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Isolation and antimicrobial resistance of *Staphylococcus* spp., enteric bacteria and *Pseudomonas* spp. associated with respiratory tract infections of sheep

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Abstract

Sheep are considered as an important part of livestock in the worldwide, particularly in Iraq, as they provide meat, milk, leather, wool, and manure. The present study aim is isolation and identification of staphylococci, enteric bacteria and *Pseudomonas* spp. Totally, 115 samples were collected from sheep (100 samples were collected from the nasal cavity of local sheep suffering from respiratory infections, and 15 samples were collected from apparently healthy local sheep). All the samples were collected from seven flocks located in Abu Ghraib and Al-Radwaniyah, Baghdad governorate, Iraq. The samples were taken during the period from October 2020 to February 2021. *Staphylococcus* spp., *Pseudomonas* spp., and enteric bacteria were detected firstly by using classical diagnostic methods, and secondly they were identified to the species level by using the corresponding analytical profile index (API 20 staph, API 20NE and API 20E) and via the Vitek2 system. Importantly, from nasal swabs, 79% bacterial isolates were obtained, including *Enterobacter cloacae*, *Escherichia coli*, *Raoutltella planticola*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* among others. The antimicrobial susceptibility test showed multi-drug resistant *S. aureus*, *P. aeruginosa* and *K. pneumoniae.* To conclude, several aerobic bacteria were isolated from the nasal cavity of diseased sheep. An extensive study is needed to determine the economic and public health impacts of these bacteria.

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Introduction

Small ruminants, especially sheep are valuable possessions for the Southeast Asian, Mediterranean, and African countries that benefited from their milk, meat, and wool (1-3). In sheep-rearing countries, infections of the respiratory tract are of major concern due to their impacts on the income and nutrition of people (4,5). The economic losses are represented by decreased growth and production, increased budgets of treatment and prevention, along with death in some cases (6). Respiratory infections, regardless of their etiology, can affect animals at any age, and contribute to 5.6% of all diseases that occur in the small ruminants (2). Disorders of the respiratory infections are classified into upper and lower respiratory tract diseases (1,2). The nature of these infections could be acute, chronic, or progressive based on the etiological, physiological, and environmental factors (2). In a study, the mortality rates due to epizootic respiratory infections were reported to range from 10-90% in the affected sheep (7).

Pathogenesis of respiratory illnesses is multifactorial, and these diseases might occur because of the interaction of many factors, including infectious microorganisms (bacteria, viruses, and fungi), environmental factors, toxicants, pollutants, host defenses, genetics, mineral loss, feed, stress induced by the mechanical factors, and other flock diseases, such as worms and fluke (2,5).

Other predisposing factors do exist, such as the propensity of these animals to crowd adding to habits of group rearing making these small ruminants prone to infectious and contagious diseases (8,9). Moreover, elder, pregnant, lactating, and immune compromised animals are more susceptible to microorganisms inhabiting the respiratory system (1).

When bacterial and viral infections occur together under bad weather conditions, this renders the respiratory infection worse (10). Such respiratory infections adversely influence international trade and eventually hindering the economy (4). Bacteria, as one of the infectious agents, have been paid more attention because of their severity of the infections, varying clinical signs, as well as recurrence of multi-drug resistant strains (11). Among the bacterial pathogens, *Mycobacterium tuberculosis, A. baumannii*, *K. pneumoniae, Strept. pneumoniae*, *H. influenzae*, *E. coli*, *Staph. aureus* and *Pseudomonas aeruginosa* are the main bacterial species that have been frequently reported to cause respiratory infections associated with morbidity and mortality in developing countries (12).

Thus, understanding bacterial causes of respiratory infections in sheep is important to improve both management and treatment of these infections (13,5). In addition, no more studies are currently available about sheep bacterial respiratory infections at least in Baghdad, Iraq. Therefore, this study aimed at investigating three main groups of bacteria associated with respiratory infections in sheep, including staphylococci, enteric bacteria and *Pseudomonas* spp. with studying their antimicrobial resistance.

Materials and methods

Study animals

Totally, 110 sheep reared in seven flocks located in Abu Ghraib and Al-Radwaniyah, Baghdad, Iraq were included in the present study, including 100 sick with respiratory infection signs and 15 apparently healthy sheep. The sheep ages ranged from 1.5 to 4 years of both genders. The infected sheep were chosen based on some clinical signs and case history, such as signs of cold, fever 40-41°C, anorexia, nasal discharge, coughing and depression.

Specimens' collection

Totally, in this study, 115 cotton swabs of the nasal cavity were collected from local sheep during the period from October 2020 to February 2021. The samples included 100 swabs collected from diseased sheep. In addition, other 15 swabs were taken from apparently healthy local sheep. All the swabs were transported to the laboratory of Microbiology Department, College of Veterinary Medicine, University of Baghdad, Baghdad, Iraq.

Isolation and identification of bacteria

The bacterial species belonging to *Staphylococcus* spp., *Pseudomonas* spp., and *Enterobacteriacea* were firstly isolated on the standard bacteriology culture media and biochemical test media using routine diagnostic methods. The media used included: brain heart infusion broth and agar, tryptone soya agar, nutrient broth and agar, blood agar base, MacConkey's agar, mannitol salt agar, staph 110 agars, and cetrimide agar. The biochemical tests involved catalase, oxidases, urease, indole production, triple sugar iron (TSI), tube coagulase, and citrate utilization tests.

The isolates were identified to the species level by using the corresponding analytical profile index (API 20 staph, API 20NE and API 20E), and instructions of the manufactured company (BioMerieux) were followed. In addition, the automated Vitek2 system (BCL identification card) was also used for confirmation of some isolates.

Antimicrobial susceptibility test

Disk diffusion susceptibility method was performed as mentioned in the clinical and laboratories standards institute (CLSI) (14). Ten different antimicrobial agents were used in this study to determine the susceptibility of each isolate of *Staphylococcus* (*S.) aureus*, *Klebsiella (K.) pneumoniae* and *Pseudomonas (P.) aeruginosa*. Applying approaches illustrated in CLSI (14), the susceptibility tests were interpreted. This test was repeated 3 times for each isolate and the average diameters were taken. The antimicrobials used to test the sensitivity of *S. aureus* isolates included: ciprofloxacin, methicillin, cefotaxime, oxacillin, amikacin, gentamicin, imipenem, ampicillin, tetracycline, tobramycin. Those used for testing *K. pneumoniae* isolates were ciprofloxacin, ceftazidime, cefotaxime, streptomycin, amikacin, gentamicin, imipenem, ampicillin, tetracycline, and tobramycin. Lastly, *P. aeruginosa* isolates were tested against each of amikacin, ampicillin, aztreonam, ceftazidime, ciprofloxacin, gentamicin, imipenem, tetracycline, levofloxacin, and tobramycin.

Results

Bacterial identification

The total number of bacterial isolates was 79. Overall, each of *Enterobacter cloacae, S. xylosus*, *Escherichia coli*, and *Raoutltella planticola* were the most frequent species isolated from sheep. Members of the *Enterobacteriaceae* family constituted the highly isolation rates (46%) among others. While staphylococci accounted for 27% of the isolates, only 6% of the bacteria belonged to *Pseudomonas* spp. (Table 1). Concerning the enteric bacteria, 15 out of 46 (32.6%) isolates were *E. cloacae*, followed by *E. coli* and *R. planticola* that represented 11/46 (23.9%) and 10/46 (21.7%), respectively, of the enteric bacteria. While *K. pneumoniae* accounted for 8.7% of the enteric bacteria, each of *Raoutltella omithinolytica* and *Pantoea* spp. had isolation rates of 6.5% among other family members (Table 1).

With respect to *Staphylococcus* spp., *S. xylosus* was isolated from 15 out of 100 samples, and the isolation rate was 55.6% compared to other staphylococci. Each of *S. sciuri* and *S. lentus* accounted for 4% of the total isolates, but constituted 14.8% of the isolated staphylococci. *S. aureus* were found in 3 out of 100 total samples, and represented 11.1% of the staphylococci. Finally, *S. capitis* was also isolated from 1% of the samples, with a prevalence of 3.7% among other *Staphylococcus* spp. (Table 1). The lowest isolation rates were reported by *Pseudomonas* spp., in which the rates among the total samples were 3%, 2%, and 1% for *P. luteola*, *P. aeruginosa*, and *P. fluorescensiputida*, respectively (Table 1). Concerning swabs taken from deep nostrils of apparently healthy sheep, bacteria such as *S. lentus*, *S. xylosus*, *Enterobacter* spp., *E. coli*, and *Pseudomonas* spp. were isolated.

Table 1: Identity, numbers, and percentages of bacteria isolated from sheep with signs of respiratory tract infections

Bacterial isolate	Bacteria spp.	No
	S. xylosus	15
	S. sciuri	4
Staphylococcus	*S. lentus*	4
	S. aureus	3
	S. capitis	1
Total		27
	E. cloacae	15
	E. coli	11
	R. planticola	10
Enterobacteriaceae	*K. pneumoniae*	4
	R. omithinolytica	3
	Pantoea spp.	3
Total		46
	P. luteola	3
Non	*P. aeruginosa*	2
Enterobacteriaceae	*P. fluorescensiputida*	1
Total		6

Antimicrobial susceptibility tests

The isolates of *S. aureus* were 100% resistant to cefotaxime and ampicillin, and 33% resisted amikacin, gentamicin, methicillin, and tetracycline. On the other hand, all the tested isolates were 100% susceptible to each of imipenem, ciprofloxacin, tobramycin, and oxacillin (Table 2). Concerning *K. pneumoniae,* 75% of the isolated bacteria were resistant to ceftazidime and tetracycline, and 25% of the isolates showed intermediate sensitivity to 5 drugs. However, 100% of the isolates revealed susceptibility to each of ciprofloxacin, gentamicin, and tobramycin (Table 3). Finally, *P. aeruginosa* isolates showed variable responses to antimicrobials. While 50% of the bacteria were resistant to ampicillin, aztreonam, ceftazidime, and levofloxacin, 100% of these microorganisms showed susceptibility to amikacin, ciprofloxacin, gentamicin, imipenem, tetracycline, and tobramycin (Table 4).

Table 2: Susceptibility tests of *S. aureus* to antibiotics

Antimicrobial	*S. aureus* no (%)		
	Resistance	Intermediate	Susceptible
Amikacin	1 (33.3%)	0	2 (66.7%)
Ampicillin	3 (100%)	0	0
Cefotaxime	3 (100%)	0	0
Ciprofloxacin	0	0	3 (100%)
Gentamicin	1 (33.3%)	0	2 (66.7%)
Imipenem	0	0	3 (100%)
Methicillin	1 (33.3%)	1 (33.3%)	1 (33.3%)
Oxacillin	0	0	3 (100%)
Tetracycline	1 (33.3%)	0	2 (66.7%)
Tobramycin	0	0	3 (100%)

Table 3: Susceptibility tests of *K. pneumoniae* to antibiotics

Antimicrobial	*K. pneumoniae*		
	Resistance	Intermediate	Susceptible
Amikacin	1 (25%)	0	3 (75%)
Ampicillin	0	1 (25%)	3 (75%)
Cefotaxime	1 (25%)	1 (25%)	2 (50%)
Ceftazidime	3 (75%)	1 (25%)	0
Ciprofloxacin	0	0	4 (100%)
Gentamicin	0	0	4 (100%)
Imipenem	0	1 (25%)	3 (75%)
Streptomycin	1 (25%)	0	3 (75%)
Tetracycline	3 (75%)	1 (25%)	0
Tobramycin	0	0	4 (100%)

Table 4: Susceptibility tests of *P. aeruginosa* to antibiotics

Antimicrobial	*P. aeruginosa*		
	Resistance	Intermediate	Susceptible
Amikacin	0	0	2 (100%)
Ampicillin	1 (50%)	0	1 (50%)
Aztreonam	1 (50%)	1 (50%)	0
Ceftazidime	1 (50%)	0	1 (50%)
Ciprofloxacin	0	0	2 (100%)
Gentamicin	0	0	2 (100%)
Imipenem	0	0	2 (100%)
Levofloxacin	1 (50%)	0	1 (50%)
Tetracycline	0	0	2 (100%)
Tobramycin	0	0	2 (100%)

Discussion

The diagnosis of respiratory tract infections is a big challenge in many countries, including Iraq (15). It has been found that aerobic bacteria isolated from the nasal cavity were higher 97.9% than those isolated from the tonsil 93.8%, trachea 79.2%, and lung 62.5%. This indicates reduction in the carrier state in the lower parts of the respiratory system (16). Therefore, this study focused on taking samples from the nasal cavity of diseased and healthy sheep. In the current research, each of *S. lentus, S. xylosus, Enterobacter* spp., *E. coli,* and *Pseudomonas* spp. were isolated from swabs collected from deep nostrils of apparently healthy sheep. This is consistent with the study of (16) who observed colonization of a wide variety of bacteria in the respiratory system of apparently healthy sheep. The same authors found that the major species isolated from apparently healthy slaughtered sheep were *E. coli* 14.2%, followed by coagulase-negative staphylococci 10.7%, while *Klebsiella* spp. 1.3% was the least genus encountered. In the present study, both *S. aureus* and *K. pneumonoiae* were not among the species isolated from healthy sheep. This partly conflicts with the observations of (17) who found that all of *S. aureus, K. pneumoniae* along with *P. aeruginosa* and *E. coli* were prevalent in the respiratory tract of apparently healthy sheep. However, gram's positive bacteria were demonstrated to be more common inhabitant than gram's negative in the respiratory tract of healthy sheep 62.2% vs. 37.8% (16).

It is notorious that infectious agents are carried in the upper respiratory tract of approximately 40% of healthy sheep, and these microorganisms can cause respiratory diseases under stress factors (17). Bacterial respiratory infection could be primary, happening in the healthy individuals or could be secondary to a large number of conditions that cause immunosuppression. Secondary bacterial infections take place, particularly, when there is a decrease in the respiratory mucosa resistance and that the growth of bacteria in the upper respiratory tract expand downwards (18). Concerning sheep with respiratory infections, in the present study, members of the *Enterobacteriaceae* family constituted the highly isolation rates 46% among others. While staphylococci accounted for 27% of the isolates, only 6% of the bacteria belonged to *Pseudomonas* spp. The variable infectious agents with different isolation percentages were documented by previous studies. For example, in a previous study performed in Baghdad, Iraq, 50 sheep suffering from respiratory infections and other 50 apparently healthy sheep were included for bacterial isolation from nasal cavity, bronchioles and the lungs tissue. Importantly, 76 different aerobic bacteria were isolated from nasal swabs of diseased sheep, among which only 6 isolates of *S. aureus* were obtained versus 8 isolates from apparently healthy sheep. Concerning *P. aeruginosa,* it was isolated from 6 and 2 cases of infected and apparently healthy sheep, respectively (19). These low isolation rates of *S. aureus* and *P. aeruginosa* are somewhat similar to those obtained in the current study. However, while *K. pneumoniae* was isolated from the nasal swabs of diseased animals in this study, it was grown from the bronchus and lung of infected sheep only but neither from the nasal swabs nor apparently healthy animals in the study of (19).

In contrast to this study, in which low isolation rates 4% and 3% were reported for *K. pneumoniae* and *S. aureus*, they accounted for higher isolation rates of 27.6% and 20%, respectively, from pneumonic lung in the study of Ugochukwu and his colleagues (20). Likewise, the data of (20) pointed to the high isolation rate around 30% of potentially pathogenic *S. aureus* from bronchopneumonia and pulmonary abscesses in sheep reared in Iran. This bacterium is considered as a major inhabitant of the upper respiratory mucosa, and plays a pathogenic role in hosts with weak immune system (21). Moreover, *S. aureus* has zoonotic implications, with opportunities for mutual transmission between human and domestic animals while natural barriers are compromised (22).

Furthermore, higher isolation rate of 11% was reported for *E. coli* from sick animals in the present study in comparison with a previous study where 2 isolates only obtained from the nasal cavity of infected sheep and 4 isolates from apparently healthy animals (20). Although in its normal habitat is usually harmless, *E. coli* has been suggested to cause respiratory and urogenital infections (23). Interestingly, *E. coli* among other bacteria were the most commonly isolated aerobic microorganism from fibrinous bronchopneumonia (20). In the same context, Ugochukwu and co-workers (20) reported that approximately 55% of the pneumonic lung were *E. coli* positive. Moreover, this pathogen was the most prevalent aerobic bacterium isolated in a similar study of (24). Both *E. coli* and *Staphylococcus* spp. were frequently isolated from catarrhal bronchopneumonia as well as interstitial pneumonia (20).

The present study also demonstrated high isolation rate 15% of *E. cloacae* from sheep respiratory cases compared to no isolate in the study of (19); nevertheless, a single isolate of *Enterobacter* spp. was cultured from apparently healthy sheep by the above authors. This inconsistency in isolating different predominant bacteria might be due to differences in feeding behaviors, in which sheep normally graze down to the soil (25), thus the risk of consuming bacteria from the ground becomes high (15).

Regarding the antimicrobial susceptibility, nearly most of the bacteria isolated from sheep with respiratory tract infection were resistant to at least two or three drugs. For instance, *S. aureus* isolates were resistant to each of cefotaxime and ampicillin, *K. pneumoniae* were resistant to ceftazidime and tetracycline, and *P. aeruginosa* isolates importantly showed multidrug resistance to ampicillin, aztreonam, ceftazidime, and levofloxacin. It is well known that these microorganisms have resistance against many antimicrobials including broad spectrum ones. Currently, *S. aureus, P. aeruginosa* and *K. pneumoniae,* among a handful of bacterial species, have been demonstrated to be the major pathogens associated with multi-drug resistance and are the leading cause of death (26). This issue might pose human health hazards because after slaughtering sheep might contain residues of these antibiotics in their meat leading to either allergic reaction conditions or emergence of drug-tolerant bacteria in persons consuming these types of meat (27). However, in this study, all of the aforementioned isolates had mutual sensitivity to ciprofloxacin and tobramycin. Therefore, the last two drugs can be used effectively to treat causes of respiratory infections in sheep regardless of the bacterial cause.

Finally, the above findings highlight the importance of isolating and identifying these microorganisms from the nasal cavity of sick sheep, this demands quick action to avoid exacerbation of the condition and progression to lower respiratory tract infections, including pneumonia. Likewise, the results of this study might help owners and veterinarians to understand the main aerobic bacteria associated with sheep respiratory infections in order to improve treatment and infection management. It also emphasizes the need to develop contemporary molecular diagnostic tools for detection of these infections.

Conclusion

In conclusion, based on the results obtained here, several bacterial species can inhabit the respiratory passage ways of apparently normal sheep. But stressful conditions, such as overcrowding, poor ventilation in the houses of these animals, and poor nutrition might turn these harmless commensals into pathogenic bacteria. This study also showed the isolation of several aerobic bacteria from the nasal cavity of diseased sheep. Thus, an extensive study is necessary to be done on isolating other aerobic and anaerobic bacteria associated with ovine respiratory tract infections, along with determining the economic and public health impacts of these microorganisms.

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Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this manuscript.

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العزل والمقاومة الميكروبية لجراثيم المكورات العنقودية والمعوية والزوائف المرتبطة بإصابات القناة التنفسية للأغنام

طيبة احمد عزيز و إنعام جاسم لفته

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الخلاصة

تعد الأغنام جزءًا مهمًا من الثروة الحيوانية في العالم وخاصة في العراق لأنها توفر اللحوم والحليب والجلد والصوف والسماد. هدفت الدراسة الحالية إلى عزل وتحديد الأنواع الجرثومية المسببة وخاصة المكورات العنقودية والجراثيم المعوية والزوائف. إجمالاً، جرى جمع ١١٥ عينة من الأغنام (جمعت ١٠٠ عينة من التجويف الأنفي للأغنام المحلية التي تعاني من التهابات في الجهاز التنفسي، و ١٥ عينة جمعت من الأغنام المحلية السليمة ظاهريا). جمعت جميع العينات من سبع قطعان تقع في أبي غريب والرضوانية، محافظة بغداد/ العراق. أخذت العينات خلال الفترة من شهر تشرين الأول ٢٠٢٠ إلى شباط ٢٠٢١. جرى الكشف عن جراثيم المكورات العنقودية الزوائف والجراثيم المعوية أولاً باستخدام طرق التشخيص التقليدية، وثانياً تم تحديدها على مستوى الأنواع باستخدام مؤشر الملف التحليلي المقابل وعبر نظام فايتك-٢. الأهم من ذلك، من مسحات الأنف، جرى الحصول على ٧٩٪ من العزلات الجرثومية، بما في ذلك الجراثيم المعوية المذراقية، الايشريكيا القولونية، كلبسيله اليفة النبات، الكلبسيلا الرئوية، الزوائف الزنجارية، والمكورات العنقودية الذهبية وغيرها. أظهرت كل من عزلات جراثيم المكورات العنقودية الذهبية والزوائف الزنجارية والكلبسيلا الرئوية مقاومة للعديد من المضادات الحيوية. في الختام، جرى عزل العديد من الجراثيم الهوائية من التجويف الأنفي للأغنام المريضة مما يدعو إلى الحاجة إلى دراسة مستفيضة لتحديد الآثار الاقتصادية والتأثيرات على الصحة العامة لهذه الجراثيم.