67.6% prevalence of gram negative and 32.4% of gram positive in consent with this study, including major species of 61.3% *Escherichia coli*, 14.8% *Streptococcus* spp., 4.9% *Staphylococcus* spp., and 6.3% of *Pseudomonas* spp. along with some minor species. Noori and Alwan (2016) detected 29.0% prevalence of *E. coli* and 6.0% of *Pseudomonas* spp., which was slightly lower than the present findings while Mwambete and Stephen (2015) reported slightly higher prevalence of *E. coli* (82.0%), *Pseudomonas* spp. (18.0%) and *Staphylococcus* spp. (44.0%). In contrary to the present and previous findings, Geidam et al. (2012) observed higher occurrence of gram positives (*Staphylococcus* spp.) in skin and feather samples as compared to gram negatives (*E. coli*). The prevalence of common pathogen in present study is justifiable since these gram negatives (*E. coli* and *Pseudomonas aeruginosa*) and gram positive (*Staphylococcus aureus*) resides as commensal as well as opportunistic pathogen in poultry systems (Quinn et al., 1994). Occurrence of *Streptococcus* spp. in tracheal and air sac sample in this study as well as previous findings indicates importance of this organism as pulmonary pathogen (Bosch et al., 2013). The higher prevalence of *E. coli* indicated a direct correlation with tissue lesions of coliobacillosis and *E. coli* is already proven to be one of the most common worldwide important microorganisms contributing to significant economic losses arising from poultry industry (Sams, 2001).

In consent to present study, multidrug resistant *Escherichia coli* were also reported by other workers such as Sharada et al. (2008) and Joshi et al. (2012) from poultry farm in India, Akond et al. (2009) from Bangladesh, El-Rami et al. (2012) from Lebanon, Talebiyan et al. (2014) from Iran and Akhtar et al. (2016) reported multidrug resistant *Escherichia coli* from Pakistan. It may indicate worldwide severity of antibiotic resistance of *E. coli*. Similar to this study, Hailu and Tefera (2016) was also observed 100% resistance against ampicillin, gentamicin, tetracycline and doxycycline by *E. coli* of poultry samples.

As compared to present study, slightly lower resistance was reported by Talebiyan et al. (2014) among broiler flocks with coli-septicemic infections in Iran and they reported 20.75% resistance against chloramphenicol, 7.55% against ciprofloxacin, 5.66% gentamicin, 71.70% erythromycin, 43.40% oxytetracycline and 39.62% resistant against sulfadimethoxine-trimethoprim. Such diverse percentage of resistance in different geographical regions may clearly demonstrated variable use of antibiotics in feed additives as growth promoters and for therapeutic management in different countries.

The higher resistance among *Pseudomonas aeruginosa* justifies the inherent antimicrobial resistant capacity of organism for many group of antibiotic. Since indiscriminate use of colistin and ceftriaxone in studied poultry farm was known through history records, it could be correlated to the higher percentage of resistance against them not only for *P. aeruginosa* but for other studied organisms as well. In consonance to our results, Elsayed et al. (2016) have also reported high resistance for initial generations of beta lactams and cephalosporins and lowered resistance for aminoglycosides by *P. aeruginosa* from poultry sample in Egypt.

In consent to present study, Owuna et al. (2015) and Yurdakul et al. (2013) reported 100% resistance to gentamicin and tetracycline and ampicillin among *Staphylococcus aureus* of poultry origin. Similar to the present investigation, Sharma et al. (2013) was also reported 100% efficacy of imipenem against *S. aureus* in samples of animal origin. Since imipenem antibiotic is one of the latest and expensive carbapenems which is not routinely employed in veterinary practice in India thus it is quite likely that resistance against imipenem is yet to occur among *S. aureus* and *Streptococcus* isolates of animal origin. In comparison to the present study, slightly lower resistance was reported by

Geidam et al. (2012) among *S. aureus* isolated from poultry in Malaysia. They reported 51.0% resistance against ampicillin, 39.0% against chloramphenicol and ciprofloxacin, while Ugwu et al. (2015) reported higher resistance against chloramphenicol (99.5%), ciprofloxacin (92.5%), gentamicin (76.5%) and trimethoprim (99.0%) against *S. aureus* from broilers, which were consent with present investigation.

CONCLUSIONS

The finding clearly demonstrates the phenomena of antimicrobial resistance to multiple antibiotics among infected poultry in India. Resistance to existing antimicrobials is widespread and is of utmost concern to poultry farmers and veterinarians alike. The low susceptibility of above microorganisms isolated from poultry can be attributed to overuse and unregulated use of antibiotics for prevention of diseases, treatment and also using them as feed additives for growth promotion. The resistance transfer among different bacteria and possible cross resistance between antibiotics used in poultry is also an area of serious concern and requires further investigations. Thus, introduction of surveillance programs to monitor antimicrobial resistance in pathogenic bacteria is strongly needed in developing countries because in addition to animal health problems, transmission of resistant clones and resistance plasmids of *E.coli* from food animals (especially poultry) to humans can occur. Hence, special emphasis is needed for judicious selection of antibiotics, preferably after antibiotic sensitivity testing and judicious use of such antibiotics at an optimum dose for sufficient duration to ensure effective treatment and control of various diseases caused by microorganisms in poultry. The sensitive drugs can be identified for use in treatment of bacterial infections. Taking ahead the indicators of present study, live market place of birds, litter, manure and beddings may also be tested as they also play a key role in spreading of resistant bacteria and further studies for decoding the genetic mechanism of resistance in these strains also needs to be carried out.

CONFLICT OF INTEREST

No conflict of interest exists among authors.

AUTHORS CONTRIBUTION

Dr. Sandeep Kumar Sharma carried out the conception and design of study, laboratory as well as field work, analysis and writing and critical revision of the manuscript. Dr. Vikas Galav and Dr. Manish Agrawal contributed in laboratory work and final version of the manuscript preparation. Ms. Farah Naz Faridi and Dr. Brajesh Kumar carried out sampling, part of laboratory work and contributed in preparation of the manuscript. All authors approved the final version of the manuscript for publication.

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