

# ISOLATION AND AMR PATTERN OF BACTERIAL ISOLATES FROM POULTRY

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# ABSTRACT

Backyard poultry rearing is becoming more and more popular in small households both in rural and urban Kerala with women being the stakeholders, without much capital investment on it. However, disease problems are the major hindrance for the small scale farmers. Bacterial diseases are more common and management of it is often a problem for the farmers. Antimicrobial resistance (AMR) has also started surfacing in this sector. A study was conducted on heart blood samples collected from 118 post-mortem cases of poultry diseases presented at SIAD, Palode. Isolation, identification and antibiogram were done on each sample. 101 cases were of fowl and 17 of duck. 52 isolates were obtained from fowls and 12 from duck. Escherichia coli was the predominant isolate obtained from all cases of fowls (46%) whereas Pasteurella spp was the major isolate obtained from duck. Klebsiella spp, Pseudomonas spp,

Riemerella spp, Streptococcus spp and Staphylococcus spp were the other major isolates obtained. Ceftriaxone was found sensitive for 90% of the isolates followed by Levofloxacin for 74% of the cases. Tetracycline and Amoxicillin was found resistant for around 70% of the isolates. Sulpha TMP along with Ceftriaxone was found sensitive for Pasteurella spp and Riemerella spp. Multidrug resistance was noticed among 3% of the isolates of which Klebsiella spp isolates was showing maximum MDR. We could conclude that hygienic practices in management and reduction of stress is important in prevention of bacterial diseases, meanwhile judicious selection and appropriate dosage of antibiotics is of utmost importance in control of AMR. This study intends to provide a lead in selection of antibiotics for treatment of bacterial diseases in poultry. However taking up similar studies at different parts of the state would give a clear picture of bacterial pathogens and AMR pattern of poultry diseases of Kerala.

**Keywords**: Isolation, Antibiogram, Poultry diseases, Antimicrobial Resistance

## INTRODUCTION

poultry Backyard rearing is becoming more and more popular in small households both in rural and urban Kerala. However, disease problems are the major hindrance for the small scale farmers. Bacterial diseases are more common and management of it is often a problem for the farmers. Over the counter antibiotic purchase and treatment has resulted in surfacing of antimicrobial resistance among the bacterial isolates. Hence a thorough understanding of the common bacteria affecting poultry of our locality and the antibiogram pattern of it is of utmost importance for maintaining the sector.

#### **MATERIALS AND METHODS**

118 cases of poultry disease problems were subjected for the study. Heart blood was collected from all poultry carcasses presented for post-mortem diagnosis at SIAD, Palode from April 2020 to November 2020. Heart blood was inoculated into Nutrient broth and incubated at 37 °C for 8-12 hours. The culture obtained was inoculated to Brain Heart Infusion Agar, Mac Conkey Agar and also to Blood Agar and incubated at 37 °C for 24-48 hours (Quinn *et al.*, 2015). Gram staining was performed to differentiate the staining characteristics. Biochemical testing was done based on Gram's staining characterstics, shape, size and motility (Cowan and Steel, 2003). Antibiotic Susceptibility Testing was performed on all isolates in Mueller-Hinton Agar based on Disc Diffusion Method (Ananthanarayan and Jayaram, 1995). Six commonly used antibiotics were used in the study.

#### **RESULTS AND DISCUSSION**

**Isolation:** Growth was obtained for all 118 cases. 12 duck isolates and 52 fowl isolates could be obtained. Details of isolation are represented in Table 1, Fig.1, Fig. 2 and Graph 1. 10 Cases revealed mixed growth of bacteria. Mixed growth of *Klebsiella spp* and *Pseudomonas* spp, *E. coli* and *Pseudomonas spp*, *E. coli* and *Pasteurella spp* (2) and *Klebsiella spp* along with *Pasteurella spp* were also obtained.

**Antibiogram:** Antibiogram pattern obtained is reported in the Table 2.

*E.coli* was the predominant isolate obtained from fowls (46%) whereas,in duck it was 25% indicating sanitation problems in watering, feeding and other management practices. It was also found concomitant to viral diseases like Avian Leukosis complex and Marek's. All the *E.coli* isolates were resistant to Tetracycline and Amoxicillin. Ceftriaxone was having



Fig. 1. Colonies of *E. coli* with metallic sheen on EMB agar

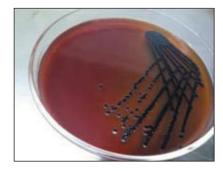
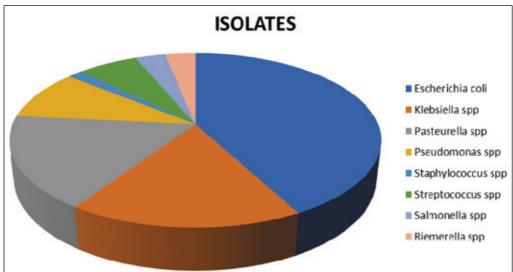


Fig. 2. Colonies of *Salmonella spp* with black centre on S.S agar



sensitivity for 87% of the *E.coli* isolates followed by Levofloxacin, Enrofloxacin and Co-Trimoxazole.

Of all the *Klebsiella spp* isolates, 16% of them showed Multi Drug Resistance (MDR) and was having maximum resistance to Amoxicillin followed by Tetracycline and were highly sensitive to Ceftriaxone and then to Levofloxacin. *Pseudomonas spp* formed 9.3% of the total isolates and all the isolates showed resistance to Tetracycline and all were sensitive to Ceftriaxone and Levofloxacin.

#### Table1: Isolates obtained

| ISOLATES           | FOWL | DUCK |
|--------------------|------|------|
| Escherichia coli   | 24   | 3    |
| Klebsiella spp     | 10   | 1    |
| Pasteurella spp    | 5    | 6    |
| Pseudomonas spp    | 6    |      |
| Staphylococcus spp | 1    |      |
| Streptococcus spp  | 4    |      |
| Salmonella spp     | 2    |      |
| Riemerella spp     |      | 2    |
| TOTAL              | 52   | 12   |

Graph1:Isolation pattern

| ISOLATES           | MULTI<br>DRUG<br>RESISTANT | Tetracycline<br>(30 mcg) | Enrofloxacin<br>(10 mcg) | Amoxicillin<br>(10 mcg) | Co-<br>Trimoxazole<br>(25 mcg) | Levofloxacin<br>(5 mcg) | Ceftriaxone<br>(30 mcg) |
|--------------------|----------------------------|--------------------------|--------------------------|-------------------------|--------------------------------|-------------------------|-------------------------|
|                    | %                          | R%                       | R%                       | R%                      | R%                             | R%                      | R%                      |
| Escherichia coli   | 6.2                        | 100                      | 40                       | 100                     | 40                             | 27                      | 13                      |
| Klebsiella spp     | 16                         | 66                       | 34                       | 84                      | 50                             | 16                      | 16                      |
| Pasteurella spp    | 0                          | 39                       | 15                       | 15                      | 15                             | 0                       | 0                       |
| Pseudomonas spp    | 0                          | 100                      | 16                       | 66                      | 50                             | 16                      | 50                      |
| Streptococcus spp  | 0                          | 40                       | 25                       | 25                      | 25                             | 25                      | 0                       |
| Staphylococcus spp | 0                          | 50                       | 50                       | 100                     | 50                             | 0                       | 0                       |
| Salmonella spp     | 0                          | 50                       | 50                       | 100                     | 50                             | 0                       | 0                       |
| Riemerella spp     | 0                          | 100                      | 100                      | 100                     | 0                              | 100                     | 0                       |
| TOTAL              | 2.75                       | 68.12                    | 41.25                    | 73.75                   | 35                             | 23                      | 9.8                     |

Table 2: Bacterial isolates obtained and *in vitro* antibiotic resistance of the isolates. (% -Percentage, R-Resistance)

*Pasteurella spp* was the most common isolate obtained from duck samples and they showed good sensitivity to Ceftriaxone, Levofloxacin and Co-Trimoxazole. Both the *Riemerella* spp isolates obtained from ducks were sensitive only to Ceftriaxone and Co-Trimoxazole. Stress may be the cause of high prevalence of Pasteurellosis and Riemerellosis among ducks. Indiscriminate use of antibiotics by the duck farmers can be incriminated for the increased resistance towards commonly used antibiotics.

Salmonella spp isolates formed only 3.1% of total isolates and 100% of them showed sensitivity to Ceftriaxone and Levofloxacin and all were resistant to Amoxicillin. *Staphylococcus spp* formed only 1.5% of the total isolates and were having good sensitivity to Levofloxacin and Ceftriaxone. *Streptococcus spp* formed 6.25% of the total isolates and all were sensitive to Ceftriaxone and had a fair sensitivity to Tetracycline.

Among the antibiotics used in the study, Ceftriaxone was having maximum sensitivity (90%) followed by Levofloxacin (74%), Co-Trimoxazole (65%) and Enrofloxacin (59%) to the isolates. Tetracycline was resistant to 68% of the isolates and Amoxicillin to 74%. Co-Trimoxazole had a better sensitivity for *Pasteurella* spp and *Riemerella spp* compared to other isolates; this is in agreement with the findings of Sivachandra et al. (2004). Combination antibiotic therapy of Cephalosporins with Quinolones would be more effective in cases of unknown bacterial aetiology. Large scale use of Tetracyclines as feed additives and also for prophylaxis might be responsible for the high resistance shown by the isolate (Nhung et al., 2017). General resistance noticed among bacterial isolates from human towards beta lactam antibiotics were seen among the poultry isolates also

# SUMMARY

Hygienic practices in management and reduction of stress is important in prevention of bacterial diseases, meanwhile judicious selection and appropriate dosage of antibiotics is of utmost importance in control of AMR. This study intends to provide a lead in selection of antibiotics for treatment of bacterial diseases in poultry. However taking up similar studies at different parts of the state would give a clear picture of bacterial pathogens and AMR pattern of poultry diseases of Kerala.

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