ISOLATION, CHARACTERIZATION AND ANTIBIOGRAM STUDY ON PATHOGENIC BACTERIA ASSOCIATED WITH RESPIRATORY AFFECTION IN POULTRY

Amita Saini*[1], Garima Rathore[2], Anita Rathor, Manisha Doot[3] and Anju Choudhary[4]
Department of Veterinary Pathology, College of Veterinary and Animal Science, Rajasthan University of Veterinary and Animal Sciences, Navania, Vallabhnagar, Udaipur 313 601, Rajasthan

Received on: 21.09.2023
Accepted on: 02.11.2023

ABSTRACT

The present investigation was carried out on poultry of organized and unorganized sector for 6 months duration at Udaipur district of Rajasthan. For bacteriological isolation, total 55 samples were collected aseptically from lungs (40), trachea (8) and heart blood (7) of poultry carcass. Isolated bacteria were *Escherichia coli* (47.27%), *Staphylococcus* spp. (21.81%), *Streptococcus* spp. (5.45%), *Klebsiella* spp. (1.81%) and mixed bacterial flora comprising of *E. coli* and *Staphylococcus* spp. (9.09%). *E. coli* and *Staphylococcus* spp. wereprime isolated organism from respiratory tract affections in poultry. Serotypes of *E. coli* isolated from lung of poultry were O7, O11, O17, O57, O83, O121 and Untypable. *In-vitro* antimicrobial sensitivity test was performed on 10 *E. coli*, 8 *Staphylococcus* spp., 3 *Streptococcus* spp. and 1*Klebsiella* spp. isolates.Results revealed that Streptomycin was found as most sensitive antibiotic, followed by Ciprofloxacin and Chloramphenicol. Azithromycin was noted as most resistant antibiotic, followed by Gentamicin and Tetracycline.

Key words: Poultry, antibiotic sensitivity, respiratory infections, bacterial culture

Introduction

Poultry industry in India is rapidly flourishing sector among the agriculture based industries (Surjagade *et al*., 2020). Poultry husbandry has emerged from backyard operation to full-fledged industry with enormous increase in egg and meat production (Lakshman *et al*., 2006). The growth of this flourishing industry is circumscribed by a number of variables i.e. management, nutrition, infection and climate etc. Respiratory tract infections are foremost component affecting poultry that leads to heavy economic losses due to high mortality in acute diseases and decreased production in subclinical and chronic cases (Roussan *et al*., 2008). A huge number of pathogens such as bacteria, virus, fungus, mycoplasma and parasites are related with respiratory infections. Various bacteria such as *Escherichia coli, Pasteurella, Klebsiella, Staphylococcus, Stereptococcus, Bordetella* and *Haemophilus* are generally involved in respiratory diseasein poultry.Infected birds express respiratory and other lesions such as cough, respiratory distress, poor growth and production leading to high economic losses. Therefore, isolation and determination of antibiotic sensitivity pattern could contribute to selection of effective therapeutic regime for respiratory infections of bacterial origin in chickens.

Materials and Methods

Present investigation was carried out at Department of Veterinary Pathology, College of Veterinary and Animal Science, Navania, Vallabhnagar, Udaipur (RAJUVAS, Bikaner). This study was done for a period of 6 months (July 2021 to December 2021). Under this study various organized and unorganized poultry farms were included.

Collection of sample for bacterial isolation

Total 55 samples were collected for bacterial isolation from poultry (*Gallus domesticus*). Out of them, 40 samples were from lung, 8 from trachea and remaining 7 were collected from heart blood. Sample were collected from either by sacrificing birds showing respiratory signs or recently dead birds having clinical sign of respiratory disease. Post mortem examination of poultry was performed. The samples were collected aseptically using sterile cotton swab from trachea.Sample from lungs and heart were collected by making a small cut over the surface using sterile scissor. The sterile swab was inserted and rotated and taken back.

Isolation and identification of bacteria

The samples collected for isolation of bacteria were inoculated in nutrient broth and incubated at 37°C for 24 to 48 hours for primary culture. Later on, sub culture was performed by streaking separately on different bacteriological agar media and plates were incubated at37°C for 24 hours. The isolation and identification of pure bacterial culture was performed as per standard method described by Cruickshank *et al.* (1975). The bacterial cultures were identified on the basis of cultural, morphological and biochemical characterization. The bacteria grew on the nutrient, blood and MacConkey agar media were observed for their colony characteristics. Gram staining was carried out to study the morphology and staining propertiesof bacteria. Biochemical tests, such as catalase, oxidase, indole, methyl red, Voges-Proskaure and Citrate utilization test were performed as per standard procedure outlined by Carter *et al.* (2004).

Serotyping of the *E. coli* isolates

After complete biochemical characterization, ten isolates of *E. coli* were sent for serotyping to the National Salmonella and Escherichia Centre, (NSEC), Central Research Institute, Kasauli (HP), India.

*[1]Assitant Professor, Department of Veterinary Pathology, MJF CVAS, Chomu, Jaipur, Rajasthan, E mail- sainiamita69@gmail.com, [2]PhD Scholar, Division of Medicine, Indian Veterinary Research Institute, Izatnagar, Bareilly; [3]PhD Scholar, Department of Veterinary Public Health, CVAS, Bikaner, Rajasthan; [4]Veterinary Officer, Department of Animal Husbandry, Government of Rajasthan

***In-vitro* antimicrobial sensitivity test**

Different strains of various organisms isolated were subjected to *in –vitro* drug susceptibility testing using antimicrobials by the disc diffusion method as suggested by Bauer *et al.* (1966). The discs of standard concentration used under present study are presented in Table 1. The sensitivity was observed on the basis of zone size interpretation chart, provided by the manufacturer. The result were recorded as sensitive (S) and resistant (R).

Results and Discussion

Out of total 55 samples, bacteria were isolated from 47 samples (85.45%). For bacterial isolation 35 samples were found positive from lungs.Out of 8 tracheal swab samples, 7 positive isolates were recorded.Among 7 heart blood samples, only 5 samples were positive for bacterial culture. Under present study, isolated bacteria were *Escherichia coli* (47.27%), *Staphylococcus* spp. (21.81%), *Streptococcus* spp. (5.45%), *Klebsiella* spp. (1.81%) and mixed bacterial flora comprising of *E. coli* and *Staphylococcus* spp. (9.09%).Similar findings were also recorded by Patel *et al.* (2017), who found highest incidence of *E. coli* 47.06%, followed by *Staphylococcus* spp. 23.53% and *Klebsiella* spp. 5.88% in broiler associated with respiratory disease complex. Popy *et al.* (2011) reported occurrence of *E. coli*, *Staphylococcus* spp. and *Klebsiella* spp. as 38.8%, 41.4% and 6%, respectively. Variation in prevalence of bacterial isolates might be due to variation in age, breeds of chickens, geographical locations and management practices.

Table 1: Concentration of different antibiotics used in *In-vitro* antimicrobial sensitivity test

S. No.	Drugs	Concentration µg/Disc
1	Ampicillin	10
2	Ciprofloxacin	5
3	Azithromycin	15
4	Streptomycin	10
5	Gentamicin	10
6	Tetracycline	30
7	Co-Trimoxazole	25
8	Amoxyclave	30
9	Chloramphenicol	30
10	Clarithromycin	15

Serotyping of *E. coli*

The various serotypes of *E. coli* that were isolated from respiratory tract affection in poultry were O7, O11, O17, O57, O83 and O121 (Table 3). Sharda and Ruban (2010) also reported O11, O79 and O111 serotypes in *E. coli* infected poultry.

***In-vitro* antimicrobial sensitivity test**

In the present study, a total of 10 *E. coli*, 8 *Staphylococcus* spp., 3 *Streptococcus* spp. and 1*Klebsiella* spp. isolates were subjected to *in-vitro* drug susceptibility testing. Under this investigation, *E. coli* isolates showed maximum (70%) sensitivity to streptomycin antibiotic followed by ciprofloxacin (60%), chloramphenicol (50%), amoxyclave and co-trimoxazole (40%). Highest drug resistance was recorded against azithromycin (90%), followed by gentamicin (80%), clarithromycin and tetracycline (70%) (Table 4).

These results of antibiogram study for *E. coli* are in accordance with Sahoo*et al*. (2012), who also found high resistance towards Ampicillin and Tetracycline and high sensitivity for Ciprofloxacin. Sultana *et al*. (2012) also reported resistance of *E. coli* for gentamicin and tetracycline. However the isolates were sensitive for chloramphenicol. the antibiotic streptomycin had the highest sensitivity (62.50%) among the isolates of *Staphylococcus* spp., followed by ampicillin and amoxyclave (50%). The most prevalent drug resistance was observed against azithromycin (87.5%), followed by 75% resistance against tetracycline, clarithromycin and chloramphenicol (Table 4).

The isolates of *Streptococcus* spp. revealed greatest (100%) susceptibility to the antibiotic Streptomycin, followed by ampicillin, chloramphenicol and tetracycline (66.66%). Highest drug resistance was recorded against azithromycin (100%). For amoxyclave, clarithromycin, co-trimoxazole and gentamicin bacterial isolates had 66.66% resistance (Table 5). Bakheet *et al*. (2018) found the higher resistance of *Staphylococcus* spp. towards Streptomycin. *Klebsiella* spp. isolates showed maximum (100%) sensitivity to ampicillin, amoxyclave, azithromycin, clarithromycin, tetracycline and streptomycin antibiotic. Highest drug resistance i.e., 100%,

Table 2: Bacteria isolated from lung, trachea and heart blood of poultry (*Gallus domesticus*) in respiratory tract affections

Bacterial Isolates	Lung (40)		Trachea (8)		Heart blood (7)		Total (55)	
	No. of cases	Percentage	No. of cases	Percentage	No. of cases	Percentage	No. of cases	Percentage
E. coli	19	47.50	3	37.50	4	57.14	26	47.27
Staphylococcus spp.	9	22.50	2	25.00	1	14.28	12	21.81
Streptococcus spp.	3	7.50	-	-	-	-	3	5.45
Klebsiella spp.	1	2.50	-	-	-	-	1	1.81
Mixed infection *(E. coli + Staph.)*	3	7.50	2	25.00	-	-	5	9.09
No. of positive isolates	35	87.50	7	87.50	5	71.42	47	85.45

Table 3: Serogroups of *E. coli* isolated from poultry having respiratory affections

S. No.	Serogroups	Number of cases (N=10)	Percentage
1.	O17	1	10
2.	O7	2	20
3.	O121	1	10
4.	O11	1	10
5.	O57	1	10
6.	O83	1	10
7.	Untypable	3	30

Table 4: *In-vitro*antimicrobial sensitivity (%) of *E. coli* and *Staphylococcus* spp. bacteria isolated from poultry (*Gallus domesticus*)

S. No.	Drug	*E. coli*		*Staphylococcus* spp.	
		Sensitive	Resistance	Sensitive	Resistance
1	Ampicillin	30% (3)	70% (7)	50% (4)	50% (4)
2	Ciprofloxacin	60% (6)	40% (4)	37.5% (3)	62.5% (5)
3	Azithromycin	10% (1)	90% (9)	12.5% (1)	87.5% (7)
4	Streptomycin	70% (7)	30% (3)	62.5% (5)	37.5% (3)
5	Gentamicin	20% (2)	80% (8)	37.5% (3)	62.5% (5)
6	Tetracycline	30% (3)	70% (7)	25% (2)	75% (6)
7	Co-Trimoxazole	40% (4)	60% (6)	37.5% (3)	62.5% (5)
8	Amoxyclave	40% (4)	60% (6)	50% (4)	50% (4)
9	Chloramphenicol	50% (5)	50% (5)	25% (2)	75% (6)
10	Clarithromycin	30% (3)	70% (7)	25% (2)	75% (6)
Number of isolates tested		10		8	

Table 5: *In-vitro* antimicrobial sensitivity (%) of *Streptococcus* spp. and *Klebsiella* spp. bacteria isolated from poultry (*Gallus domesticus*)

S. No.	Drugs	*Streptococcus* spp.		*Klebsiella* spp.	
		Sensitive	Resistance	Sensitive	Resistance
1	Ampicillin	66.66% (2)	33.33% (1)	100% (1)	0% (0)
2	Ciprofloxacin	33.33% (1)	66.66% (2)	0% (0)	100% (1)
3	Azithromycin	0% (0)	100% (3)	100% (1)	0% (0)
4	Streptomycin	100% (3)	0% (0)	100% (1)	0% (0)
5	Gentamicin	33.33% (1)	66.66% (2)	0% (0)	100% (1)
6	Tetracycline	66.66% (2)	33.33% (1)	100% (1)	0% (0)
7	Co-Trimoxazole	33.33% (1)	66.66% (2)	0% (0)	100% (1)
8	Amoxyclave	33.33% (1)	66.66% (2)	100% (1)	0% (0)
9	Chloramphenicol	66.66% (2)	33.33% (1)	0% (0)	100% (1)
10	Clarithromycin	33.33% (1)	66.66% (2)	100% (1)	0% (0)
Number of isolates tested		3		1	

was recorded against ciprofloxacin, co-trimoxazole and gentamicin (Table 5).

The overall sensitivity was found highest to Streptomycin and followed by ciprofloxacin and chloramphenicol. The resistance was observed to azithromycin, gentamicin and tetracycline. The variation in findings of antibiogram of bacterial isolates might be result of indiscriminate and injudicious uses of antimicrobials, development of resistant strains with passage of time and differences in geographical area. Antibiotics which are used as feed additives also contribute to multi drug resistance.

Conclusion

It was concluded that, *E. coli*, *Staphylococcus* spp. were most commonly observed bacteria in association with respiratory affections in poultry. *Streptococcus* spp. and *Klebsiella* spp. were also found at lower rate in Udaipur district of Rajasthan. These isolated pathogens showed high resistance against some tested antibiotics and only few antibiotics are effective. Indiscriminate use of antibiotics for prophylactic and curative purpose in poultry leads to development of antibiotic resistant, which creates serious threat for public health. Resistance against commonly prescribed antibiotics should be assed regularly and alternative therapeutic approach should be utilized.

Acknowledgement

This research work was done with financially assistance by College of Veterinary and Animal Sciences, Navania, Vallabhnagar, Udaipur (RAJUVAS, Bikaner).

References

Bauer AW, Kirby MM, Sherris JC and Truck M (1966) Antibiotic susceptibility testing by a standardized single disk method. *Am. J. Clinc. Path*. **45**: 493-496.

Carter ME, Quinn PJ, Markey B and Carter GR (2004) Text book of Clinical Veterinary Microbiology: Elsevier. 1-646.

Cruickshank R, Duguid JP, Marsion BP and Swain RHA (1975) Medical Microbiological Volume II 12th edition, Churchill Livingstone, Edinburgh, London and New York.

Lakshman M, Shashikumar M and Rajendranath N (2006) Pathology of lung affections in poultry-A field study. *Indian J. Vet. Pathol*. **30**(1): 42-45.

Patel JG, Pate BJ, Joshi DV, Patel SS, Raval SH, Parmar RS, Chauhan HC and Chandel BS (2017) Culture based isolation of pathogenic bacteria associated with respiratory disease complex in broiler with special reference to *Ornithobacterium rhinotracheale* from India. *J. Pure Appl. Microbiol*.**11**: 1919-1924.

Popy N, Asaduzzaman M,Miah MS, Siddika A, Sufian MA and Hossain MM (2011) Pathological study on the upper respiratory tract infection of chickens and isolation, identification of causal bacteria. *Bangladesh Vet.* **28**(2): 60-69.

Roussan DA, Haddad R and Khawaldeh G (2008) Molecular survey of avian respiratory pathogens in commercial broiler chicken flocks with respiratory diseases in Jordan. *Poult. Sci.* **87**(3): 444-448.

Sahoo TK, Sahoo L, Sarangi LN, Panda SK and Panda HK (2012) Prevalence, isolation, characterisation and antibiogram study of pathogenic *Escherichia coli* from different poultry farms of Odisha. *J. Adv. Vet. Res*. **2**(3): 169-172.

Sharada R and Ruban SW (2010) Isolation, characterization and antibiotic resistance pattern of *Escherichia coli* isolated from poultry. *Am. Eurasian J. Sci. Res*. **5**(1): 18-22.

Surjagade S, Joshi BP, Mathakiya RA, Kapadiya B, Bansod AP, Jadhao AD, Prajapati R, Ghodasara DJ, Dave CJ andJani P (2020) Mortality pattern and pathological study of *E. coli* infection in broiler chickens. *J. Entomol. Zool. Stud*. **8**(6): 867-872