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Diagnosis of Bovine Respiratory Infection by Isolation and Identification of *Kl. PNEUMONIA* in Karbala city

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Abstract

To isolate the Klebsiella pneumonia nasal swab samples were dependent for the current study this purpose collects 50 Nasal swab samples from 1-2-year-old seasick cows showing respiratory exhibitions. Swabs samples were collected from different farms located in the Karbala governorate. The study was carried out from the period 20/12/2021 to 15/2/2021. The swabs were subjected to routine bacterial isolation procedures, by culture in nutrient broth, then Maconkey and blood agar, then Gram stain test and determination of the general morphological physiognomies of the colony. Then submitted to further micromorphological examination oxidase, and indole test, then confirmed by Vitek 2 system. The results of the current study revealed the growth of more than one type of bacteria in a diverse medium. *Klebsiella pneumonia* was isolated from 2 cases in pure form while another species mingled with (*E. coli, proteus, staph,* and *pseudomonas*).

Keyboard: Respiratory Infection, Bovine, *Kl. PNEUMONIA.*

Introduction

Klebsiella pneumonia (K. pneumonia) is extensively present in surface waters, wastewaters, soil, plants, and mammalian mucosal surfaces, because of the broad spectrum of virulence factors, K. pneumonia infection plays the main role in the pathogenesis of pneumonia, bacteremia a, n pyogenic hepatic abscesses in animals. (1) Domestic animals infected with K. pneumonia not only pose a threat to livestock production but also affect potential public health threats because these animals can act as a reservoir for multidrug-resistant strains of K. pneumonia. Despite the prevalence of antibiotic therapy to treat invading K. pneumonia, antibiotic resistance to pathogenic bacteria from food-producing animals and environmental sources is a global public health problem so the K. pneumoniae isolates often appear drug-resistant phenotypes, creation difficult to select subtle antibiotics for curative. (2).

The economic losses are reduced growth and production, increased budgets for treatment and prevention, along with death in some cases (3). All respiratory diseases can occur regardless of their etiology, can affect animals of any age, and 5.6% of all diseases occur in small ruminants. (4) Infectious respiratory diseases are categorized into upper and lower respiratory diseases (1, 4). The idea of this disease can be severe, constant, or moderate depending on the etiological, physiological, and natural variables (4). In one survey, mortality rates as a result of an epizootic respiratory illness are conveyed to rate from 10-90% in infected sheep. (5). The pathogenesis of respiratory infection has many factors and the etiology of its arias of the relationship between these factors, so the infectious pathogens such as (bacteria, viruses,

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fungi), ecological agents, toxic substances, pollutants, immunologic, inheritance, physiologic(starvation) and fatigue, mechanical factors, and another herd diseases, such as worms and fluke (6,5). The predisposing agents like animals tend to group, and this makes small ruminants more susceptible to infections and contagious diseases. (7). Furthermore, aging, pregnant, lactating, and immunocompromised mammals are very sensitive to respiratory microorganisms. (8). After bacterial and viral infections happen together, bad weather worsens, making the respiratory infection worse (9). Respiratory infections negatively affect worldwide jobs and delay the economy (6). More concern has been paid to bacteria, like a contagious agent, due to the acuteness of the infection, and the variability of clinical findings, in addition to the frequency of highly drug-resistant strains (10). Among the bacterial pathogens, are Mycobacterium tuberculosis, A. baumannii, K. pneumonia, and Strep. Pneumoniae, H. influenza, E. coli, Staph. Aureus and Pseudomonas aeruginhey are the major bacterial strains considerably recorded as respiratory infections related to morbidity and mortality in evolving countries.

The goal of the study

To investigate the spreading of *K pneumonia* among bovine

Materials and methods

Samples collection:

The duration of the research work (collecting samples - laboratory examination) through the 2021-2022 year. 50 nasal swabs samples are gathered from 1 to 2 years old from infected cows with respiratory manifestations. All samples are gathered from various flocks from a place in Karbala governorate, and these samples are placed in a cold container and transferred to the bacterial laboratory. In Vet Med. collage to isolate and identify pneumonia.

Specimens culturing

After gathering nasal swabs they should be grown as stripes on blood agar and MacConkey agar and then incubated at 37 °C for 24 h. The project plan involved the following;

• Smear or loop striping on blood agar and MacConkey agar

• The read of a polished colony on blood agar depending on hemolysis, form, and stickiness and then colored and look into the microscope.

• Colony growth on MacConkey agar is partitioned into lactose fermented and lactose non-fermented.

Microorganisms that ferment lactose are incubated in urease agar under aseptic procedures and then incubated at 37°C for 24 hours. No change in color or pink of urease agar indicates a positive result.

Identification of the isolates

The Suspected isolates are specified depending on the shape of the colony, micros, copy, and biochemical tests (11). After that proved by two Vitek techniques (12).

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Morphological Characterization

The initial determination of each isolate is based on common cultural features (colour, form, structure, and size) of the colony on MacConkey agar and blood agar after overnight incubation at 37 °C. Other properties such as fermentation of lactose have been noted.

Microscopic examination: -

One colony was picked up after the bacteria were isolated and stained with Gram stain, then examined under an optical microscope for its shape, length, and Gram-reactivity microscopically according to (12).

Biochemical tests: -

There are many biochemical tests used to confirmation of any dubious isolate, depending on **(**11).

Identification of bacteria using the Vitek system (a confirmation test)

The Vitek technology is utilized to screen two isolates via the standard protocol demanding 0.5 McFarland inoculum and based on the opinion of the manufacturer's directions and interpretations delivered by the software version. This technique occurs by loop culture of stock agar and cultured on agar overnight then the bacterial suspension was put in normal saline (1-2 x 10 8) CFU/ML as described by (13)

RESULT and DISCUSSION

Klebsiella pneumonia is a bacterial microbe that was classified as an opportunistic microorganism due to the incidence of diseases hhumanshumand animals. The highly virulent strains that developed due to the characteristics of high mucosal viscosity causing severe contagions such as a hepatic abscess in immunosuppression in young persons with high abilities to obtain new MAR characteristics additional to complicate the problems facing these strains. Bovine respiratory disease (BRD) is one of the most significant health problems. (14)

In bovine medicine worldwide [15]. The respiratory disease is lead to major economic losses in all areas of beef and dairy production [16]. In terms of its influence on US feedlots, BRD is the greatest significant disease, with a yearly frequency of 44%, leading to a loss of US$13.90 per animal due to therapy budgets and reduced weight gain. [17]. In dairy cattle, the BRD is more influenced in calves pre-weaned [16]. Moreover, pregnancy rates, milk production, and durability of dairy cows are negatively affected by this syndrome. 50 whole nose samples that were cold from different areas of Karbala city, only one of these samples (2%) can synthesize pink mucous colony in MacConkey agar which was diagnosed as Klebsiella pneumonpneumoniaesult which conveyed by (18) and (19). Some scientists are ++wo++rking on isolating Klebsiella pneumonia through nasal swabs and bronchial mucous membranes of calves with bronchopneumonia and these samples were initially diagnosed as Klebsiella pneumonia via morphological and cultural features such as those conveyed by (20). Microscopic investigation of each isolate discovered that they were wholly having lone Gram-

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negative active short rod, and non-spore-forming. Morphological features of bacteria grown on various media where they remain inside an incubator at 37°C for 24 hours so MacConkey agar colonies are detected as a high viscosity lactose fermenter in the shape of the large dome and pink color, and in EMB the colonies appeared large size, mucous, confluent deep pink light violet the color. Fig (1), (table 1)the .

The whole 79 raw milk wastes wwerecool from different regions in Baquba city, Among the total isolates, only 2 (2.53%) isolates were capable to yield red color, whiprovideside a pointer that these isolabelonglong to Serratia this same result which reported by

Fig. (1) *Klebsiella pneumonia*, on MacConkey agar

The Biochemical tests of Klebsiella pneumonia exhexhibit follow Catalase positive, oxidase negative, Gelatin hydrolysis negative, lactose fermenter, non-motile, Indole negative, citrate utilization negative.

TABLE (1) the Klebsiella isolates overturn the colour of the slant and butt, were generated acidic slant (yellow) and acid butt (yellow) escort with gas generation (bubbles formation), but without black residues formation and this signal for lactose and glucose leavening had happened and no H2S was created. These results agreed with those avowed by (11).

whilst urease tests used to recognize Klebsiella spp from Enterobacter spp were positive for Klebsiella and negative for Enterobacter (21). the breakdown of urea occurs via Urease enzyme catalyzes, so the bacteria were able to create such enzyme and detoxify the wastefulness and utilization the metabolic energy where alteration the medium colour from yellow to purple-pink, this main the urease positive test so the Klebsiella can yield urease enzyme and appear urease positive test (12).

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Table (1): cultural characters of Klebsiella Pneumonia in selective media

bacterial isolate	**EMB**	**Triple Sugar Iron(TSI) Slant/butt**	**MacConkey agar**
Klebsiella pneumonia	Purple	y/y, gas, no H2S	pink colonies

Y=yellow. H2S=sulfur hydrate production, EMB=Eosin Methylene Blue

Table (2) Biochemical tests to distinguish the Klebsiella pneumonia

Characteristics	*Klebsiella pneumoniae*
Capsule	+ve
Catalase	+ve
Citrate	+ve
Oxidase	-ve
Gas	+ve
Gelatin Hydrolysis	-ve
Urease	+ve
VP (Vogues Proskauer)	+ve
H2S	-ve
Indole	-ve
Lactose	+ve
Nitrate Reduction	+ve
MR (Methyl Red)	-ve

Depending on the species scale, the measures of the indole test for K. pneumonia differ from K. oxytoca, where give positive for K. oxytoca and negative for the rest. (12). At a

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temperature of 10 ° C, K. oxytoca and K. terrigenous can survive while K.pn cannot survive at the same temperature (22).

Confirmatory diagnosis:

The Vitek of 2 methods revealed that two of the suspected isolates had a 100% match with al. pneumonia with a possibility of 98%.

This method is used to detect the final diagnosis of K. pneumonia so the discovery of bacteria faster, more efficiently, and away from contamination can impede the detection options of pathogens.

Tables (3) and (4) Affords biochemical and antimicrobial sensitivity testing were presented positive results from *K. pneumonia* in Vitek 2 system

Table (3) Show the antibiotic susceptibility tests for Klebsiella pneumonia

No	Antimicrobial	MIC	Interpretation	Antimicrobial	MIC	Interpretation
1	Ticarcillin	≥128	R	Amikacin	≥64	R
2	Ticarcillin/Clavulanic acid	≥128	R	Gentamicin	2	S
3	Piperacillin	≥128	R	Tobramycin	≥16	R
4	Piperacillin/ Tazobactam	≥128	R	Ciprofloxacin	≥4	R
5	Ceftazidime	16	R	Pefloxacin		
6	Cefepime	≥64	R	Minocycline	≥16	R
7	Aztreonam	≥64	R	Colistin		
8	Imipenem	1*	R*	Rifampicin		
9	Meropenem	≥16	R	Trimethoprim/ sulfamethoxazole	≥320	R

*=AES modified **= User modified

Table (4) Show the antibiotic susceptibility tests for Klebsiella pneumonia

No	Antimicrobial	MIC	Interpretation	Antimicrobial	MIC	Interpretation
1	Ticarcillin	≥128	R	Amikacin	≥64	R
2	Ticarcillin/Clavulanic acid	128≥	R	Gentamicin	4	S
3	Piperacillin	128≥	R	Tobramycin	16≥	R
4	Pippiperacillin/ Tazobactam	128≥	R	Ciprofloxacin	4≥	R
5	Ceftazidime	16	R	Pefloxacin		
6	Cefepime	64≥	R	Minocycline	16≥	R
7	Aztreonam	64≥	R	Colistin		
8	Imipenem	2	1	Rifampicin		

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9	Meropenem	16≥	R	Trimethoprim/ sulfamethoxazole	320≥	R

Conclusions:

1. 1. Bovine respiratory disease (BRD) reasons huge economic havoc on dairy and meat farms.
2. Klebsiella pneumonia is a bacterial microorganism that is a zoonotic pathogen that causes affecting diseases in humans and animals.
3. The results of Biochemical identification of Klebsiella pneumonia showed that bacteria were Catalase positive, oxidase negative, Gelatin hydrolysis negative, lactose fermenter, non-motile, Indole negative, citrate utilization negative.

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