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Identification of predominant pathogenic bacteria isolated from respiratory manifested small ruminants in western north Egypt with regard to their susceptibility to antibiotics

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***Abstract*---**Respiratory illnesses continue to be a persistent issue threatens small ruminants as sheep and goat flocks which constitute an important section of the livestock economy in Egypt. The current study have conducted survey among a total of 432 acquiring respiratory manifested sheep (n≈ 380) and goat (n≈ 85) flocks scattered in Western North regions in Egypt during September 2020 to June 2021. Bacterial screening was proceeding through classical cultivation and biochemical identification as well as screening of their antibiogram. The bacterial isolation revealed that the most prevalent pathogen was Klebsiella pneumoniae from 123 cases (27.15%), followed by *Mannheimia haemolytica* 108 (23.84%), *Escherichia coli* 75(16.5%); *Staphylococcus* spp. 36 (7.9%) and *Streptococcus* spp. 14(3.1%). The sensitivity to various antimicrobial agents was tested and demonstrated multi-drug resistant. In conclusion, the study supported the concept of the multi-bacterial etiology of the respiratory infections among sheep and goat flocks. There were high incidences of

multidrug resistance behavior of the bacterial isolates which may constitute a public health importance.

***Keywords*---**sheep, goat, respiratory, bacteria, Egypt.

Introduction

Small ruminants especially sheep and goats share remarkably in the economy on farmers' scale in Mediterranean countries. These worthy assets supply meat, milk, and wool output, as well as their nature to replicate and grow rapidly. So keeping these livestock healthy is usually represents important challenge (Lacasta et al. 2008). Respiratory illness has been an enduring issue for the revival of small ruminants; sheep and goat, due to its prolonged impact and depression in rest of life (Besser et al. 2012). Depending upon this concern, the management and control of respiratory diseases in small ruminants represented a great importance to avoid the large economic losses due to lowered productivity, reduced number of offspring and high mortalities (Drew et al. 2014).

One of the most substantial strategies for combating respiratory illness is the precise diagnosis and detection of the causative agents; however, the causes are uncertain. The illness has been ascribed to numerous bacterial pathogens including either Gram positive or Gram negative species (Wood et al. 2017). The task of this current study was confronting the challenge concerning poly-microbial nature of respiratory illness through assessing the prevalence of common bacterial causes associated to respiratory manifested sheep and goat population in three rural Bedouin governorates located in Western North of Egypt.

Material and Methods

Ethical approval

As per CPCSEA guidelines, ethical approval was not needful as the study including clinical samples.

Samples collection

A number of forty hundred and fifty three collected samples included nasopharyngeal and tracheal swabs aseptically taken from respiratory manifested sheep and goat in three governorates located in Western North Egypt as shown in table (1) from September 2020 to June 2021.

Table 1: the number and location of samples gathered from sheep and goat

Governorate	Sheep	Goat	Total
El-Behira	189	36	225
Matrouh	126	33	159
Alexandria	54	15	69
Total	369	84	453

The clinical pictures of involved diseased animals comprised hyperthermia, nasal or ocular discharging, cough, redness in mucous membranes, mouth breath, increasing in respiratory rate, dis-appetite, emaciation and loss of weight.

Bacteriological examination

The collected samples were inoculated in Brain Heart Infusion broth and kept in ice box and transferred to laboratory. The swabs were streaked onto the following cultural media; sheep blood agar (5%), MacConkey, eosin methylene blue (EMB) and mannitol agar. The cultures were incubated for 24 to 72 hours at 37°C in aerobic condition. After incubation, the plates were noticed for growth of bacterial colonies. Observations on the blood agar constituted size of the colony, presence or absence of hemolysis, type of hemolysis and pigment production. On the other hand the morphological appearance of colonies grown on MacConkey and mannitol agars indicated the fermentation of lactose or mannitol respectively.

Afterwards, sole obtained colonies were aerobically sub cultured on tryptose soya agar slant and kept at 4°C for subsequent Gram staining, biochemical and fermentative identification tests (Quinn et al. 2002). The pure colonies were identified by API 20E system and API STAPH IDENT 32 (BioMerieux, Marcy l'Etoile, France) as well as VITEK-2 compact system (BioMerieux, Craponne, France), according to manufacturer's instruction.

Antimicrobial sensitivity assay

The susceptibility of the isolates to various antibiotics was achieved using the diffusion technique on Mueller-Hinton agar (CLSI 2018), the following antibiotic discs were used; Amikacin (AK)30 µg, Ampicillin (AM)10 µg, Amoxicillin + Clavulanic acid (AMC) 20+10 µg, Ampicillin + Sulbactam (SAM) 10+10 µg, Aztreonam (ATM) 30 µg, Ceftriaxone (CRO) 30 µg, Cefalexin (CN) 30 µg, Cefotaxime (CTX) 30 µg, Ceftazidime (CAZ) 30 µg, Ceftriaxone (CTR) 30 µg, Cefeprime (FEP) 30 µg, Ciprofloxacin (CIP) 5 µg, Colistin (CL)10 µg, Co-trimoxazole (COT) 25 µg, Doxycycline (DC)30 µg, Erythromycin (E)15 µg, Gentamicin (GM)10 µg, Imipenem (IPM)10 µg, Novobiocin (NV) 30 µg, Neomycin (N) 30 µg, Norfloxacin (NOR) 10 µg, Oxicillin(OX) 10 µg Rifampicin (RF) 5 µg, Tetracycline (TE) 30 µg and Trimethoprim/ sulfamethoxazole (SXT) 1.25+23.75=25 µg.

Results

The obtained colonies were noted for their morphology, Gram stained and screened for biochemical tests as shown in tables 2 and 3.

Table 2: the biochemical activities of isolated Gram negative bacteria

Biochemical tests	*Klebsiella pneumoniae*	*Escherichia coli*	*Mannheimia haemolytica*
Oxidase	-ve	-ve	+ve
Catalase	+ve		+ve
Indole	-ve	+ve	-ve

Citrate utilization	+ve	-ve	
Urease production	+ve	-ve	
H_2S production	-ve	-ve	
Methyl Red (MR)	-ve	+ve	
Voges-Proskauer (VP)	+ve	-ve	
Glucose fermentation	+ve	+ve	
Sucrose fermentation	+ve	+ve	
Lactose fermentation	+ve	+ve	
Mannitol fermentation	+ve	+ve	
Sorbitol fermentation	+ve	+ve	

Table 3: the biochemical activities of isolated Gram negative bacteria

Biochemical tests	*Staphylococcus aureus*	*Staphylococcus epidermidis*	*Staphylococcus saprophyticus*	*Streptococcus* spp
Oxidase	-ve	-ve	-ve	-ve
Catalase	+ve	+ve	+ve	-ve
Coagulase	+ve	-ve	-ve	
Nitrate reduction	+ve	+ve	-ve	
Voges-Proskauer (VP)	+ve	+ve	-ve	-ve
Urease production	-ve	+ve	+ve	-ve
H_2S production	-ve	+ve	+ve	
Methyl Red (MR)	+ve	-ve	-ve	
Glucose fermentation	+ve	+ve	+ve	+ve
Sucrose fermentation	+ve	+ve	+ve	+ve
Lactose fermentation	+ve	+ve	+ve	+ve
Fructose fermentation	+ve	-ve	+ve	-ve
Mannitol fermentation	+ve	-ve	+ve	-ve

Based on the cultural growth, morphological and biochemical criteria; out of 453 collected nasopharyngeal and tracheal samples, *Klebsiella pneumoniae* represented the most prevalent pathogen that isolated from 123 cases (27.15%), followed by *Mannheimia haemolytica* 108 (23.84%), *E. coli* 75(16.5%); *Staphylococcus* sp. 36 (7.9%) and *Streptococcus* spp. 14(3.1%) table 3.

Table 3: the incidence of various bacterial pathogens isolated from both respiratory affected sheep and goat among the three screened Governorates

Species	Sheep			Goat			Total
Governorate	El-Behira	Matrouh	Alexandria	El-Behira	Matrouh	Alexandria	
K. pneumoniae	42	23	17	20	16	5	123 27.15%
E. coli	24	21	7	9	10	4	75

							16.5%
M. haemolytica	16	24	8	38	15	7	108 23.84%
S. aureus	6	4	1	15	7	4	26
S. epidermidis	3	3	-	-	2	-	8
S. saprophyticus	-	2	-	-	-	-	2
							36 7.9%
Streptococcus spp	7	6	-	-	1	-	14 3.1%

Presumptive Gram –ve stained *Klebsiella pneumoniae* isolates exhibited circular, mucoid pink (lactose fermenter) colonies on Mac-Conkey agar (Fig. 1a), greyish white non hemolytic colonies on blood agar (Fig. 1b), also the same colonial appearance was presented EMB agar. Furthermore the other Gram –ve stained isolates which represented presumptive *E. coli* showed greyish colored circular colonies (alpha hemolysis) on blood agar while flat lactose fermenter pink circular colonies on Mac-Conkey agar (Fig. 2), and black with metallic green sheen colonies on EMB agar.

(a)   (b)

Figure (1):
a) *Klebsiella pneumoniae* isolates showed mucoid pink (lactose fermenter) colonies on Mac-Conkey agar .
b) *Klebsiella pneumoniae* isolates showed greyish white non hemolytic colonies on blood agar.

Figure (2): *E. coli* isolates showed lactose fermenter pink colonies on Mac-Conkey agar

The presumptive *Mannheimia haemolytica* isolates displayed small gray circle colonies with narrow zone of complete hemolysis (β-hemolysis) on blood agar plate and grew on MacConkey agar plate. On the other hand, mannitol salt agar was a selective for growth of Gram positive stained bacteria "inhibit growth of Gram negative ones". The intuitive isolates which represented *Staphylococcus aureus* showed yellow mannitol fermenters colonies surrounded by yellow zone. Also they showed golden yellow colored colonies with zone of hemolysis (β- hemolysis). This hemolytic activity could differentiate *Staphylococcus aureus* isolates from that non hemolytic ones belonged to *Staphylococcus saprophyticus* which also can ferment mannitol and showed yellow colored colonies on mannitol salt agar. The other isolates which presumptively belonged to *Staphylococcus epidermidis* neither mannitol fermenter (red colonies) nor have hemolytic activity on blood agar (white colonies). Finally the isolates represented *Streptococcus* spp. alpha-hemolytic gray colonies on blood agar. In our study the various isolates were tested for their susceptibility to groups of antibiotics as followed.

The isolates of *Klebsiella pneumoniae* were highly resistant to many antibiotics as ampicillin, amoxicillin+ clavulanic acid, oxicillin, doxycycline, erythromycin, rifampicin and tetracycline, less resistant to neomycin, cefalexin, novobiocin and trimethoprim/ sulfamethoxazole but sensitive to ciprofloxacin, norfloxacin, ceftriaxone and amikacin (Fig. 3). While, *E. coli* isolates showed great resistant to ampicillin, amoxicillin + clavulanic acid, oxicillin, doxycycline, novobiocin erythromycin and rifampicin, medium resistant to neomycin, cefalexin, tetracycline and trimethoprim/ sulfamethoxazole also sensitive to ciprofloxacin, norfloxacin, ceftriaxone and amikacin (Fig. 3).

Regarding, isolates belonging to *Mannheimia haemolytica* were resistant to ceftazidime, cefotaxime, colistin, co-trimoxazole, doxycycline, trimethoprim/sulfamethoxazole while sensitive to cefeprime, ciprofloxacin, ceftriaxone and gentamicin.On the other hand most of isolates referred to *Staphylococcus* spp. and *Streptococcus* spp. were highly resistant to ampicillin, amoxicillin + clavulanic acid and ampicillin + sulbactam, oxicillin, doxycycline but sensitive to ciprofloxacin, amikacin, aztreonam and imipenem.

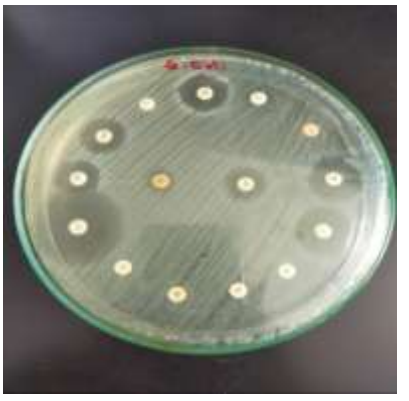
a)  b) 

Figure (3):
a) *K. pneumoniae* isolates antibiogram
b) *E. coli* isolates antibiogram

Discussion

Domestic small ruminants keep being substantial in the daily life and economic status of certain human populations that live in rural regions in Egypt due to meat, milk, and wool and hair production [7](Metawi et al., 2019). The current study was conducted on respiratory manifested sheep and goat and our findings displayed numerous bacterial pathogens have been implemented and deemed to be part of the etiology of respiratory illness. The obtained data revealed that *K. pneumoniae* was the most isolated pathogen by 27.15% (123/ 453).This result was coincided with other investigations mentioned that *K. pneumoniae* was often associated with respiratory illness in sheep and goat (Al-Agha et al., 2017; Patel et al., 2017). Also, Mansour et al. (2014) mentioned nearly similar isolation of *K. pneumoniae* from sheep and goats flocks in Taif, KSA by 24.2, 21.2% respectively. Another study in Al-Qadisiyah province, Iraq, revealed high isolation of *K. pneumonia* by 30% from 60 post-mortems pneumonic sheep (Abdulkareemsalman et al. 2019). But opposite of our data, Loncaric et al. (2016) and Franco et al. (2019) reported a low incidence (3.1 and 6.2%) among examined respiratory ill sheep in Austria and Brazil respectively.

A recent Egyptian investigation was carried out on 30 nasal swabs and 80 lungs obtained from live and dead sheep suffered from respiratory signs and different types of pneumonia in Sharkia Governorate's farms revealed isolation of *K. pneumoniae* by 10.9% (Rania et al., 2021). Another Egyptian study reported a lower result at Qalyuobia Governorate among slaughtered sheep's lungs; 3.84% (El-Mashad et al., 2019). Our data revealed that another member of *Enterobacteriacae*; *E. coli* was isolated from 75 cases (16.5%). Near data were obtained by Husseein and Alrodhan, (2011) who reported 20.78% isolation of *E coli* from respiratory infected sheep in Diwaniya Governorate, Iraq. *E. coli* O95 serotype was isolated from 17 lambs' carcasses died from septicemia and pneumonia (19%) in Rajasthan, India (Sonawane et al., 2012). *E.coli.*was isolated as a sole pathogen (25%) from lung obtained from slaughtered bronchopneumonic goat and as a mixed infection 20% in Bangladesh (Rashid et al., 2013). On the other hand in Egypt, El-Mashad et al. (2019) reported isolation of the organism by15.38 and 7.69% among sheep and goats' lungs respectively. While, a little higher prevalence was recorded in another study as *E. coli* of different serotypes were isolated by 21.8%, (Rania et al., 2021).

In our study *Mannheimea heamolytica* represented the second etiology and isolated from108 cases (23.84%). There were dissimilar data; a high isolation incidence (56.14%) as 64 out of 114 of 6-month-old lambs suffered or died from pneumonia in Konya, Turkey (Oruç, 2006).On the other side a low prevalence (4.46%) was obtained among respiratory infected sheep in Baghdad Province, Iraq (Ahmed et al., 2017). In Nigeria, *M. heamolytica* was recovered from 116 pneumonic lungs (98 were caprine and 18 were ovine) by 30 and 19% respectively, (Ugochukwu, et al., 2017). Very close results were obtained in Iran abattoirs; 38 isolates were recovered from 161 sheep's lungs that showed hepatization and consolidation (23.6%) (Tabatabaei and Abdollahi 2018) as well as (18.8% and 22.4%) reported from 197 sheep and 152 goat's pneumonic lungs respectively (Gharib Mombeni, et al., 2021).

Concerning previous Egyptian studies; the isolation rate of *M. heamolytica* varied bacteriological examination of 54 samples from ill sheep demonstrated an isolation rate of 22.2% while the rate reached 26.70% among diseased goats (Kaoud et al., 2010). On opposite side another very recent study showed no recovery of *M. heamolytica* in deep nasal swabs collected from sheep (n≈80) and goat (n≈18) suffering respiratory manifestations from different localities in the Giza Governorate (Bahr et al., 2021). *Staphylococcus* sp. were isolated from 36 cases by (7.9%) in our study, this isolation rate was greatly lower than other Egyptian investigation; El-Mashad et al. (2019) mentioned that *S. aureus* was the most prevalent isolated strain from the examined grossly pneumonic sheep and goats' lungs by isolation rate 38.46% as a sole cause and 7.69% as mixed infection at Qalyuobia Governorate. Furthermore, Husseein and Alrodhan, (2011) reported *S. aureus* at percentage of16.85% in Diwaniya Governorate, Iraq. Rashid et al., (2013) isolated *Staphylococcus sp.*by (40%) from purulent pneumonic, focal pneumonic and emphysemated sheep lungs, Bangladesh. An isolation rate of 22.2 % was obtained from examined respiratory ill sheep in Brazil (Franco et al., 2019).

Lastly, *Streptococcus pneumoniae* was isolated from examined ill sheep and goat in14cases (3.1%) which was harmonized with another previous study (3.9%) (Husseein and Alrodhan, 2011). On the contrary our data was greatly lower than other investigations; 26.92% reported by El-Mashad et al. (2019) as well as15.5% mentioned by Franco et al. (2019). Concerning, the susceptibility to various groups of antibiotic commercially used in the field, the different isolates showed a general multi drug resistance pattern. This phenomenon may constitutes a public health importance due to the hazard of consumption of affected slaughtered animals which their meat contained antibiotic residues (Kakoullis et al. 2021).

In the same time most of isolates were sensitive to certain drugs; for instance: *K. pneumoniae* and *E. coli* isolates were sensitive to ciprofloxacin, norfloxacin, ceftriaxone and amikacin. Also, isolates belonged to *Staphylococcus* spp. and *Streptococcus* spp. were found to be sensitive to ciprofloxacin, amikacin, aztreonam and imipenem. While, *M. haemolytica* isolates were sensitive to cefeprime, ciprofloxacin, ceftriaxone and gentamicin. So for-mentioned antibiotics may be used as drugs for choice in treatment process of respiratory illnesses affected sheep and goat flocks. These results are consistent with previous findings; *K. pneumoniae* (Mansour, et al. 2014; Ou et al. 2017; Patel et al. 2017; Yang et al. 2019 and Rania et al. 2021). *E. coli* (Sonawane et al. 2012; Rashid et al. 2013 and Rania et al. 2021). *M. haemolytica* (Marru et al. 2013; Sahay et al. 2020 and Bahr et al. 2021), *Staphylococcus* spp. (Rashid et al. 2013 and Aziz and Lafta 2021) and finally *Streptococcus* spp. Awosile et al. 2018.

Conclusion

The study revealed that there were many bacterial pathogens could be implemented in respiratory illnesses in sheep and goat flocks in Egypt and give the attention to their isolation and identification. Also the study directed the veterinarians and owners for the proper therapy to avoid the economic losses and achieve the public health.

Conflict of interest
The authors declared that there is no conflict of interest

Authors Contribution

Ehab A Fouad designated the study and collected the samples and supervised all study steps. Doaa D Khalaf, and Engy Farahat assisted in the cultural and antibiotic sensitivity test. Doaa D Khalaf and Ashraf S Hakim assisted in biochemical identification of the isolates. Ehab A Fouad and Ashraf S Hakim assisted in writing of manuscript. All authors revised the manuscript and shared in the publication.

Novelty statement: screening of sheep and goat suffered from respiratory illnesses in three non-studied before rural and Bedouin governorates located in Western North of Egypt. Moreover, identification of the most prevalent bacterial causative agents as well as their antibiogram to assess their resistance.

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