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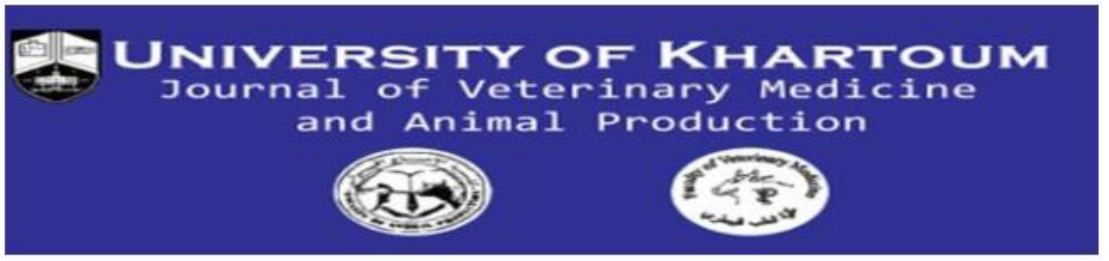

Isolation and Identification of Aerobic Bacteria Associated with Respiratory Tract Infection in Chechens (Baladi Types) in Khartoum State, Sudan

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Abstract

This study was carried out in Khartoum State (Khartoum, Bahri and Odurman) from January to July, 2014 to isolate and identify the aerobic bacteria associated with the respiratory tract infections of chickens (Baladi Type). Fourty five samples were collected from different infected chickens (male and female from one month to ten months) showing clear respiratory symptoms. The samples were collected from Large Baladi (30 samples, 66.7%) and Bare-neck (15 samples, 33.3%). The samples were nasal, conjunctival, infra-orbital sinuses and tracheal swabs and specimen from lungs. Different types of media (Nutrient broth, Nutrient agar, Blood agar, Chocolate agar, Peptone water) were used for bacterial culturing. Identification of all isolates was carried out on the basis of morphology, staining reaction, cultural characteristics and biochemical reactions. All collected samples showed bacterial growth and yielded 55 isolates. Thirty four (61.8%) of the isolates were Gram negative bacteria and the remaining 21 (38.2%) isolates were Gram positive bacteria. The Gram negative bacteria isolated were *Escherichia coli* (16.4%), *Pseudomonas spp* (9.1%), *Klebsiella spp* (5.5%), *Shigella spp*(3.6%), *Proteus spp* (9.1%), *Pasteurella multocida* (7.3%), *Bordetella spp* (3.6%), *Haemophilus paragallinarum* (3.6%) and *Yersinia pseudotuberclosis* (3.6%). The Gram positive bacteria isolated were *Staphylococcus spp* (12.7%), *Micrococcus spp* (9.1%), *Bacillus spp* (10.9%) and *Streptococcus spp* (5.5%). It was concluded that the bacterial respiratory tract infections constitute one of the constraints to poultry production (Baladi Type) in Khartoum State, Sudan.

Keywords: Aerobic bacteria, Respiratory tract infections, chickens, Sudan

المستخلص

أجريت هذه الدراسة بولاية الخرطوم (الخرطوم، بحرى وأم درمان) فى الفترة ما بين شهر يناير حتى يوليو 2014 لعزل وتعريف البكتريا الهوائية التى تصاحب اصابات الجهاز التنفسى فى الدواجن (النوع البلدى). جمعت خمس وأربعين عينة من أنواع مختلفة من الدجاج (ذكور واناث وأعمارهم تتراوح بين شهر وعشرة شهور) التى تظهر عليها اعراض الجهاز التنفسى. العينات جمعت من النوع البلدى الكبير (30 عينة، 66.7%) والعارى الرقبة (15 عينة، 33.3%). العينات هى مسحات من الأنف، ملتحمة العين، الجيب تحت الحجاج، القصبة الهوائية وبالاضافة إلى عينات من الرئة. أنواع متعددة من الوسائط المغذية (المرق المغذى، الأجار المغذى، أجار الدم، أجار الشوكولاتة، ماء البيتون) استخدمت لزراعة البكتريا. والتعرف على المعزولات تم بدراسة الشكل، تفاعل الصبغة، نموها فى الأوساط والتفاعلات الكيميائية الحيوية. كل العينات التى جمعت أظهرت نمواً واضحاً وأنتجت 55عزلة من البكتريا. أربعة وثلاثون (61.8%) عزلة وجدت سالبة لصبغة جرام و21 (38.2%) عزلة المتبقية موجبة لصبغة جرام. البكتريا سالبة الجرام التى تم عزلها فى هذه الدراسة هى أنواع الإشريكية القولونية (16.4%)، أنواع الزائفة (9.1%)، أنواع االكلبسيلة (5.5%)، أنواع الشيغلة (3.6%)، أنواع المتقلبة (9.1%)، الباستيوريلة ملتوسيدا (7.3%)، أنواع البوردتيلا (3.6%)، الهيموفيلاس باراقالنيرم ويرسينيا السل الكاذب (3.6%). البكتريا موجبة الجرام التى تم عزلها فى هذه الدراسة هى أنواع العنقودية (12.7%)، أنواع المكورة الدقيقة (9.1%)، أنواع العصوية (10.9%) وأنواع العقدية (5.5%). فى الختام وجد أن أمراض الجهاز التنفسى البكتيرية تشكل عائقا لانتاج الدواجن (النوع البلدى) فى ولاية الخرطوم، السودان.

Introduction

Among infectious diseases, respiratory infections are the most serious diseases affecting poultry and cause heavy economic losses in the poultry industry worldwide. Diseases of the respiratory tract are a significant component of the overall disease incidence in poultry. In many cases, respiratory disease observed in a flock may be a component of a multi-systemic disease or it may be the predominant disease with lesser involvement of other organ systems.

Bacterial pathogens play an important role in causing respiratory disease in domestic poultry species. In many cases, the bacterial component of a respiratory disease colonizes the respiratory system only after a primary viral or environmental insult. In some cases, such as infectious coryza or infectious laryngotracheitis, the disease may be limited to the respiratory system, at least initially. Various pathogens may initiate respiratory disease in poultry, including a variety of viruses, bacteria and fungi. Environmental factors may augment these pathogens to produce the clinically observed signs and lesions. In avian host, several microorganisms of the genus *Pasteurella* (*P. multocida, P. gallinarum, P. haemolytica* and *P. anatipestifer*); *Bordetella* (*B. avium*) and *Haemophilus (H. paragallinarum)* are involved in respiratory disease complex (Hafez, 2002*)*.

Village chickens in Sudan (Baladi type) have the same importance for the villagers in rural areas. Out of a total population of 45.3 million chickens in Sudan the conventional sector comprises around 30 million from which the annual meat and egg production is 20.1 million birds and 900 million eggs, respectively (Sulieman, 1996). Desai (1962) classified the indigenous breed (Baladi) into three types that include Large Baladi, Bare-neck and Betwil. The Large Baladi is the most common type and distributed all over the country. The birds are of medium size (adults weighting 1.350-1.362 kg), with a small crushed comb and a lot of plumage of varying colors. The Bare-neck type is smaller and characterized by a featherless neck. It occurs in various colors, is very active and comparatively more resistant to diseases than the other two types (Desai, 1962). The Betwil type is the smallest in body size. The adult body weight averages 0.681-0.908 kg, with tiny black legs and a compact body (Desai, 1962). The native Baladi hen lay on average 40-50 eggs per year while the Betwil is considered the best layer producing 70-80 eggs per year (Sulieman, 1996). However, under controlled conditions and improved management the average egg production can increase to 172-177(Sulieman, 1996 and Mekki, 1998).

Among the diseases of village chickens, respiratory infections were ranked as the most important diseases. Although traditional poultry production has been present throughout Sudanese villages and rural areas as well as in some towns, little information about the production system and respiratory diseases is available in the literature.

Up to now, there was no detailed study on the epidemiology of respiratory infections in village chickens in Sudan elucidating the exact effect of the diseases on the chicken production. The present study is intended to throw light on the situation of respiratory infections in village chickens in Khartoum

State, Sudan by isolation and identification of aerobic bacteria associated with respiratory tract infections in village chickens (Baladi type).

Materials and methods

Collection of samples

A total of 45 samples were collected from sick village chickens with clinical symptoms of respiratory tract infections. These signs include mucoid or serous nasal discharges, sneezing, lacrimation, conjunctivitis and facial swelling. The samples were collected from the Baladi (Local or indigenous) breed in Khartoum State. All samples were collected from houses and markets where chickens are always not vaccinated against infectious diseases due to lacking organized breeding systems for this breed.

Sampling

Nostril

Sterile cotton wool swabs were used for sampling the nostril of live chickens.

Trachea

Sterile cotton wool swab was used for taking samples from the inside of the trachea of live and recently slaughtered chickens.

Lung

The lung of a recently slaughtered chicken was cut into pieces with sterile scalpel and a small piece was taken by a sterile forceps.

Conjunctival sac

The eyes of the live and dead chickens were opened with sterile forceps and sterile cotton wool swab was used for sampling the conjunctival sac.

Infra-orbital sinus

The infra-orbital sinuses of the live swelled head chickens were opened with sterile forceps and sterile cotton wool swab was used for sampling the infra-orbital sinuses.

Culture methods

The collected samples were cultured onto a blood agar, MacConkey's agar and chocolate agar medium. The inoculated plates were incubated for 24-48 hr at 37^{o}C. The colonies characteristics were observed. Smears were made from each type of colony, stained by Gram's method and examined under light microscope for cell morphology, cell arrangement and staining reaction.

Purification and preservation of culture

Purification of culture was done by subculturing part of typical well separated colony on the corresponding medium. The process was repeated several times. The purity of the culture was checked by examining stained smears. Pure culture was then inoculated into cooked meat medium and incubated overnight at 37^{o}C. The pure culture was then stored at 4^{o}C. The pure culture was used for studying cultural and biochemical characteristics.

Microscopic examination

Smears were made from each type of colony on primary culture and from purified colonies. Then fixed by heating and stained by Gram stain method according to Barrow and Feltham (2003**)**, and examined microscopically under oil immersion lens. The smear was examined for cell morphology, cell arrangement and staining reaction.

Identification of isolates

The purified isolates were identified according to criteria described by Barrow and Feltham (2003). This included staining reaction, cell morphology, growth condition, colonial characteristics on different media, haemolysis on blood agar and biochemical characteristics.

Results

Isolation and Identification

All the 45 collected samples showed clear bacterial growth in all type of media. The 45 samples gave 55 isolates, 34 (61.8%) of them were Gram-negative bacteria, and the rest 21 (38.2%) isolates were Gram-positive bacteria (Table, 1).

The 55 isolates were identified as: 5(9.1%) *Pseudomonas* species, 9(16.4%) *E.coli*, 3(5.5%) *Klebsiella* species, 2 (3.6%) *Bordetella* species, 2(3.6%) *Shigella* species, 5(9.1%) *Proteus* species, 2(3.6%) *Haemophilus paragallinarum*, 2(3.6%) *Yersinia pseudotuberculosis*, 4(7.3%) *Pasteurella*

multocida, 7(12.7%) *Staphylococcus* species, 3(5.5%) *Streptococcus* species, 5(9.1%) *Micrococcus* species and 6(10.9%) *Bacillus* species (Tables 4 and 5).

Bacteria isolated from nostrils

The number of samples collected from nostrils was 10 samples (22.2%). All samples showed positive growth when cultured and gave 23 isolates (41.8%). The 23 bacterial isolates comprised both Gram-negative and Gram-positive bacteria. Eighteen isolates (78.3%) were found to be Gram-negative bacteria while 5 isolates (21.7%) were Gram-positive bacteria. The 18 of Gram-negative bacteria were one isolate of *Pseudomonas aeruginosa* (5.6%), one isolate of *Pseudomonas fluorescence* (5.6%), 2 isolates of *E.coli* (11.1%), 3 isolates of *Klebsiella aerogenes* (16.7%), 2 isolates of *Bordetella avium* (11.1%), 2 isolates of *Shigella flexineri* (11.1%), 5 isolates of *Proteus vulgaris* (27.8%), 2 isolates of *Yersinia pseudotuberculosis* (11.1%).

The 5 isolates of Gram positive bacteria were 2 isolates *Staphylococcus gallinarum* (40%), one isolate of *Micrococcus varian* (20%), one isolate of *Bacillus cereus* (20%) and one isolate of *Bacillus megaterium* (20%) (Tables, 1, 4 and 5).

Bacteria isolated from conjunctiva

The samples collected from conjunctiva were 5 samples.All samples showed positive growth when cultured and gave 6 isolates. Three isolates (50%) were Gram-positive bacteria, one isolate was *Staphylococcus gallinarum* (33.3%), one isolate was *Micrococcus varian* (33.3%) and one isolate was *Bacillus megaterium* (33.3%). The other 3 isolates (50%) were Gram-negative bacteria, 2 isolates were *E. coli* (66.7%) and the other one isolate was *Haemophilus paragallinarum* (33.3%) (Tables 1, 4 and 5).

Bacteria isolated from trachea

Twenty samples were collected from trachea. All samples showed positive growth and gave 17 isolates. Nine of them (52.9%) were Gram-positive bacteria, one isolate *Staphylococcus aureus* (11.1%), one isolate *Staphylococcus gallinarum* (11.1%), 3 isolates *Streptococcus pneumoniae* (33.3%), one isolate *Bacillus cereus* (11.1%) and 3 isolates *Micrococcuslentus* (33.3%). The other 8 isolates (47.1%) were Gram-negative bacteria, 3 isolates *Pseudomonas areuginosa* (37.5%), 2 isolates *Pasteurella multocida* (25%) and 3 isolates *E. coli* (37.5%) (Tables 1, 4 and 5).

Bacteria isolated from lungs

The samples collected from lung were 5 samples. All samples showed positive growth when cultured and gave 5 isolates. Three of them (60%) were Gram-negative bacteria, 2 isolates (66.7%) were *Pasteurella multocida* and one isolate (33.3%) was *E. coli*. The other 2 isolates (40%) were Gram positive bacteria, one isolate (50%) was *Staphylococcus areus* and other one isolate (50%) was *Bacillus cereus* (Tables, 1, 4 and 5).

Bacteria isolated from infra-orbital sinuses

The samples collected from infra-orbital sinus were 5 samples. All samples showed positive growth when cultured and gave 4 isolates. Two of them (50%) were Gram-negative bacteria, oneisolate (50%) was *E. coli* and other one isolate (50%) was *Haemophilus paragallinarum.* The other 2 isolates (50%) were Gram-positive bacteria, one isolate (50%) was *Staphylococcus gallinarum* and other one isolate (50%) was *Bacillus megaterium.*

Cultural, microscopic and biochemical reactions of the isolates

***Staphylococcus* species isolates**

On blood agar medium, colonies of *Staphylococcus* species appeared round, smooth and glistening with golden pigmentation. Gram-positive, non spore-forming, cocci occurred in pairs or clusters were seen when Gram's stained smear was examined microscopically.

All isolates of *Staphylococcus* were oxidase negative and catalase positive. There was no H_2S production and was indole negative. In *Staphylococcus aureus* all sugar reactions were found positive except xylose. In *Staphylococcus epidermidis* all sugar reactions were negative except lactose, mannose and sucrose. *Staphylococcus gallinarum* was positive in all sugar reactions. *Staphylococcus aureus* was coagulase positive while other species were negative.

Micrococcus species isolates

On blood agar medium, colonies of *Micrococcus* species appeared round, smooth and glistening. Gram-positive, non spore-forming cocci, occurring in pairs or clusters were seen when Gram's stained smear was examined under the microscope. *Micrococcus* species isolates were oxidase, catalase and VP positive and attacked sugar oxidatively, did not produced H_2S and non motile.

Streptococcus species isolates
On blood agar medium, the colonies of *Streptococcus* species were small in diameter, appeared round, smooth, glistening and looking like dew drops. It was Gram-positive, non spore-forming cocci, occurring in pairs or in chains when examined under microscope. *Streptococcus* species were oxidase negative, catalase negative, and attacked carbohydrate fermentively. Gas was not produced also H_2S and was indole negative. *Streptococcus lentus* was mannitol and VP positive. *Streptococcus pneumonia* was VP and mannitol negative and non motile.

Bacillus species isolates
On blood agar medium and Mac Conkey's agar medium the colonies of *Bacillus* species looked roughs, flat, gray and mucoid. The *Bacillus* isolated were Gram-positive and large spore forming rods. They were catalase positive, oxidase, indole, urease and VP negative except *Bacillus cereus*. Bacillus isolated gave weak positive reaction in citrate test.

***Yersinia pseudotuberculosis* isolates**
On Mac Conkey's agar medium mucoid pink colonies of *Yersinia pseudotuberculosis* were seen. It was Gram-negative, it was indole, VP, oxidase and sucrose negative, but MR, urease and salicin positive and motile.

***Pasteurella multocida* isolates**
On blood agar medium grayish round and large mucoid colonies of *Pasteurella multocida* were seen. *Pasteurella multocida* was Gram-negative, small rod when examined under the microscope. It was oxidase and indole positive, urease negative and non motile.

Bordetella species isolates
The colonies of *Bordetella* species isolated in this study, appeared large flat glistening on blood agar medium. On Gram's stained smear, small Gram negative, coccobacilli were seen when examined microscopically. The isolated *Bordetella* was oxidase, lactose, urease and citrate positive; H_2S negative and did not ferment carbohydrate.

***Haemophilus paragallinarum* isolates**
On chocolate agar medium, the colonies of *Haemophilus paragallinarum* were dew drop like and grayish in color. On microscopic examination, Gram- negative, coccobacilli that occurred in pairs or short chains were seen. *Haemophilus paragallinarum* isolates of this study were catalase, oxidase, indole, urease and lactose negative.

***Escherichia coli* isolates**
The isolates of *Escherichia coli* appeared on Mac Conkey's agar medium as large rose colored colonies indicating lactose fermentation. Gram- negative, non spore forming rods were seen under microscope. All isolates were lactose fermenters, indole and MR positive, and they were VP, oxidase, citrate, urease and H_2S negative. Acid and gas were produced from glucose.

***Shigella flexineri* isolates**
On Mac Conkey's agar medium the isolated *Shigella flexineri* gave non lactose fermenting colonies. Non spore forming, non capsulated, Gram negative rods were seen microscopically. This isolate was indole positive, urease negative, and produced acid from majority of sugars tested, and was non motile.

Pseudomonas species isolates
On Mac Conkey's agar medium *Pseudomonas* species isolated in this work produced pale-colored colonies. Gram negative, non spore forming and non capsulated rod cells were observed microscopically. *Ps. aeruginosa* fermented only glucose and xylose, and on nutrient agar medium the isolates gave blue green pigment. *Pseudomonas diminuta* was citrate and urease negative. *Pseudomonas fluorescence* fermented all sugars except salicin. All *Pseudomonas* species isolates were oxidase positive.

Proteus species isolates

On Mac Conkey's agar medium *Proteus* species isolated in this work produced pale-colored non-lactose fermenting colonies. On nutrient agar medium distinctive smell colonies with swarming appearance were detected. Gram-negative, non spore forming and non capsulated rods were seen under microscope. All *Proteus* species were urease positive and lactose fermenters.

Klebsiella species isolates

On Mac Conkey's agar medium *Klebsiella* species isolated in this study produced large mucoid pink colonies indicating lactose fermentation. Gram-negative, non spore forming, capsulated rods were seen under microscope. The *Klebsiella* isolated were VP and MR negative. *Klebsiella pneumoniae* was indole negative.

Biochemical methods for identification of isolated bacteria

All biochemical tests were performed as described by Barrow and Feltham, (2003).

Table (1): Isolation of aerobic bacteria from respiratory tract of infected village chickens (Baladi type), in Khartoum State.

Sample source	No. (%) of samples examined	Total No. (%) of isolates	No. (%) of Gram positive isolates	No. (%) of Gram negative isolates
Nostrils	10 (22.2%)	23 (41.8%)	5 (21.7%)	18 (78.3%)
Trachea	20 (44.5%)	17 (30.9%)	9 (52.9%)	8 (47.1%)
Conjunctiva	5 (11.1%)	6 (10.9%)	3 (50%)	3 (50%)
Lung	5 (11.1%)	5 (9.1%)	2 (40%)	3 (60%)
Infraorbital sinuses	5 (11.1%)	4 (7.3%)	2 (50%)	2 (50%)
Total	45 (100%)	55 (100%)	21 (38.2%)	34 (61.8%)

(The percentage calculated from number of samples examined).

Table (2): Characters and biochemical reactions of Gram negative bacteria isolated from respiratory tract of infected village chickens (Baladi type) in Khartoum State.

Bacterial species	Character															
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**
Pseudomonas aeruginosa	+	+	+	+	-	-	-	-	+	+	+	+	+	-	-	-
Pseudomonas fluorescence	+	+	+	+	+	+	-	-	+	+	+	+	+	-	-	-
Escherichia coli	+	-	-	+	+	+	+	+	+	+	-	+	+	-	+	+
Klebsiella pneumonia	-	-	-	+	+	+	+	+	+	-	+	-	+	+	-	W
Proteus vulgaris	+	-	+	-	-	-	+	+	+	+	+	+	+	+	+	+
Shigella flexineri	-	-	-	-	-	+	-	-	-	+	-	-	-	+	-	+
Pasteurella multocida	-	+	W	+	W	+	-	+	-	-	-	+	+	+	-	-
Haemophilus paragallinarum	-	-	W	+	-	**W**	-	+	-	-	-	+	-	-	-	+
Bordetella avium	+	+	+	-	-	-	W	+	-	-	+	+	-	-	-	+
Yersinia pseudotuberculosis	+	-	N	N	N	N	N	-	N	N	+	-	+	-	N	N

1 = Motility, 2 = Oxidase, 3 = Catalase, 4 = Citrate, 5 = Glucose, 6 = Lactos,

7 = Maltose, 8 = Sucrose, 9 = Xylose, 10 = OF, 11 = Urease, 12 = Indole,

13 = MR, 14 = VP, 15 = H_2S and 16 = Nitrate.

(+) = Positive, (-) = Negative, (W) = Weak reaction, (N) = Not done.

Table (3): Characters and biochemical reactions of Gram positive bacteria isolated from respiratory tract of infected village chickens (Baladi type) in Khartoum State. (O = Oxidative, F = Fermentative, C = Central spore)

Characters	Bacterial species						
	Bacillus cereus	***Bacillus megaterium***	***Staphylococcus aureus***	***Staphylococcus gallinarum***	***Streptococcus pneumoniae***	***Streptococcus lentus***	***Micrococcus varian***
Gram reaction	+	+	+	+	+	+	+
Motility	+	+	-	-	-	-	-
Spore forming	C	C	Non	non	Non	Non	Non
Catalase	+	+	+	+	+	+	+
Oxidase	-	-	-	-	-	-	-
Oxidation fermentation (O/F)	O	F	F	F	O	F	O
Coagulase	W	W	+	-	-	-	-
Glucose	+	+	+	+	+	+	+
Lactose	-	-	+	+	-	+	+
Maltose	-	-	+	+	-	+	W
Mannitol	-	-	+	+	-	+	+
Mannose	-	-	-	-	+	-	+
Xylose	-	-	-	-	-	+	-
Nitrate	-	-	+	+	-	+	+
VP	-	-	+	+	-	-	-

Table (4): Gram-negative bacteria isolated from respiratory tract of infected village chickens (Baladi type), in Khartoum State.

Bacterial species	**Number (%) of isolates from:**				
	Nostrils	**Trachea**	**Conjunctiva**	**lung**	**Infraorbital sinus**
Pseudomonas aeruginosa	1 (5.6%)	3 (37.5%)	-	-	-
Pseudomonas fluorescence	1 (5.6%)	-	-	-	-
Escherichia coli	2 (11.1%)	3 (37.5%)	2 (66.7%)	1 (33.3%)	1 (50%)
Klebsiella aerogenes	3 (16.7%)	-	-	-	-
Proteus vulgaris	5 (27.8%)	-	-	-	-
Shigella flexineri	2 (11.1%)	-	-	-	-
Pasteurella multocida	-	2 (25%)	-	2 (66.7%)	-
Haemophilus paragallinarum	-	-	1 (33.3%)	-	1 (50%)
Bordetella avium	2 (11.1%)	-	-	-	-
Yersinia pseudotuberculosis	2 (11.1%)	-	-	-	-

(The percentage was calculated from total number of isolates).

Table (5): Gram-positive bacteria isolated from respiratory tract of infected village chickens (Baladi type), in Khartoum State.

Bacterial species	Number (%) of isolates from:				
	Nostrils	**Trachea**	**Conjunctiva**	**lung**	**Infra-orbital sinus**
Staphylococcus aureus	-	1 (11.1%)	-	1 (50%)	-
Staphylococcus gallinarum	2 (40%)	1 (11.1%)	1 (33.3%)	-	1 (50%)
Streptococcus pneumoniae	-	3 (33.3%)	-	-	-
Bacillus cereus	1 (20%)	1 (11.1%)	-	1 (50%)	-
Bacillus megaterium	1 (20%)	-	1 (33.3%)	-	1 (50%)
Micrococcus varian	1 (20%)	-	1 (33.3%)	-	-
Micrococcus lentus	-	3 (33.3%)	-	-	-

(The percentage was calculated from total number of isolates).

Discussion

This work was carried out to isolate and identify aerobic bacteria infecting respiratory tract of village chickens (Baladi Breed) in Khartoum State, Sudan. Fourty five samples were collected from infected chickens and cultured. In this study, the bacterial isolates were obtained from nostril, conjunctiva, trachea, infra-orbital sinuses and lungs. All these samples were showed bacterial growth and gave fifty five isolates.

In the study *Haemophilus paragallinarum* was isolated from conjunctival sacs and infra-orbital sinuses of infected chickens. This agrees with previous reports (Shigidi, 1971; Linzitto *et al*., 1988; and Nour, 2007). *Haemophilus paragallinarum* causes infectious coryza in chickens which is an acute contagious upper respiratory tract infection in chickens (Hirsh *et al*., 2004). The economic importance of the disease relates to; loss of condition in broilers and reduce egg production in laying birds (Quinn *et al*., 2002).

Pseudomonas species were isolated from nostrils of infected chickens. Other studies reported the isolation of *Pseudomonas* species from nostrils of infected chickens (Saad *et al*., 1981) In Sudan *Pseudomonas aeruginosa* was isolated from cases of substantial deaths among young chicks (Iman, 1997 and Mohamed *et al*., 1996). *Pseudomonas aeruginosa* causes a wide range of opportunistic infections (Quin *et al*., 2002). This reveals that *Pseudomonas* infections could be a cause of heavy losses among chickens.

Pasteurella multocida was isolated from trachea and lung of chickens in this study. Also Linzitto *et al*. (1988) and Nour (2007) isolated *Pasteurella multocida* from respiratory tract of chickens. *Pasteurella multocida* causes fowl cholera (avian pasteurellosis) in poultry (Quinn *et al*., 2002). The disease is highly contagious and affects both domestic and wild birds. The sub acute form of the disease is mostly respiratory and manifested by rales and mucopurulant nasal discharge (Hirsh *et al*., 2004).

Escherichia coli cause opportunistic infections in almost all animal species. In poultry *E. coli* causes colibacillosis of fowl. Fowl may also be infected by respiratory tract and develop respiratory or septicemic disease (Hirsh *et al*., 2004). In this study *E. coli* was isolated from nostril, trachea, conjunctiva, lung and infra-orbital sinuses of infected chickens. Several authors reported the isolation of *E. coli* from respiratory tract of infected chickens, (Khogali, 1970; Eis *et al*., 1985; Mahgoub, 1986 and Iman, 1997). Abdellah (2003) reported the isolation of *E. coli* from lung and air sacs. Nour (2007) also isolated *E. coli* from respiratory tract of poultry. Respiratory diseases, vaccination, high or low temperature, absence of feed or water, high egg production, accumulation of contaminated dust, stress of crowding and movement to strange environment are among predisposing factors to respiratory tract infections by *E. coli*. History of infectious bursal disease might have had a rule in *E. coli* infections (Pages and Costa, 1985). Nighot *et al*., (2002) reported that faulty management and lack of routine vaccination against some viral diseases may lead to activation of commensally living bacteria forming the normal flora and challenges the natural immunity and defense mechanism. Sometimes the lesions and symptoms of respiratory infections may be aggravated when secondary *E. coli* invasion occurred. Seetha (1988) reported the complication of infactious coryza with *E.* coli which leads to chronic form of the disease.

Other *enterobacteriacae* were also isolated in this study. *Klebsiella* species was isolated from nostrils of infected chickens. This confirms the previous finding of Iman (1997) and Elhassan and Elsanousi (2002) who isolated *Klebsiella* species from respiratory tract of chickens in Sudan. *Klebsiella* is found in mucosa of upper respiratory tract, intestine and urigenital tract of man and other animals and causes pneumonia, nasal infection, urinary tract infections and pyogenic infections in man (Hirsh *et al*., 2004).

Shigella was found in the intestinal tract in man and affect human and non human primates (Hirsh *et al*., 2004). However, in this study, it was isolated from nostrils of infected chickens.

Proteus species was isolated from respiratory tract (nostrils) of infected chickens in this investigation. Proteus is reported to produce pyogenic lesions and infections of respiratory tract in man (Hirsh *et al*., 2004).

Staphylococcus species were isolated from nostrils, trachea, conjunctiva, lungs and infra-orbital sinuses of infected chickens, also Iman (1997) isolated Staphylococcus species from respiratory tract of infected chickens. Staphylococcus species are present in the upper respiratory and upper epithelial surface of the warm-blooded animals (Hirsh *et al*., 2004). Transmission of *Staphylococcus aureus* between animals and human occurs infrequently (Hirsh *et al*., 2004). In man *Staphylococcus aureus* infection results 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 broiler meat and cause food poisoning.

In the present investigation Streptococcus species were isolated from trachea of infected chickens this is in agreement with finding of Linzitto *et al*. (1988) who isolated *Streptococcus* species from respiratory tract of infected chickens. *Streptococcus* species (*Streptococcus zooepidemicus*) was reported to cause suppurative conditions and septicaemia in poultry (Domermuth and Gross, 1975). As, the healthy animals may carry Streptococci, may be infections are probably endogenous and stress related (Hirsh *et al*., 2004).

Bacillus species were also isolated from nostrils, trachea, conjunctiva, lungs and infra-orbital sinuses of infected chickens in this investigation. Bacillus cereus is known as cause of opportunistic infections and causes abortion and mastitis in cattle (Hirsh *et al*., 2004) and it also responsible for human food poisoning. Thus poultry meat contaminated with Bacillus cereus might be a source of human food poisoning.

Conclusion

In conclusion the result of this study demonstrated and identified, both Gram positive and Gram negative bacterial pathogens were isolated from respiratory tract of infected village chickens, respiratory tract bacterial infections could be an important constrains in poultry industry in Khartoum State, Sudan. High prevalence of *Escherichia coli* associated with respiratory tract infections in village chickens was observed. These bacteria may get transferred to humans, animals and other birds through direct contact or via food chain resulting in complications in them. Data of this study would be helpful for undertaking prevention and control measures against respiratory tract bacterial infections in village chicken (Baladi type breed).

Recommendations

1-The high prevalence of Echerichia coli associated with respiratory infections of village chickens (Baladi breed) needs further studies.
2- Attention should be paid for the breeding system of the Baladi type.
3- Maintaining ideal environment (ventilation and temperature) is important in control chickens respiratory diseases and supply well balanced feed, there for to minimize the spread of respiratory diseases.
4-Apply vaccination programme and monitoring of immune response are essential for respiratory diseases control.
5-Continued surveillance of the major bacterial diseases of respiratory system is necessary to ensure availability of sufficient information which can be used for planning proper control measures.

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