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# Isolation and identification of bacterial agents causing respiratory infection in native chicken

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**Abstract**
The present research work was undertaken for the isolation and identification of the etiological agents present in the respiratory tract of infected native chicken. Totally of 276 samples were collected from infected native chickens from various locations of Salem district, Tamil Nadu, India. The samples were nasal, conjunctiva and tracheal swabs. Twenty three of these collected samples did not show any bacterial growth in any type of growth media despite of the clear clinical respiratory symptoms. The remaining 253 samples gave 337 isolates, 258 (76.5%) of them were Gram negative bacteria, and the rest 79 (23.4%) isolates were Gram positive bacteria. The 337 isolates were 23 (6.82%) *Pseudomonas* species, 116 (34.42%) *E. coli* species, 14 (4.15%) *Klebsiella* species, 51 (15.13%) *Pasteurella* Species, 79 (23.4%) *Staphylococcus* species and 54 (16.0%) *Salmonella* species. Motility test and biochemical test viz. sugar fermentation test, indole test, Methyl red (MR) test, Voges-proskauer (VP) test, Triple sugar iron (TSI) test, catalase and coagulase tests were performed to differentiate motility and identification of isolated bacteria. Our findings will help to understand the bacterial organisms associated with respiratory distress in native chickens.

**Keywords:** Bacterial organisms, respiratory infection, native chicken, isolation of bacteria

## 1. Introduction
Diseases of the respiratory tract are a significant component of the overall disease incidence in poultry [1]. It affects almost all sub species of poultry namely chicken, turkey, quail, duck, geese etc [2]. Various pathogens may initiate respiratory disease in poultry, including a variety of viruses, bacteria, and fungi [3, 4] Environmental factors may augment these pathogens to produce the clinically observed signs and lesions [1]. Bacterial infections of the respiratory tract are of major importance in poultry production as it can cause around 30% of mortality per year [5]. A wide variety of bacteria are found in the respiratory tract. Important bacterial respiratory diseases of poultry are fowl cholera, infectious coryza, pullorum disease and colibacillosis [1] which are responsible for high percentage of morbidity and mortality. Incidence of various pathogenic microbes such as *Escherichia coli*, *Salmonella* spp., *Pasteurella* spp., *Streptococcus* spp. and *Staphylococcus* spp. have been implicated to reduce the growth of poultry including native chicken [6]. All of these organisms have been reported to be associated with upper respiratory disease under certain conditions such as stress, viral infections etc [7].
The isolation, identification and characterization of microorganisms like *E. coli*, *Salmonella* spp., *Staphylococcus* spp., *Pasteurella* spp. in broilers and layers have been accomplished from the clinical cases. But the present study considering the distribution of bacterial isolates from respiratory tract of infected native chicken has still remained uncertain [8]. Ensuring proper treatment and control of any bacterial disease, the isolation, identification and characterization of normal bacterial flora is very much essential, because it will be helpful for the selection of antibiotics and vaccine therapy. Keeping in mind the above facts, the present research work was undertaken to isolate bacterial pathogens from various samples of respiratory tract of infected native chickens and to ascertain the degree of sensitivity of the isolated bacteria against a panel of antimicrobial agents.

## 2. Materials and Methods
### 2.1. Collection of samples
The study was carried out in commercial native chicken farms of Salem district in Tamil Nadu, India. The farms selected were maintained under backyard and intensive farming system, with

uniform management practices. A total numbers of 276 samples were collected from infected chicken with clinical symptoms of respiratory tract diseases. These symptoms include mucoid or serous nasal discharge, sneezing, lacrimation, conjunctivitis and facial swelling. All samples were collected from farms where chickens are vaccinated against Newcastle disease and Fowl pox. Nasal swabs were collected from secretions of nostril and Tracheal swabs were collected from pharyngeal region after opening the mouth using sterile cotton swab. After collecting aseptically the samples were transferred to the fresh nutrient broth for isolation and characterization of bacterial organisms. Conjunctival swab also collected from the live birds from the purulent lacrimal discharge.

### 2.2. Isolation of bacteria

The samples were inoculated nutrient broths were incubated at 37 °C for 24 hrs and then streaked onto different bacteriological media such as Nutrient agar (NA), MacConkey Agar (MAC), Brilliant Green Agar (BGA), Methylene Blue Agar (EMB), Mannitol Salt Aagar (MSA) and Bismuth Sulphite Agar (BSA) (Himedia, India) and incubated at 37 °C for 24 hrs to obtain pure culture of the bacteria.

### 2.3. Identification of bacteria

Identification of bacteria was performed on the basis of colour, size, shape, texure and edge elevation of colony growth. Motility test was performed to differentiate motile bacteria from non-motile one [9]. Isolated bacteria from each sample were biochemically identified by sugar fermentation test, indole test, Methyl red (MR) test, Voges-proscure (VP) test, Triple sugar iron (TSI) test, catalase and coagulase tests [10].

## 3. Results

### 3.1. Isolation of bacterial agents causing respiratory infection in Native chicken

Out of 276 samples, twenty three samples did not show any bacterial growth in any type of media despite of the clear clinical respiratory symptoms. The remaining 253 samples gave 337 isolates, 258 (76.5%) of them were Gram negative bacteria, and the rest 79 (23.4%) isolates were Gram positive bacteria. The 337 isolates were 23 (6.82%) *Pseudomonas* species, 116 (34.42%) *E. coli* species, 14 (4.15%) *Klebsiella* species, 51 (15.13%) *Pasteurella* Species, 79 (23.4%) *Staphylococcus* species and 54 (16.0%) *Salmonella* species (Table 1; Figure 1)

**Table 1:** Prevalence of bacteria isolated from all respiratory samples in Native chicken

| Name of The Sample | Number of samples | Types of bacteria isolated | | | | | |
|---|---|---|---|---|---|---|---|
| | | *E. coli* | *Salmonella* spp. | *Pasteurella* spp. | *Klebsiella* spp. | *Pseudomonas* spp. | *Staphylococcus* spp. |
| Tracheal swab | 108 | 57 | 16 | 36 | 4 | 3 | 29 |
| Nasal swab | 98 | 34 | 22 | 13 | 3 | 13 | 37 |
| Conjunctival swab | 70 | 25 | 16 | 22 | 7 | 7 | 13 |
| Total | 276 | 116 | 54 | 51 | 14 | 23 | 79 |

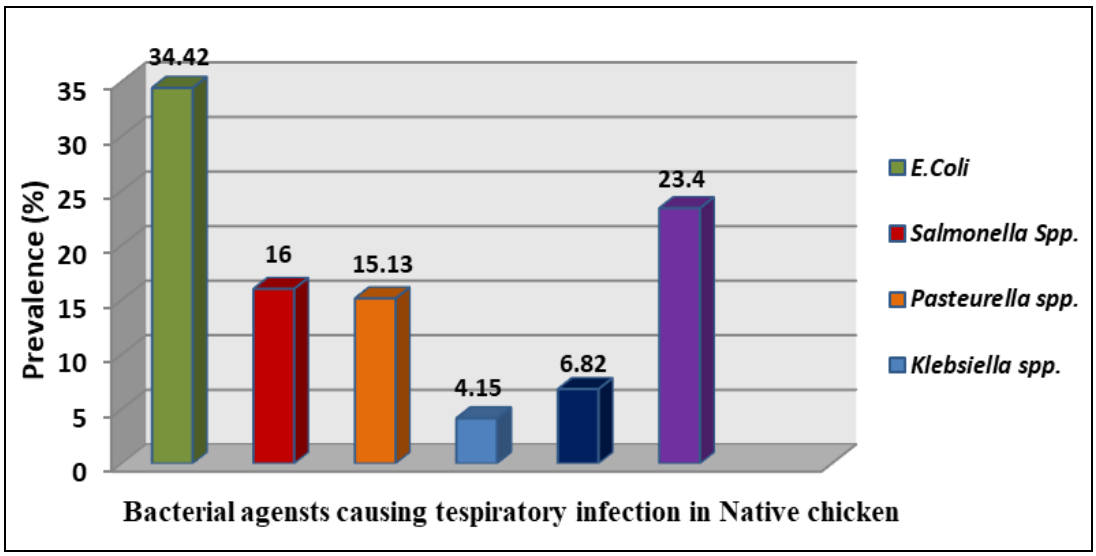


**Fig 1:** Prevalence of bacteria isolated from all respiratory samples in Native chicken

### 3.2. Identification of bacterial agents causing respiratory infection in Native Chicken

#### 3.2.1. Identification through Cultural methods and motility test

The purified isolates were identified according to growth condition, colonial characteristics on different media, haemolysis on blood agar, and biochemical characteristics and sensitivity of the isolates (Table 2). The isolated bacteria were *E. coli*, *Salmonella* spp., *Pasteurella* spp., *Staphylococcus* spp., *Klebsiella* spp. and *Pseudomonas* spp. (Fig. 2-7)

**Table 2:** Cultural characteristics of isolated bacterial organisms.

| Bacterial organisms | Agar | Characteristics of bacterial culture | Motility test |
|---|---|---|---|
| *E. coli* | EMB agar | Smooth, circular, black colour colonies with metallic sheen were produced | The organisms were motile |
| | Brilliant green agar | Green colour colony | |
| *Salmonella spp.* | Bismuth sulphide agar | Black colour colony | The organisms were motile |
| | EMB agar | Pinkish circular smooth small colony translucent, amber coloured or colourless. | |
| *Pasteurella spp.* | Blood agar | Whitish, opaque colonies were produced with musty odour and there was no hemolysis. | - |
| | Nutrient agar | Whitish, opaque, circular, translucent appearance | - |
| *Klebsiella spp.* | MacConkey agar | Pink colour mucoid colony | - |
| *Pseudomonas spp.* | Nutrient agar | Green colour colony | - |
| *Staphylococcus spp.* | Nutrient agar | Gray, white or yellowish colony | - |
| | MSA agar | Yellow colour colony | |

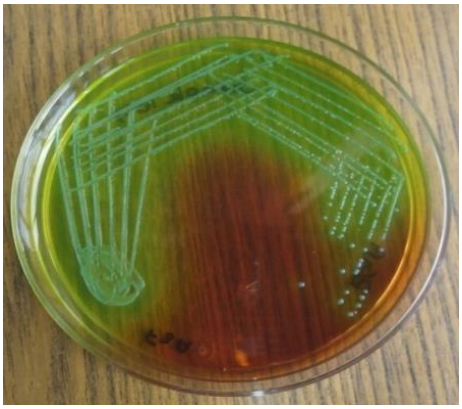

**Fig 2:** Green colour colony in Brilliant green agar shows the positive growth of *E. coli*

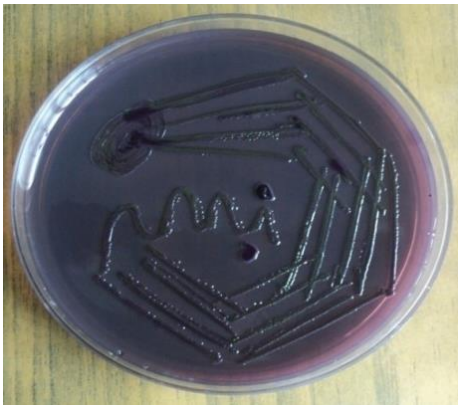

**Fig 3:** Green metallic sheen colour colony in EMB agar shows the positive growth of *E. coli*

**Fig 4:** Pink colour mucoid colony in MacConkey agar shows the positive growth of *Klebsiella*

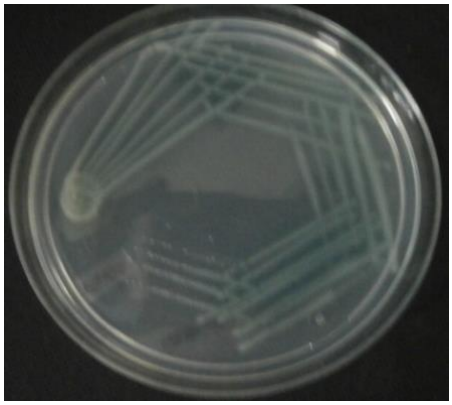

**Fig 5:** Green colour colony in Nutrient agar shows the positive growth of Pseudomonas

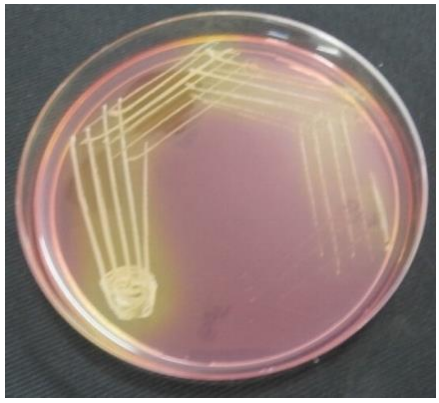

**Fig 6:** Yellow colour colony in Mannital salt agar shows the positive growth of *Staphylococcus spp.*

**Fig 7:** Whitish colonies without hemolysis of *Pasteutella* spp. on Blood agar.

### 3.3. Biochemical characteristics of isolated bacteria

The results of biochemical test of isolated bacteria's are presented in Table 3. All the isolates of *E. coli* fermented 5 basic sugars and produce acid and gas. *E. coli* also shows positive reaction in MR and Indole test but negative to catalase, coagulase and VP reaction. Similarly, with *Salmonella* spp. all the isolates fermented 5 basic sugars and produce acid and gas except sucrose and lactose. *Salmonella* spp. only showed positive reaction in MR test otherwise it produce negative reaction. On the other hand, all the isolates of *Pasteurella* spp. fermented all sugar and produce acid except maltose and lactose. It only produces positive reaction in Indole production test. Among the isolated bacteria, *Staphylococcus* spp. fermented all the five basic sugar producing only acid. It shows positive reaction in the cases of catalase, Indole and MR test but was negative to coagulase test and VP reaction. All the isolates of *Klebsiella spp*. fermented all five sugars, thereby produce acid except lactose and sucrose. In case of *Klebsiella* spp. Catalase test, Citrate test and VP test are positive but oxidase, indole and MR reaction negative.

**Table 3:** Biochemical confirmation of the organisms

| Sl. No | Name of the test | | | | | | | Inference |
|---|---|---|---|---|---|---|---|---|
| | Indole | MR | VP | Citrate | Oxidase | Catalase | TSI | |
| 1 | + | + | - | - | - | - | A/AG | Positive for *E. coli* |
| 2 | - | + | + | - | - | + | K/A | Positive for *Staphylococcus spp.* |
| 3 | - | - | + | + | - | + | A/AG | Positive for *Klebsiella spp.* |
| 4 | + | - | - | - | - | - | A | Positive for *Pasteurella spp.* |
| 5 | - | - | - | + | + | + | K/K | Positive for *Pseudomonas spp.* |
| 6 | - | + | - | - | - | - | A/AG | Positive for *Salmonella spp.* |

(A-Acid; G-Gas; AG- Acid and gas; + - Positive; - - Negative)

## 4. Discussion

### 4.1. Isolation of bacterial agents causing respiratory infection in Native chicken

In this study out of 276 samples, 253 samples gave 337 isolates, 258 (76.5%) of them were Gram negative bacteria, and the rest 79 (23.4%) isolates were Gram positive bacteria. Six different types of bacteria were isolated from respiratory samples of native chicken. The isolated bacteria were *E. coli*, *Salmonella* spp., *Pasteurella* spp., *Klebsiella* spp., *Pseudomonas* spp. and *Staphylococcus* spp. The results of isolation are in agreement with the findings reported by Hirsh *et al*. [11], Elhassan *et al*. [12] and Quinn *et al*., [13].

In this study *E. coli* was isolated from nostrils, trachea and conjunctiva of infected chickens which is similar to findings described by Hofstad *et al*. [14]; Rajashekar *et al*. [15]; Hasan *et al*. [16]; Poppy *et al*. [7] and Hossain *et al*. [5]. *E. coli* was the more prominent pathogen isolated in this study, which is an agreement with the report of Rajasekar *et al*. [15], who reported *E. coli* was the most frequently isolated from the poultry species. The higher occurrence of *E. coli* probably from contaminated poultry feeds and with faeces during lay in unhygienic condition or also from infected poultry. This was supported by Islam *et al*. [17] who reported *E. coli* was a common microflora in raw feeding materials and poultry feeds.

*Pseudomonas* species were isolated from nostrils of infected chickens. Similar finding were also recorded by Mrden [18]. *Pseudomonas* is considered to be an opportunistic organism [13] that produces respiratory infection, sinusitis, Keratitis or keratoconjunctivitis and septicemia and it becomes an infection when it is introduced into tissues of susceptible hosts [19]. This reveals that Pseudomonas infections could be a cause of heavy losses among chickens.

*Klebsiella* species was also isolated from respiratory infected chickens in this study. This confirms the previous finding of Dashe *et al.* [20] and Elhassan *et al*. [12] who isolated *Klebsiella* species from respiratory tract of chickens. *Klebsiella* is found in mucosa of upper respiratory, intestine and urogenital tract of man and other animals and cause pneumonia, nasal infection, urinary tract infection and biogenic infection in man [11]. Fielding *et al.* [21] also opined that members of the genus *Klebsiella*, especially *K.pneumonia* and *K. oxytoca*, are opportunistic pathogens associated with severe nosocomial infections such as septicaemia, pneumonia and urinary tract infections in animals and birds.

*Pasturella* species was isolated from chicken in this study. Also Linzitto *et al*. [22] isolated *Pasturella multocida* from respiratory tract of chickens. *Pasturella multocida* causes fowl cholera / avian pasteurellosis in poultry [13]. The disease is highly contagious and affects both domestic wild birds. The sub acute form of the disease is mostly respiratory and manifested by rales and mucopurulant nasal discharge [11].

*Staphylococcus* species were isolated from trachea, Nostril and conjunctiva of infected chickens, also Bibersein *et al*. [23] and Linzitto *et al*. [22] isolated *Staphylococcus* species from respiratory tract of infected chickens. *Staphylococcus* species are present in the upper respiratory tract and upper epithelial surface of the warm-blooded animals [11]. Transmission of *Staphylococcus aureus* between animal and human occurs infrequently [11]. In man, *Staphylococcus aureus* infection result in several infections such as otitis externa, urinary tract and wound infection. In addition, it also causes staphylococcal food poisoning which result from consumption of contaminated food. Hence *Staphylococcus aureus* may contaminate chicken meat and cause food poisoning.

### 4.2. Identification of bacterial agents causing respiratory infection in Native Chicken

#### 4.2.1. Identification through Cultural methods and motility test

In this study, colony characteristics of *E. coli* observed in EMB and Brilliant green agar were similar to the findings of Nazir *et al.* [24], Sharada

*et al.* [25]. The colony characteristics of *Salmonella* spp. observed in Bismuth sulphide agar and EMB agar were similar to the findings of Rahman *et al.* [26] and Khan *et al.* [27]. The motile *salmonella* isolated in this study might belong to serovar other than *S. pullorum and S. gallinarum* [28]. The colony characteristics of *Pasteurella* species observed in blood agar and nutrient agar which was supported by Woo and Khim [29], Cowan [9] and Cheesbrough [10]. Similarly the colony characteristics of *Klebsiella* species, *Pseudomonas*

species and *Staphylococcus* species were observed in MacConkey agar, Nutrient agar and MSA agar respectively. *Pasteurella* spp. and *Staphylococcus* spp. were found non motile due to absence of peritrichous flagella [30].

#### 4.2.2. Biochemical characteristics of isolated bacteria

The *E. coli* isolates revealed a complete fermentation of 5 basic sugars by producing both acid and gas which was supported by Thomas [31], Sandhu *et al*. [32]. The isolates also revealed positive reaction in MR test and Indole test but negative reaction in VP test [33, 34].

Sugar fermentation tests profile *Salmonella* spp. in the present study showed similarities with the findings of other researchers [34]. However, differentiation of *Salmonella* into species level was difficult based on fermentation reaction as there are many serotypes of *Salmonella* namely *S. typhimurium, S. enteritidis, S. agona, S. Newport, S. hador* etc [35].

The *Pasteurella* spp. revealed a complete fermentation of dextrose, sucrose and mannitol completely and production acid without gas but no fermentation was recorded in case of maltose and lactose. These biochemical properties were closely correlated with the findings of Choudhury *et al.* [36] and Calnek *et al.* [37].

Isolates of *Staphylococcus* spp. was revealed a complete fermentation of 5 basic sugars and production of acid which was supported by Beutin *et al*. [38]. Coagulase test of *Staphylococcus* spp. was performed to determine whether the organism is pathogenic or not pathogenic. It was found that the isolated *Staphylococcus spp.* were coagulase negative i.e. they were nonpathogenic. Beutin *et al*. [38] found *Staphylococcus spp*. is both coagulase-positive and coagulase negative. But *Staphylococcus aureus* are commonly coagulase positive. So this isolated may be other species of *Staphylococcus spp.*

All the isolates of *Klebsiella spp.* fermented dextrose, sucrose, lactose, maltose and mannitol with the production of acid within 24- 48 hrs of incubation. Results of *Klebsiella* spp. were positive as reported by Honda *et al.* [33] and Buxton and Fraser [34]. The isolates also revealed negative reaction in VP test, positive reaction in MR and Indole test which was supported by Honda *et al.* [33] and Buxton and Fraser [34].

The results obtained in this study will help to better understand the bacterial organisms associated with respiratory distress in native chickens and enable the veterinarians to ensure the proper treatment and control therapy and farmers to take adequate measures to control the spread of infection.

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