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# Identification of *Klebsilla Spp.* From Different Ruminant Secretion in Thi-Qar Province

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**Abstract**; The current study explained that detection of Klebsiella species from different infected cattle secretions. 120 samples and swabs were collected divided in to (40 as nasal secretion, 40 as vaginal discharge and finally 40 as a milk samples). These sample cultured on different culture media and identified by using cultural characteristic and microscopic appearance. klebsiella pneumonia Identified using ViteKII system and Confirm the final diagnosed of those organism by using polymerase chain reaction(PCR)assay (16S rRNA). Polymerase chain reaction used to detected some gene Kl,K2(which is part of several genes responsible for capsule formation), and also blaSHv gene which is one of the genes related with multy drug résistance.

Results show that Klebsiella pneumoniae were isolated in rate (10%) from nasal discharge, while isolated in rate (10%) from milk samples, on the

other hand, vaginal secretion not appeared positive result for Klebsiella

pneumonia. the result involved that Kl,K2 genes were not detected in all samples of K. pneumonia. Also, completely isolates of K. pneumonia harbored blaSHv gene (100%), Also the result included that Klebsiella pneumoniae can be consider as major pathogen responsible for different medical case affected bovine in Al-Shatrah distric in ThiQar province.

**Keywords: Klebsiella pneumoniae, milk, vagial secretion, nasal discharge.**

## Introduction

Klebsiella spp. are significant zoonotic pathogens globally, posing a risk of transmission between people and dairy cattle. (Song et al., 2023). Klebsiella spp. are opportunistic pathogens colonizing mucosal surfaces without causing pathology, It may spread to other tissues causing threat to life (Paczosa and Mecsas, 2016). Gram-negative, non-ciliated, non-spomlating, bacilliform bacteria with a thick cell wall, Klebsiella spp. are virulent in vivo and produce mucous colonies in solidstate in vitro cultures. Of the several Klebsiella species and subspecies, Klebsiella pneumonia is thought to be the most clinically significant in both humans and animals. Plants, soil, wastewater, surface water, and other ecosystems are all considered to be contaminated by Klebsiella species. Klebsiella pneumonia is pathogen capable of causing a different infections. Classically, Klebsiella pneumonia is known the main causes of pneumonia, Urinary tract infections and bacteremia (Choby ,et al.,2020). Antibiotics are frequently given to at-risk calves to avoid bacterial pneumonia, which increases stress and viral infection and generates significant financial losses for the cattle sector. The mass of administration of antibiotic lead to negative result in meat production and that perhaps lead to increase of resistant pathogen (Vulikh et al.,2019). This study amed to detection and Identification of klebsiella pneumonia using culturing, using ViteKII system and Confirm the final diagnosed of those organism by using polymerase chain reaction(PCR)assay.

## Material &Methods

### Isolation and primary identification of bacteria:

One hundred and twenty swabs and samples was collecting from the cattle, from Thi -Qar province, the information including age and location were fixed. The samples collected m sterilized container and swabs put in transport media then transport in ice box to microbiology laboratory in veterinary collage within period less than 24 hours. The, the samples and swab incubated in brain heart infusion in $37^0$C for 24 hours then subcultured on MacConky agar. incubated at 37 $^0$C for 24-48 hours, according to first identification included size color and shape of colony on MacConky agar, . Under a microscope, the Klebsiella pneumoniae isolates were confirmed to be non-motile, Gram-negative.

### Identification by using automated methods [Viteklll system

There were 30 individual wells on the plastic reagent cards that made up the Vitekll system each of which contain a microliter quantitative sample of various biochemical test medium needed for diagnosing a given organism, An inoculum was taken from cultured sample and put automatically on the card and the card's color changes as a result of the microbe's metabolism were periodically measured by a photometer. Gram-negative (GN) and Gram positive (GP) identification cards were generated after the information was entered in to a computerized data for analysis and storage(Maina and Kagotho, 2014).

Molecular studies

Bacterial DNA extraction

The Presto$^{TM}$ Mini gDNA Bacteria Kit was used to extract the genomic DNA from all Klebsiella pneumoniae isolates in accordance with the manufacturer's procedure, which may be summed up as follows:

1. Sample Preparation

After being transferred to a 1.5 ml micro-centrifuge tube, the overnight BHI broth culture of bacterial isolates (up to I x 109) was centrifuged for I minute at 14—16,000 rpm, and the supernatant was disposed of. added 180 gl of GT Buffer, and then used a pipette or vortex to resuspend the cell pellet. put 20 gl of Proteinase K in. Incubate for at least 10 minutes at $60^0$C. Every three minutes during incubation, flip the tube.

2. Lysis

Two hundred microliters of GB Buffer were added to the sample and mixed by vortexing for 10 sec, then incubated at $70^0$C for at least 10 mm to ensure the sample lysate is clear. During incubation, inverted the tube every 3 min. At this time, pre-heat the required Elution Buffer to $70^0$C (for step 5 DNA Elution).

3. DNA Binding

The sample lysate was immediately combined with two hundred microliters of 100% ethanol and shaken briskly. If precipitation forms, use a pipette to break it up as much as you can. Next, put a GD Column in a 2 ml Collection Tube. After that, move the mixture—including any precipitate that is insoluble—to the GD Column and spin it for two minutes at 14—16,000 x g. Place the GD Column in a fresh 2 ml Collection Tube after discarding the 2 ml Collection Tube that contained the flow-through.

4. Wash

The GD Column was filled with 400 microliters of WI Buffer, centrifuged for 30 seconds at 14— 16,000 rpm, and the flow-through was disposed of. reinserted the GD Column into the 2 ml Collection Tube and filled it with 600 gl of Wash Buffer (ensurmg that ethanol was supplied). After 30 seconds of centrifuging at 14—16,000 rpm and discarding the flow-through, replace the GD Column in the 2 ml Collection Tube. Lastly, the column matrix was dried by centrifuging it one more for three minutes at 14—16,000 rpm.

5. Elution

After transfen-ing the dried GD Column to a sterile 1.5 ml microcentrifuge tube, 100gl of preheated Elution Bufferl was added to the middle of the column matrix. The mixture was then centrifuged for 30 seconds at 14—16,000 rpm to elute the purified

Statistical Analysis

Statistical analysis was conducted using descriptive statistics; nominal variables (source of isolation) were expressed as percentages (%). The age variable was categorized into five age groups and considered ordinal variables. All the associations and goodness of fit between variables were analyzed by Chi-square and Fisher's exact tests. The Eta test measured the correlation between interval variables and numerical vanables. The Alpha significance level was considered, and values less than 0.05 were considered statistically significant.

The result

The bacterial isolation rate of the different cow samples(120) samples, 58(48.3%) showed positive growth (20 milk, 20 nasal swabs, and 18 vaginal swabs). Only 4/58 (6.9%) isolates were identified as Klebsiella pneumonia. The isolation rate of Klebsiella pneumonia was equal in milk and s nasal

secretion 2/20(10%) for each. Nevertheless, Klebsiella pneumonia could not be isolated from all vaginal secretion 0 18(0%). The statistical analysis showed no significant differences (P>0.05) in the bacterial isolation as showed m table (l).

Table (1): Isolation rate of different cow samples

| No. of swabs | Sours | No. | Positive growth | Positive to Klebsiella and ercent |
|---|---|---|---|---|
| 120 | Mllk | 40 | 20 | 2 (10%) |
| | Nasal secretion | 40 | 20 | 2 (10%) |
| | Vaginal secretion | 40 | 18 | 0 |
| Total | | 120 | 58(48.3%) | |
| P-value | 0.876 | | | |

## Discussion

Klebsiella pneumoniae is recorded one of the most important opportunistic pathogen in animals causing mainly respiratory infections and mastitis (Ribeiro et al; 2022), In a current study the samples were examined for presence of Klebsiella isolates. A total of 120 clinical samples and swabs were included (40from milk, 40from nasal discharge and40 from vagma discharge) about 62 of these samples and swabs are no growth and 58(48%) of bacteria culture belong to different Gram negative bacteria.

From all milk samples were examined the results, showed that 20 (50%) which contamed bacterial growth and 20 (50%) of these samples were negative for bacterial growth. From positive cultures of milk samples several Gram negative bacterial isolates were identified such as E.coli 5 (25%),Enterobacter cloacae complex 3(15%), Leclerciaa decarboxylata2( 10%), Klebsiella pneumoniae2(10%) Acinetobacter spp. 2(10%)Stenotrophomonas maltophillia 2 (10%),while other bacterial isolates were Pseudomonas alcaligenes, , Providenciarettgeri, Pseudomonasstutzeri, and Pseudomonas aemgiosa I (5%) .

From 40 nasal swabs 20 showed no growth and 20 showed growth that include E.coli 13(65%), Burkholderiacepacia 1(5%), Coronobactersakazakii 1(5%), Achromobacterdenitrificans 1(5%), Pseudomonasalcaligenes I (5%), Burkholderiagaldioli 1(5%) and Klebsiellapneumonia 2(10%).

From 40 vaginal swabs isolated the present study involved no growth were in 22 swabs,while the positive numbers were 18(45%) included E.coli 13(72,5%), Escherichia hermannii 1(5,5%), Achromobacter lowffii 1(5,5%), Aeromonas hydrophilal(5,5%), Pseudomonas aemgiosa 1(5,5%) and Enterobaxter cloacaecomplex 1(5,5%).

Klebsiella pneumonia were isolated from millk samples in rate (2\20 (10%) . On the other hand, its isolated from nasal discharge in rate 2\20(10%) Nevertheless, K. pneumonia could not be isolated from all vaginal secretion 0/18(0%).

This result agree to Ramadan (2023) which was not recorded of Klebsiella pneumoniae from vaginal secretion cattle and isolated Klebsiella pneumonia number was in nasal swaps 24/233(10.3%) and 5/60(8.33%) milk and. The current study which agreement to other study done by Chiaverini and his group (2022), who examined samples from wild animals and the Klebsiella pneumonia isolate recorded13/130 in rate 13%. In addition to that, he detected the same cultural characters of klebsiella species and recorded that the isolated bacteria appeared as pink, large ,mucoid colomes on McConkey agar for 24 h at 37 C.

Higher isolation rate of klebsiella species from different sources most of them were from pig, cattle, milk and the rest of isolates were isolated from vegetables, pets, livestock and farm animals (Kiaper et al., 2021).

Vitekll system for identification of Klebsiella isolates:

In vitekll only 4/58 (6.9%) isolates were identified as Klebsiella pneumonia. This result which convenant to the results of (Yang 2021)when he isolated (4/66, 6.06%) .

Molecular discussion

The variation of 16SrRNA gene sequence in K. pneumoniae, 4 samples were included, which showed approximately 1500bp amplicons length., it was made sure that all the amplified amplicons had shown sharp, specific, and clean bands

The 16SrRNA gene sequencing used to identified the K. pneumoniae that isolated from various sources as samples obtained from animal, or from clinical isolates such as urme, blood, feces, sputum, which were aligned and compared to the sequence of strains that deposited in GenBank database (Budiarso et al., 2021).

Despite their potential health risks, street meals are popular in developing nations due to their low prices and preference among the lower and middle classes. According to Rowbotham and Ruegg (2016) and Fuenzalida and Ruegg (2019), Klebsiella pneumomae and Klebsiella oxytoca are commonly found in farm surroundings, cow skin and milk, teet-end swabs, and clinical mastitis. Additionally, the Klebsiella pneumoniae species was a significant pathogen linked to both human and ammal health. It was known to cause urinary tract infections, liver abscesses, and pneumonia (Bengoechea and Pessoa 2019; Fuenzalida and Ruegg 2019).

It is crucial to stress that different bacterial species have different 16S rRNA genes. Although shorter sequences can yield findings similar to those of the entire genome, the 16SrRNA gene sequencing was carried out on the chosen hyper-variable areas inside the 16S gene. Unfortunately, according to Liu et al. (2007), there is currently no one hypervariable area that can distinguish between every known species of bacterium.

The present results documented that high genetic variation in nucleotide positions of 16SrRNA gene in Klebsiella pneumoniae isolated from milk and nasal secretion of cattle that may be related isolated source of this microorganism. Budiarso et al., (2021) recorded 13 nucleotide polymorphic sites among Klebsiella pneumoniae group, Because the sequences of those isolates had a relatively high value of genetic distance, nucleotide blast, and isolates' strong similarity with Citrobacter sp., some isolates had two distmct nucleotide polymorphisms that differ from all isolates in nucleotide locations.

## Conclusion

Klebsiella pneumonia considered an important pathogen that can isolated from different animal samples. Vitekll system can be reliable way to diagnose klebsiella pneumonia isolates similar to polymerase chain reaction.

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