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## Bacterial respiratory tract infection in dog

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### Abstract

The research entitled “Bacterial Respiratory Tract Infection in Dog” was undertaken *w.e.f.* October 2022 to June 2023 in the Veterinary Clinical Complex, College of Veterinary Science, Assam Agricultural University, Khanapara, and Guwahati with the objective of isolation and identification of bacterial isolates along with therapeutic regimen based on *in-vitro* antibiotic sensitivity test. Nasal swab samples were collected by using sterile swabs aseptically from the suspected animals for isolation, identification of the bacteria and performed *in-vitro* antibiotic sensitivity test and rendered treatment in the three treatment groups (Group B, Group C and Group D). The most common clinical findings observed were moist cough, inappetence and wheezing sound of the lung elicited through thoracic auscultation. *Staphylococcus* spp. concurrently with *Escherichia coli* (30.00%) were common isolates. In *in-vitro* antibiotic sensitivity test *Staphylococcus* spp., *Klebsiella pneumoniae*, *Pseudomonas* spp. and *Bacillus* spp. showed highest sensitive to Ceftriaxone. *Escherichia coli* and *Bacillus* spp. showed highest sensitive to Doxycycline. Ceftriaxone sodium was found (100.00%) effective followed by Doxycycline hydrochloride and Enrofloxacin (90.00%).

**Keywords:** Ceftriaxone, *Staphylococcus* spp., isolation, wheezes, crackles

### Introduction

Dog (*Canis familiaris*) is an affectionate and faithful pet animal which can learn easily and is obeyed by the master. Dogs are the first domesticated animal and have interaction with human (Freedman and Wayne, 2017) <sup>[10]</sup>. In a research study by Pescini *et al.*, 2019 explained that dogs as well as human both release oxytocin when they spend time together and they inferred that oxytocin is the main reason for creating a strong social bond between human and pet animal. Dogs are affected by both infectious as well as non-infectious diseases. Respiratory tract infections (RTI) is caused by viruses, bacteria, fungus, parasites, mycoplasma etc and out of all, bacterial involvement is one of the common reasons for the onset of RTI. *Staphylococcus* spp., *Streptococcus* spp., *Escherichia coli*, *Pasteurella* spp., *Klebsiella* spp., *Pseudomonas* spp., *Bordetella bronchiseptica* etc. are the common bacteria associated with RTI (Qekwana *et al.*, 2020) <sup>[21]</sup>. During the infection, patient exhibit clinical manifestation like dyspnea, fever, coughing, sneezing, loss of appetite, nasal discharge, lethargy, weight loss etc. Doxycycline and Amoxicillin-clavulanic acid are preferred for first line systemic antibiotic therapy; Fluoroquinolones, Azithromycin etc. can be indicated for second line antibiotic therapy. But in the present scenario due to indiscriminate use of antibiotics, they become ineffective against pathogens.

In North Eastern Region of India, investigation on bacteria associated respiratory tract infection in canine has not been studied so far. Hence, an attempt was made to study the association of bacteria in respiratory tract infection of dogs and to generate proper therapeutic management for the affected animals. Considering the importance of canine respiratory tract infection present study was undertaken considering of few objectives namely isolation and identification of the bacteria associated with respiratory tract infection, *in vitro* antimicrobial sensitivity pattern of the isolates and therapeutic management of the affected dogs.

### Materials and Methods

From October, 2022 to June 2023, dogs presented to the Veterinary Clinical Complex (VCC), College of Veterinary Science, Assam Agricultural University, Khanapara from

different parts of Assam and neighboring states of the North Eastern Region, irrespective of their age, sex and breed having clinical signs like coughing, sneezing, presence of nasal discharge, fever, respiratory distress, loss of body weight etc were included in the study. Nasal swab samples were collected by using sterile swabs aseptically for isolation, identification of the bacteria up to genus level. The samples which were collected by sterile cotton swabs kept into the Brain Heart Infusion Broth and incubated at 37°C overnight. The incubated broth sample was streaked over Brain Heart Infusion Agar and further incubated for 24 hours aerobically to obtain a single colony for primary isolation. After that the smear was prepared from the pure colonies and subjected to Gram’s staining.

On the basis of the characteristic of Gram’s staining the single colony was streaked on selective media *i.e.* Eosin Methylene Blue agar (EMB) and Mannitol Salt Agar (MSA). Then the plate was incubated at 37°C for 24 hours for the growth of the organism. Further confirmation series of biochemical test was done *viz.* catalase test, IMViC test etc (Quinn *et al.*, 2011) [22].

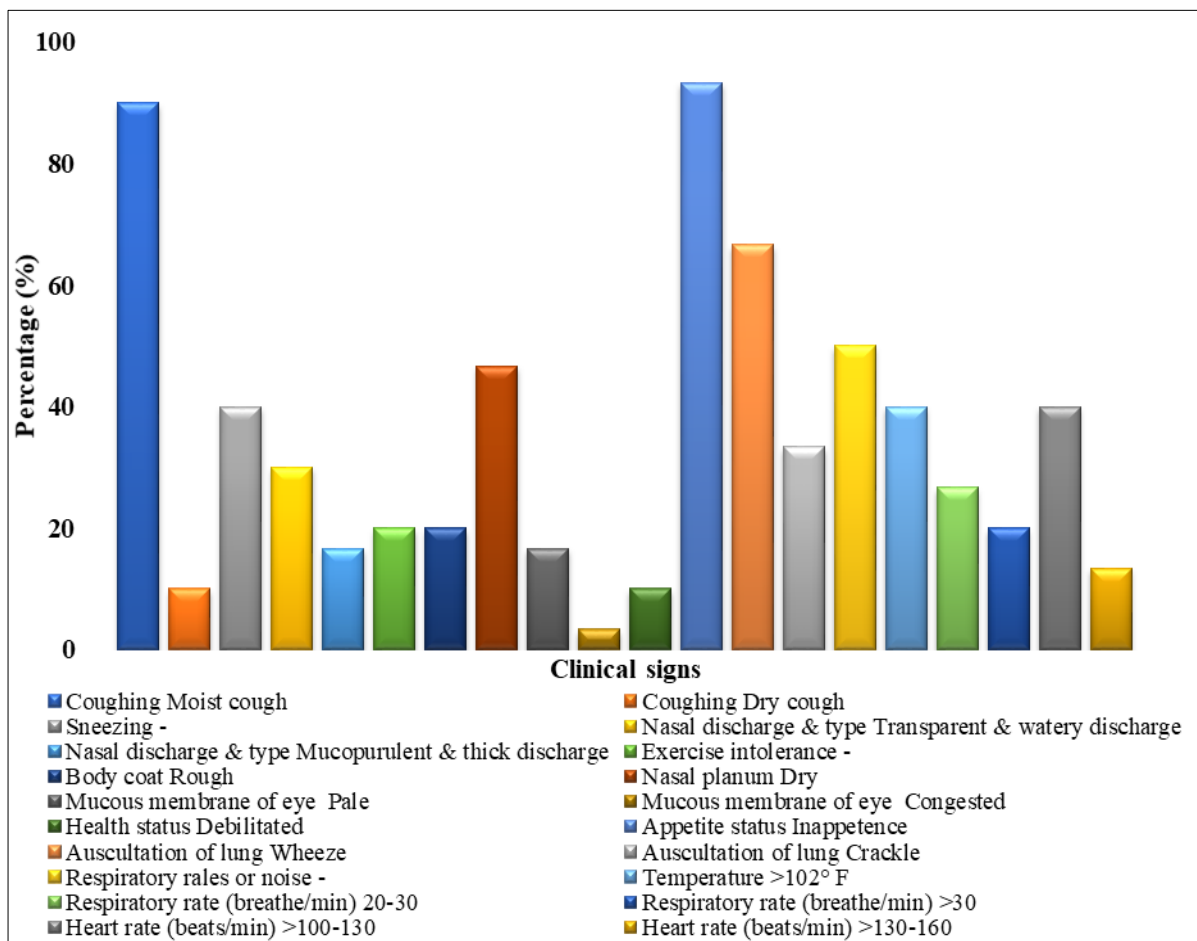
After isolation and identification of the organisms *in-vitro* antibiotic sensitivity test was done by disc diffusion techniques in Mueller Hinton Agar (Quinn *et al.*, 2011) [22]. A single isolate of pure culture was inoculated into 5 ml of Nutrient Broth and incubated overnight at 37°C. The Mueller Hinton Agar plates were inoculated with the pure culture grown in the Nutrient Broth with the help of a sterile swab. The antibiotic discs were placed over the agar plates and then incubated for 24 hours before reading the sensitivity (CLSI, 2022). The antibiotic discs used for the

antibiotic sensitivity test were Amoxicillin-Clavulanic acid, Ceftriaxone, Ceftriaxone-Tazobactam, Enrofloxacin, Doxycycline and Gentamicin. Discs were procured from Hi-Media Pvt. Ltd., Mumbai

In the current study, for therapeutic management, dogs with bacteria-associated respiratory tract infections were divided into three therapeutic trial groups (Group B, Group C and Group D) comprising of 10 animals in each group. Each group *viz.* Group B, Group C and Group D was administered three different antibiotics *i.e.* Doxycycline, Enrofloxacin and Ceftriaxone respectively based on *in-vitro* antibiotic sensitivity test and supportive treatment was also rendered based on animal condition. The dogs were evaluated on the 10<sup>th</sup> day. A therapeutic trial was conducted for 10 days according to Lappin *et al.* (2017) [12] and Vientòs-plotts *et al.* (2021) [25]. Babesiosis, Canine distemper, Chronic liver disease along with ascites and Heart related disease with bacteria-associated respiratory tract infection was treated accordingly as per requirements of the affected dogs. Further statistical analysis of data was done with Microsoft excel.

**Results**

**Clinical Manifestation of Bacteria Associated Respiratory Tract Infection in Dogs:** In the present study, different clinical manifestations are recorded in the Fig 1. The most common clinical findings observed were moist cough (90.00%), sneezing (40.00%), transparent and watery nasal discharge (30.00%), exercise intolerance (20.00%), inappetence (93.33%) and wheezing sound of the lung (66.67%) elicited through thoracic auscultation.

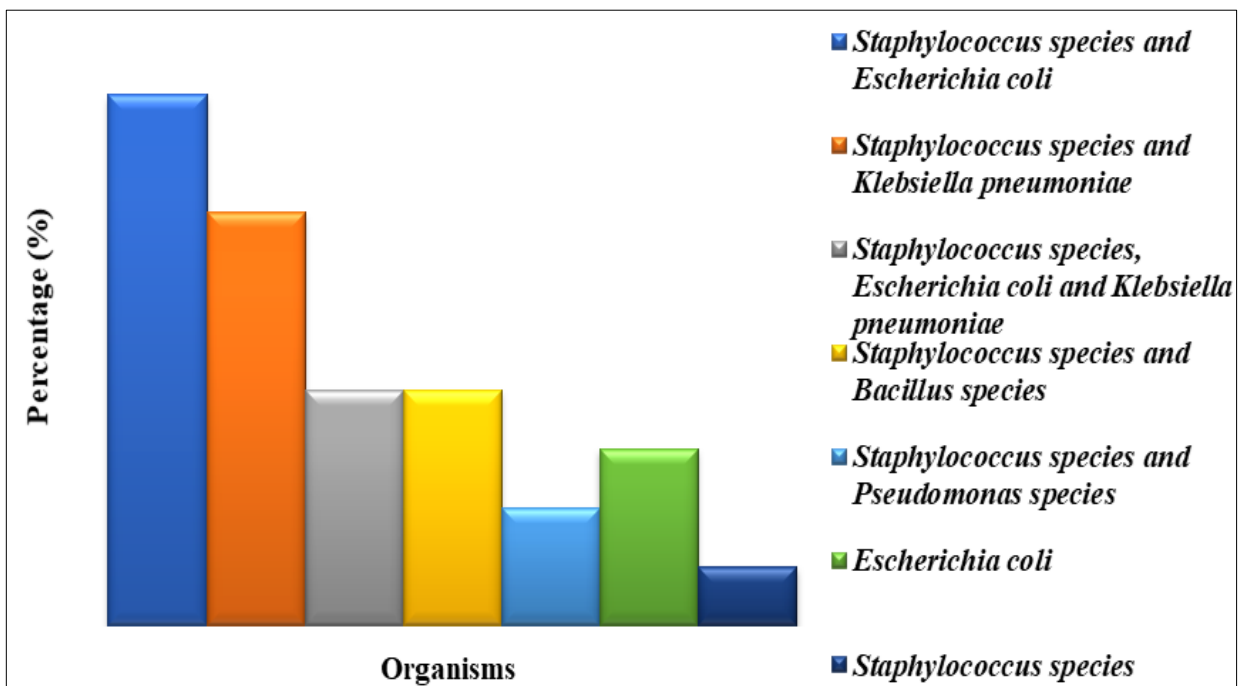


**Fig 1:** Bar diagram showing clinical findings observed in bacteria associated respiratory tract infection in dogs

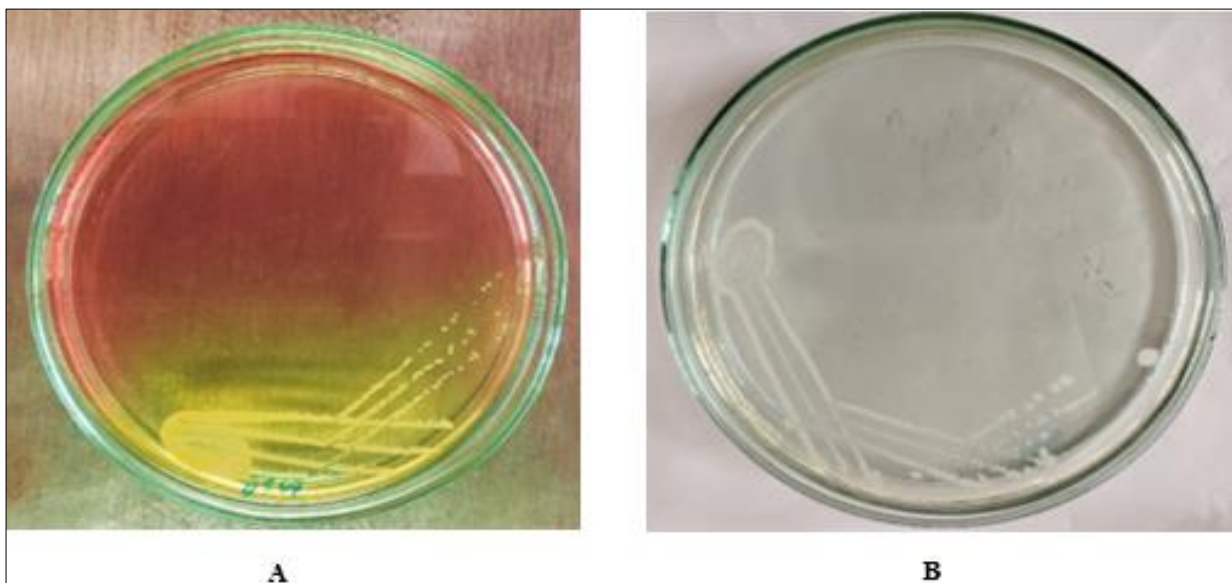
**Isolation and identification of bacteria**

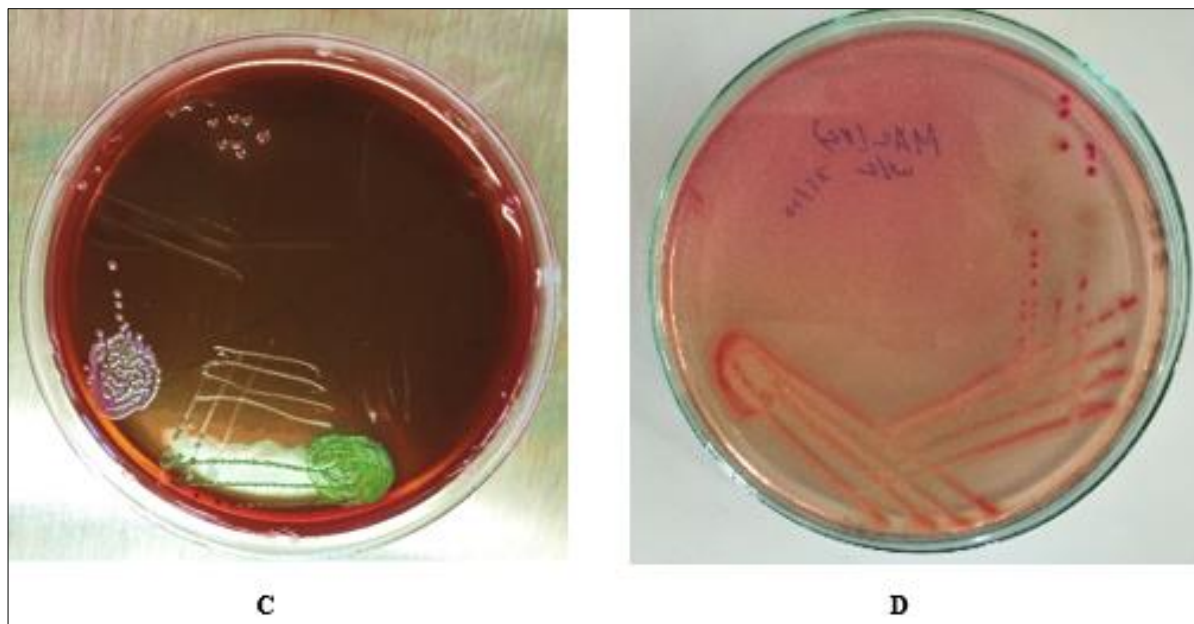
In the current study different bacterial species of the clinically affected dogs were isolated from the nasal swab and presented in the Fig 2, Fig 3, Fig 4 and Fig 5. Under the category of multiple isolates, (30.00%) samples were positive for *Staphylococcus* spp. concurrently with *Escherichia coli* and similarly (23.33%) for *Staphylococcus* spp. concurrently with *Klebsiella pneumoniae* (13.33%) for *Staphylococcus* spp. concurrently with *Escherichia coli* and *Klebsiella pneumoniae*, (13.33%) for *Staphylococcus* spp. concurrently with *Bacillus* spp. and (6.67%) positive for the presence of *Staphylococcus* spp. Concurrently with *Pseudomonas* spp. Under single organism the samples were found to be positive for *Escherichia coli* (10.00%) and *Staphylococcus* spp. (3.33%). Identification of *Staphylococcus* spp. and *Bacillus* spp. were executed based on the Gram's staining properties. Microscopically, *Staphylococcus* spp. were resembled like an irregular clusters on the other hand *Bacillus* spp. were rod-shaped

purple colour in appearance. In mannitol salt agar all the isolates of *Staphylococcus* spp. produce a small yellow-coloured colony. In biochemical test all the isolates of *Staphylococcus* spp. and *Bacillus* spp. showed a catalase positive reaction. *Escherichia coli* were confirmed based on colony characteristics on MacConkey agar (bright pink colour colonies) and metallic sheen were observed in EMB agar. The further confirmatory diagnosis was done by biochemical test i.e. IMViC test. *Escherichia coli* showed a positive reaction for the indole and methyl red test. *Klebsiella pneumoniae* on Gram's staining showed gram-negative rods and produced lactose fermenting mucoid colony on MacConkey agar. On biochemical test, organisms showed positive reaction for Voges-proskauer and Citrate utilization test. *Pseudomonas* spp. on Gram's staining showed gram-negative rods. Further confirmation was done by IMViC test and in the result, the Citrate utilization test revealed a positive reaction whereas all other exhibited a negative reaction.

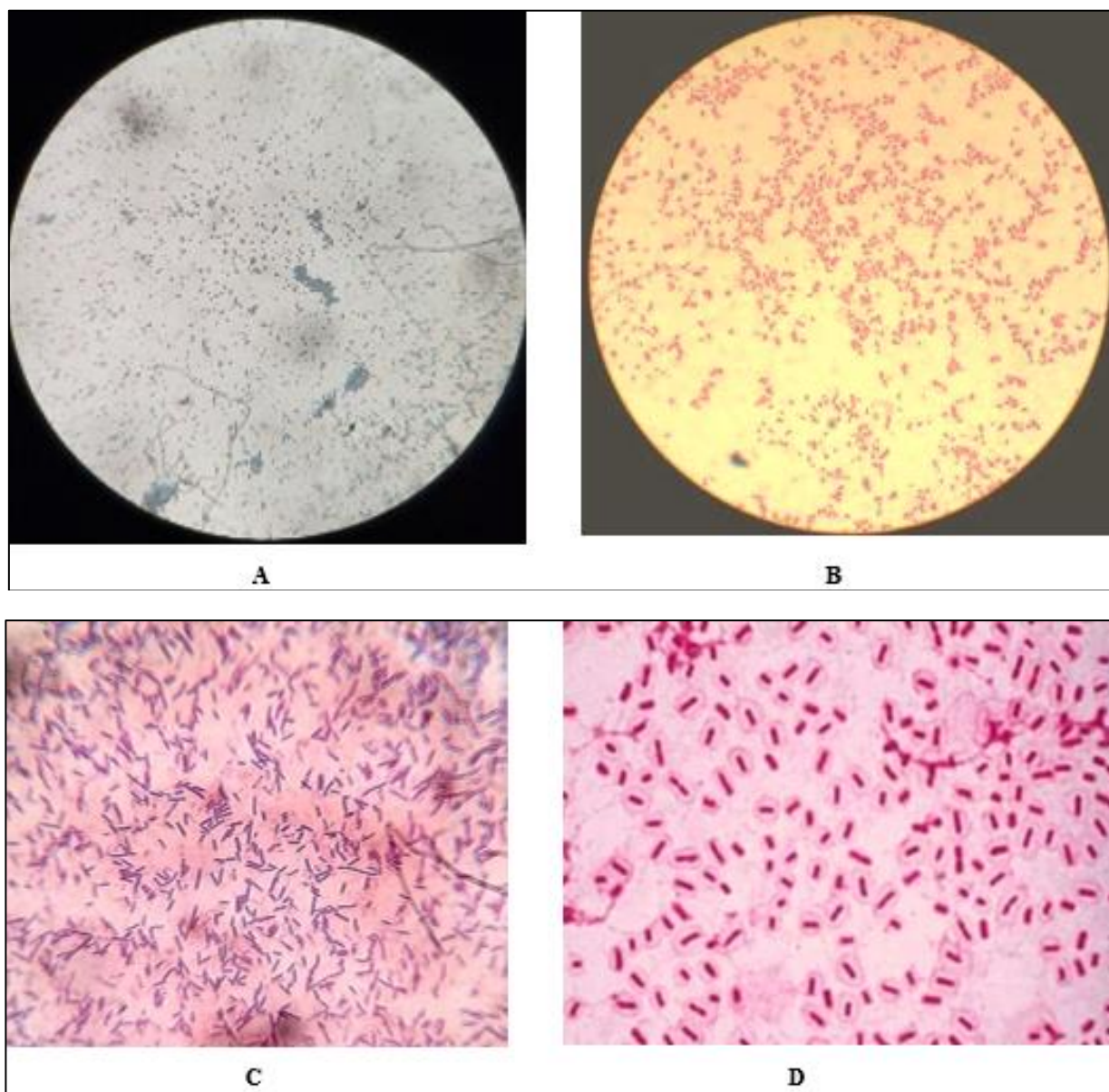


**Fig 2:** Bar diagram indicating the percentage of bacterial isolates in the respiratory tract infected dogs





**Fig 3:** Photograph showing growth of *Staphylococcus* species on MSA (A), *Bacillus* species on Nutrient Agar (B), *E. coli*; *K. pneumoniae* on EMB agar (C) and *E. coli* on MLA (D)



**Fig 4:** Photomicrograph showing *Staphylococcus* species (A), *E. coli* (B), *Bacillus* species (C) and (D) *K. pneumoniae* after gram's staining

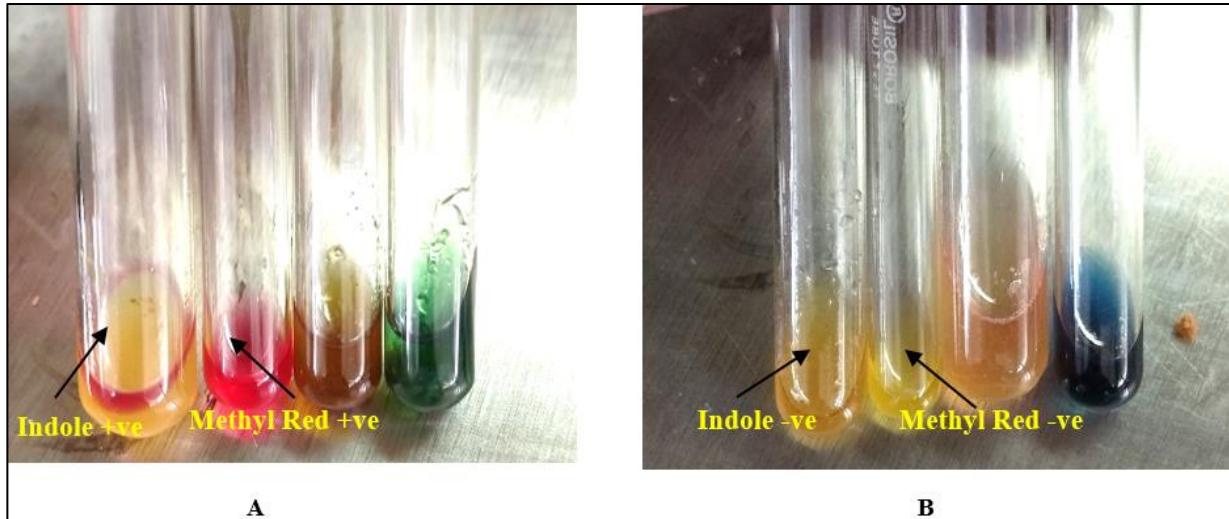


Fig 5: Photograph showing IMVIC test for *E. coli* (A) and *K. pneumoniae* (B)

**In-vitro Antibiotic Sensitivity Test (ABST)**

In the recent study, an antibiotic sensitivity test was done by disc diffusion technique in Mueller Hinton Agar (Fig 6 and Fig 7). In the result, for *Staphylococcus* spp. the highest sensitivity was observed on Ceftriaxone (100%) followed by Enrofloxacin (81.48%), Doxycycline (77.77%), Ceftriaxone potentiated with tazobactam (11.11%) and Gentamicin (11.11%) but found resistant to Amoxicillin potentiated with clavulanic acid. Antibiotic sensitivity test for *Escherichia coli* revealed the highest sensitivity towards Doxycycline (62.50%) followed by Enrofloxacin (56.25%), Ceftriaxone (50.00%) and Gentamicin (37.50%) but found resistant to Ceftriaxone potentiated with tazobactam and Amoxicillin potentiated with clavulanic acid. *Klebsiella pneumoniae* was found highest sensitive to Ceftriaxone (90.91%) followed by

Doxycycline (72.73%), Enrofloxacin (36.36%) and Ceftriaxone potentiated with tazobactam (18.18%) but found resistant to Gentamicin and Amoxicillin potentiated with clavulanic acid respectively. An antibiotic sensitivity test was done on isolates of *Pseudomonas* spp. ABST revealed (100.00%) sensitivity to Ceftriaxone, Doxycycline, Enrofloxacin and Ceftriaxone potentiated with tazobactam and (50.00%) sensitivity to Gentamicin but found resistant to Amoxicillin potentiated with clavulanic acid. *Bacillus* spp. was found to be the highest sensitivity to Doxycycline (100.00%) and Ceftriaxone (100.00%) followed by Enrofloxacin (75.00%) but found resistant to Ceftriaxone potentiated with tazobactam, Gentamicin and Amoxicillin potentiated with clavulanic acid.

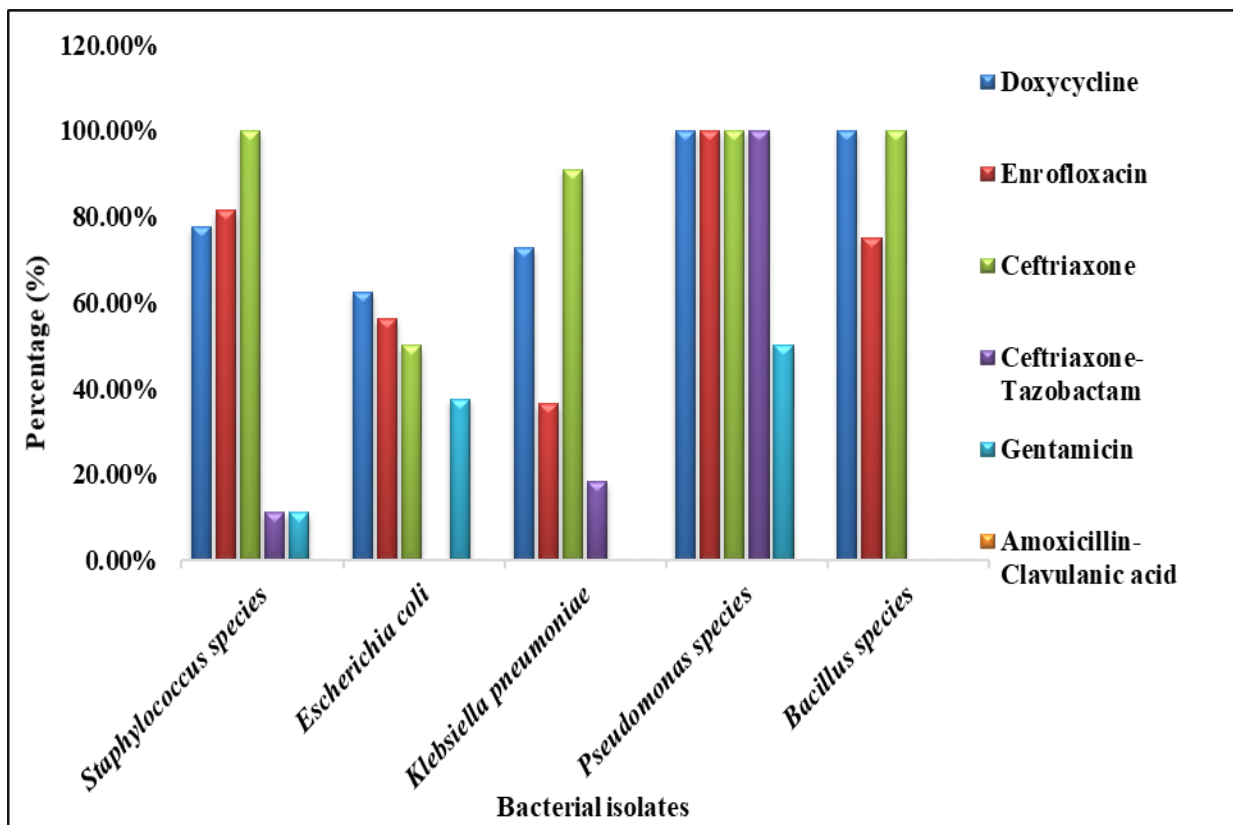
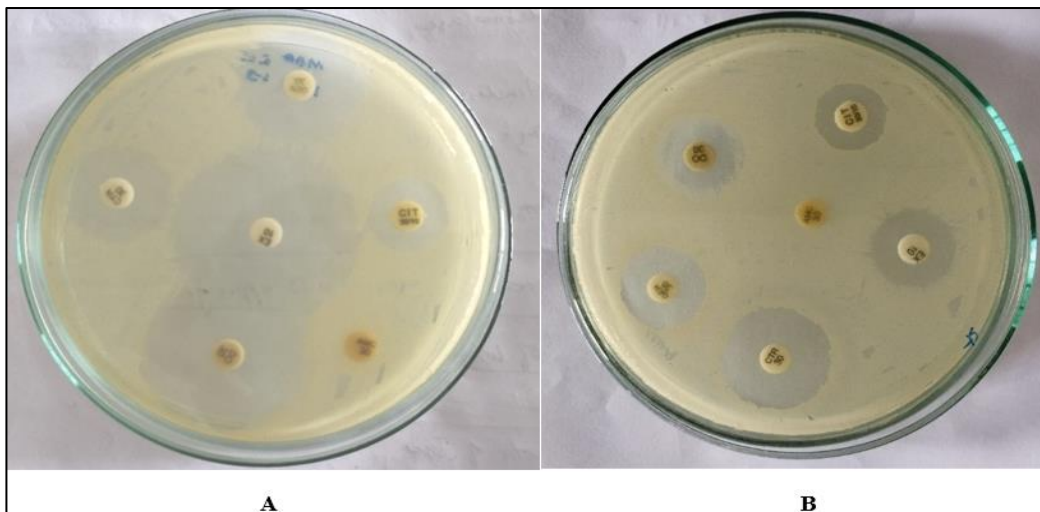


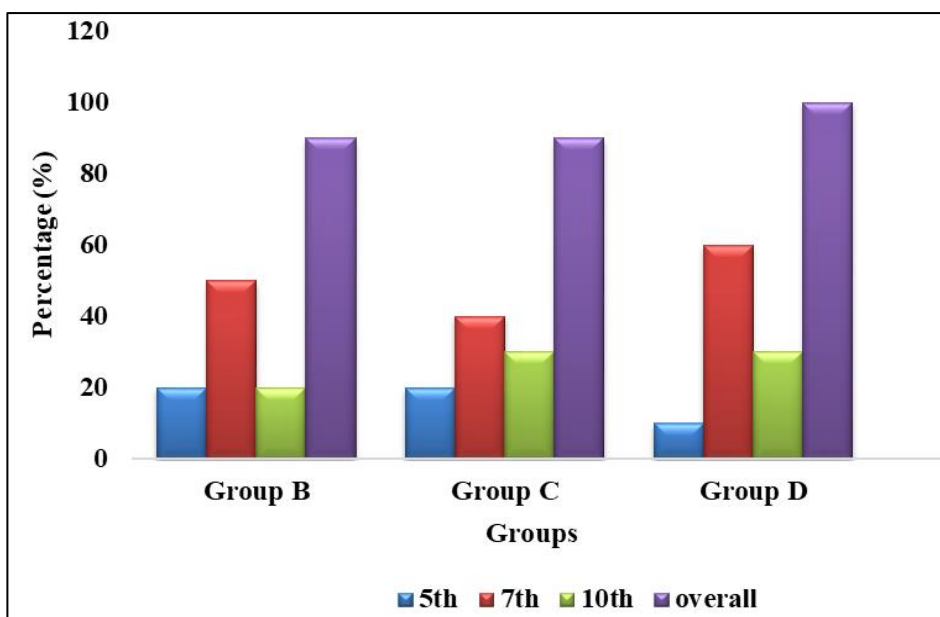
Fig 6: Bar diagram showing antibiotic sensitivity of isolates in bacteria associated respiratory tract infection in dogs



**Fig 7:** Photograph showing (A) and (B) *in-vitro* antibiotic sensitivity test

**Therapeutic Management:** The post-treatment clinical evaluation of signs and symptoms was done for 10 days. On the basis of an *in-vitro* antibiotic sensitivity test, three antibiotics namely Doxycycline hydrochloride, Enrofloxacin and Ceftriaxone sodium were administered to the animals along with supportive therapy for the treatment of bacteria-associated respiratory tract infections in dog. Overall recovery is illustrated in the Fig 8, Fig 9 and Fig 10. In Group B, ten dogs were treated with pantoprazole, 20% mannitol, furosemide and vitamin supplements. Among ten dogs parenteral administration of Doxycycline hydrochloride was given to eight dogs and the remaining two dogs were treated with oral administration of Doxycycline hydrochloride. One dog was given ramipril, pimobendan and a cardio-supportive drug. Four dogs were given imidocarb along with atropine sulphate at 14 days intervals. Haematonic was given to four dogs. Liver supportive and pheniramine maleate was given in two dogs. Prednisolone was given parenterally in six dogs for 5 days. Meloxicam was also given parenterally in one dog for 3 days. Nebulization was performed in three dogs. In Group B two dogs recovered on 5<sup>th</sup> day, five dogs recovered on 7<sup>th</sup> day and another two dogs recovered on 10<sup>th</sup>

day for respiratory tract infection and remission of clinical signs. In Group C, ten dogs were treated with 20% mannitol, furosemide, pantoprazole and vitamin supplements parenterally. Among ten dogs eight dogs were treated with parenteral administration of Enrofloxacin and two dogs were given Enrofloxacin tablet orally. Homeopathic medicine rhus toxicodendron, vitamin C, canine distemper immunoglobulin and nervine tonic were given to one dog. Pheniramine maleate was given in one dog and nebulization was performed in five dogs. In Group C, two dogs recovered on 5<sup>th</sup> day, four dogs recovered on 7<sup>th</sup> day and three dogs recovered on 10<sup>th</sup> day. In Group D, ten dogs were treated with 20% mannitol, furosemide, Ceftriaxone sodium and vitamin supplements parenterally. Meloxicam tablet was given orally to two dogs. Pimobendan and cardio-supportive drugs were given orally in two dogs. Ramipril was given in three dogs through the oral route. Fructodex in saline was given to one dog through the parenteral route. Prednisolone was given through parenterally in three dogs for 5 days and liver supportive and hematonic were given in two dogs. Nebulization was performed in three dogs. In Group D, only one dog recovered on 5<sup>th</sup> day, six dogs recovered on 7<sup>th</sup> day and three dogs recovered on 10<sup>th</sup> day.



**Fig 8:** Bar diagram showing therapeutic response between groups



**Fig 9:** Photograph showing presence of mucopurulent nasal discharge in pre-treatment period



**Fig 10:** Photograph showing disappearance of mucopurulent nasal discharge in recovery period

### Discussion

In the present study, the most common clinical findings observed in dogs were analogous to Elgalfy *et al.* (2022)<sup>[8]</sup>, Arsevska *et al.* (2018)<sup>[2]</sup>, Ellis *et al.* (2016)<sup>[9]</sup>, Sanchez-Viscaino *et al.* (2016)<sup>[24]</sup>, Dear (2014)<sup>[7]</sup>, Ayodhya *et al.* (2013)<sup>[3]</sup>, Vieson *et al.* (2012)<sup>[26]</sup> and Peeters *et al.* (2000)<sup>[19]</sup>. Whenever the causative organisms enter in to the respiratory tract by inhalation, they invade the mucosal layer and multiply in the epithelium. As a result of this different types of inflammatory mediators, bacterial toxins and enzymes release and lead to inflammation and copious exudates production as well as a rise of body temperature. There is a loss of mucociliary movement because of the

destruction of respiratory epithelium and followed by sloughing off. In the later stage, necrotic material oozes out with a mucopurulent consistency (Baron, 1996)<sup>[4]</sup>. Finally, to evacuate the exudates from the tract cough is generated in the affected dogs.

In the current study, different bacteria were isolated from the samples and similar observations were also recorded by various researchers Menard *et al.* (2022)<sup>[14]</sup>, Moon *et al.* (2022)<sup>[15]</sup>, Elgalfy *et al.* (2022)<sup>[8]</sup>, Yadav *et al.* (2020)<sup>[28]</sup>, Ayodhya *et al.* (2013)<sup>[3]</sup>, Moonsun *et al.* (2004)<sup>[16]</sup>, Moyaert *et al.* (2019)<sup>[17]</sup> and Köse *et al.* (2021)<sup>[11]</sup>. *Staphylococcus* spp., *Escherichia coli* and *Bacillus* spp. are present as a normal flora in the respiratory tract in the dog

according to Ajuwape *et al.* (2006) <sup>[1]</sup>. Due to immunodeficiency or other stress factor organisms may flare up and leads to respiratory tract infection in dogs as mentioned by Lappin *et al.* (2017) <sup>[12]</sup>. *Pseudomonas* spp. and *Klebsiella pneumoniae* are gram negative opportunistic bacteria in dogs. Infections mainly develop when the animal is immune compromised or normal barriers are breached as described by Nielsen *et al.* (2022) <sup>[18]</sup> and Zhang *et al.* (2022) <sup>[29]</sup>.

In the recent study, *Staphylococcus* spp. isolates were sensitive to Ceftriaxone (100.00%) followed by other antibiotics *in-vitro* antibiotic sensitivity test. These observations were in accordance with Moyaert *et al.* (2019) <sup>[17]</sup>, Yadav *et al.* (2019) <sup>[27]</sup> and Rheinwald *et al.* (2015) <sup>[23]</sup>. *Klebsiella pneumoniae* isolates were sensitive to Ceftriaxone (90.91%) followed by other antibiotics and these observations were in accordance with Köse *et al.* (2021) <sup>[11]</sup> and Yadav *et al.* (2019) <sup>[27]</sup>. The present finding revealed *Escherichia coli* had the highest sensitivity towards Doxycycline (62.50%) followed by Enrofloxacin, Ceftriaxone and Gentamicin. Similar observations were also recorded by Moyaert *et al.* (2019) <sup>[17]</sup>, Yadav *et al.* (2019) <sup>[27]</sup> and Rheinwald *et al.* (2015) <sup>[23]</sup>. *Pseudomonas* spp. was found to be 100.00% sensitivity to Ceftriaxone, Doxycycline, Enrofloxacin and Ceftriaxone potentiated with tazobactam and 50.00% sensitivity to Gentamicin. These observations were in accordance with Yadav *et al.* (2019) <sup>[27]</sup> and Rheinwald *et al.* (2015) <sup>[23]</sup>. *Bacillus* spp. isolates were highest sensitive to Doxycycline and Ceftriaxone followed by Enrofloxacin and resistant to Ceftriaxone potentiated with tazobactam, Gentamicin and Amoxicillin potentiated with clavulanic acid. These findings were similar to Yadav *et al.* (2019) <sup>[27]</sup>. High and moderate level of resistance was exhibited by the isolates to Amoxicillin potentiated with clavulanic acid, Ceftriaxone potentiated with tazobactam and Gentamicin in the present study might be due to indiscriminate and over use of antibiotics in the treatment of animal (Mavrides *et al.*, 2021) <sup>[13]</sup>.

Three groups of dogs, Groups B, C and D comprising 10 dogs in each group were treated with Doxycycline hydrochloride, Enrofloxacin and Ceftriaxone sodium respectively along with supportive therapy. A similar report on the administration of antibiotic therapy in bacteria associated respiratory tract infection was made by Lappin *et al.* (2017) <sup>[12]</sup>. The antibiotic therapy should be chosen based on antimicrobial susceptibility tests (Vieson *et al.*, 2012) <sup>[26]</sup>. Carbone *et al.* (2001) <sup>[5]</sup> reported that the clinical efficacy of different antibiotics may vary from *in-vitro* antibiotic sensitivity test results.

### Conclusion

The most common clinical sign observed was moist cough (90.00%). *Staphylococcus* spp. concurrently with *Escherichia coli* (30.00%) were common isolates. In *in-vitro* antibiotic sensitivity test *Staphylococcus* spp., *Klebsiella pneumoniae*, *Pseudomonas* spp. and *Bacillus* spp. showed highest sensitive to Ceftriaxone. *Escherichia coli* and *Bacillus* spp. showed highest sensitive to Doxycycline. Ceftriaxone sodium was found 100.00% effective followed by Doxycycline hydrochloride and Enrofloxacin (90.00%).

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