Animal Health Research Journal Vol. 7, No. 5, December 2019 pp. 57-67

ISSN: 2356-7767

# Study on *Klebsiella pneumonia*e causing respiratory infection in small ruminants

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Received in 12/11/2019
Accepted in 18/12/2019

**Abstract**

A total of 100 samples of nasal swabs and lungs (50 of each) collected from diseased sheep and goats were examined for bacterial causes of respiratory syndrome. Samples were collected from Cairo and Giza abattoirs. Bacteriological examination recovered 6 types of different bacteria which were *K. pneumoniae* (36%), *K. oxytoca* (16%), *S. aureus* (26%), *Proteus* spp. (20%), *E. coli* (18%), and *P. aeurginosa* (15%). The results of antibiotic sensitivity pattern of 36 *K. pneumoniae* against eleven commonly used antibacterial agents showed that the isolates were highly sensitive to ceftriaxone, enrofloxacin, norfloxacin (100% of each) and sensitive to cefotaxone and ciprofloxacin (66.7% of each) while all isolates resistant to sulphamethaxozle/trimethoprim (88.9%), oxytetracycline (86.1%), ampicillin (83.3%), amoxicillin (83.3%), chloramphincol (66.7%) and nalidexic acid (66.7%). Molecular studies using PCR were applied on five strains of *K. pneumoniae* isolated from sheep and goats for detection of antibiotic resistance genes (*blaCTX, Sul1, blaTEM, tetA* and *blaSHV*). The results of antimicrobial resistance genes *blaCTX* (40%), *Sul1* (100%), *blaTEM* (60%), *tetA* (80%) and *blaSHV* (80%) were detected among *K. pneumoniae* strains.

***Keywords:*** *Respiratory diseases, small ruminants, Klebsiella pneumonia, PCR.*

## Introduction

Sheep and Goats play vital economic roles as they are raised mainly for lamb production followed by milk, wool and hair production for large section of population especially in village and desert area. They can support the survival of millions of people in many countries all over the world including Egypt **(Hatem *et al.*, 2003** and **Ali *et al.*, 2009**).

Pneumonia is an important disease and major problem of sheep and goats. Several causative agents and factors appear to be involved. The main cause of pneumonia are bacteria, then fungi and viruses whereas poor hygienic measures are the most factors to infection with *Klebsiella* **(Hafez, 2002**).

*K. pneumonia*eis a Gram-negative, medium in size, non-motile, rod shaped, which differentiated according to biochemical reactions **(Seaton, 2000),** which are found in nature and as a part of the normal flora in tissues of the respiratory tract **(Umeh and Berkowitz, 2006)**.

*K. pneumonia*e infection may occur at almost all body sits but the highest incidence is found in the respiratory and urinary tract and is an important nasocomial pathogen , most frequently causing pneumonia, urinary tract (**Brisse *et al.*, 2009**), also *K.pneumoniae* were reported as the most common cause of lung abscess in the western chain **(Takayanagi *et al.*, 2010**).

Antibiotics resistance is a serious problem in clinical medicine, the efficacy of treatment with the widely used $\beta$-lactamase antibiotics is constantly challenged by the emergence of new resistant bacteria strains $\beta$-lactamase production is the predominant mechanism for resistance to $\beta$-Lactamase antibiotics in Gram negative bacteria. In the recent years, a substantial increase in antibiotic resistance has been observed mainly in developing countries, because of self-medication suboptimal quality of bacteria can be transferred to pathogenic

species **(Doucet *et al*., 2001).**

The genotypic method using specific PCR amplification of resistance genes seems to have 100% specificity and sensitivity in detection of ESBL when compared to phenotypic methods which lacks the constant sensitivity (**Krishnamurthy *et al.,* 2013).**

Extended-Spectrum-*β*-Lactamase (**ESBL**) are enzymes that are often plasmid mediated were first described in 1980 and they have been detected in *Klebsiella* spp. and later in other gram -negative bacteria **(Kiratisin *et al*., 2008**).
Extended Spectrum β-Lactamases (ESBLs) genes were found in 93.4% of *K.pneumoniae* strains of which *blaTEM* was the most common (93.4%), followed by *CTX-M* and *SHV* were 57.6% and 39.4%, respectively **(Cheng *et al*., 2018**).

Determination of *blaTEM* and *blaSHV* genes by molecular techniques in ESBL producing bacteria and their pattern of antimicrobial resistance can supply useful data about their epidemiology **( Jain and Mondal, 2008**).
The present study aimed to characterized *K. pneumoniae* isolated from small ruminants, applying antibiogram pattern and molecular study of isolates to detect antibiotic resistance genes by PCR.

## Materials and Methods

### Collection of samples

A total of 100 samples from diseased sheep and goat (**50** of each), suffering of respiratory manifestation. Samples represented by nasal swabs before slaughter and lung samples after slaughter (**25** of each) were collected from Cairo and Giza abattoirs. Samples were collected in clean and sterile polyethylene bags and send to laboratory as quick as possible.

### Bacteriological examination

**Preparation of samples:** according to **I.CM.S.F. (1978)**
**Nasal swabs:** Nasal swabs were inoculated in buffer peptone water and incubated at 37$^{o}$ C for 24hrs. and inoculated onto MacConky agar and Baird Parker agar. In addition, a loop full of buffer peptone water was cultivated onto blood agar. All cultivated plates were incubated at 37$^{o}$ C for 24hrs. Suspected colonies were picked up for further identification.

**Lungs samples:**
**Direct method**: The surface of the lungs seared with hot spatula then incised using sterilized scalpel and the exudates from lung was taken by swabs and directly inoculated to MacConky agar, blood agar and Baird Parker agar , then incubated at 37$^{o}$C for 24hrs .

**Indirect method** :Twenty five grams of deep tissues under aseptic conditions after sterilization of lung surface by hot spatula were inoculated into **225** ml buffer peptone water in sterile stomacher bags and homogenized for **2** min. to provide 1/10 dilution (Original dilution).
1ml. of the original dilution to **9** ml of sterile peptone water and incubated at **37$^{o}$C** for **24**hrs then cultured onto MacConky agar and Baird Parker agar, also a loop full from original dilution was cultivated on Baird Parker agar and blood agar and incubated at **37$^{o}$C** for **24hr**s. Suspected colonies were subculture for further identification.

The prepared samples were streaked on the surface of Baird Parker agar (Oxoid-UK) plates for identification of *S. aureus* according to **FDA (200)** and incubated at **37$^{o}$C** for **24**hrs. Suspected colonies were picked up into slant of nutrient agar for biochemical identification and Coagulate activity.

**Biochemical identification of isolates**, according to **Quinn *et al.,* (2002**) the following biochemical tests: Indole, Methyl red, Voges-Proskauer, Simmon's citrate., Urea, Triple Sugar Iron, Sugar fermentation, Oxidase and Catalase in addition API 20 E were used for identification of *K. pneumoniae.*

**Antibiogram pattern:** according to **Quinn *et al*, (2002**)
Antibiogram pattern was applied upon the isolated strains of *Klebsiella pneumoniae* which were isolated from lungs and nasal swabs of sheep and goats, in vitro using Disc Diffusion Technique. Eleven different discs of chemotherapeutic agents (Oxoid), were used (ampicillin, amoxicillin, cefotaxime, ceftriaxone, chloramphinicol, ciproflxacin, enrofloxa-

cin, nalidixic acid, norfloxacin, oxytetracycline and sulphamethoxazole/trimethoptim. The results were interpreted according to **CLSI (2010) and EUCAST (2016**).

**PCR Technique:**

DNA extraction from samples was performed using the QIAamp DNA Mini kit (Qiagen, Germany, GmbH) with modifications from the manufacturer's recommendations. Briefly, 200 µl of the sample suspension was incubated with 10 µl of proteinase K and 200 µl of lysis buffer at 56ºC for 10 min. After incubation, 200 µl of 100% ethanol was added to the lysate. The sample was then washed and centrifuged following the manufacturer's recommendations. Nucleic acid was eluted with 100 µl of elution buffer provided in the kit.

**Oligonucleotide Primer**

Primers used were supplied from Metabion **(Germany)** are listed in Table (1).

**PCR amplification.**

PCR primers were utilized in a 25- µl reaction containing 12.5 µl of Emerald Amp Max PCR Master Mix **(Takara, Japan)** 1 µl of each primer of 20 pm of concentration, 4.5 µl of water, and 6 µl of DNA template. The reaction was performed in an applied bios system 2720 thermal cycler.

**Analysis of the PCR Products:**

The products of PCR were separated by electrophoresis on 1.5% agars gel (Applichem, Germany, GmbH) in 1x TBE buffer at room temperature using gradients of 5V/cm. For gel analysis, 20 µl of the PCR products were loaded in each gel slot. Gene ruler 100 bp ladder (Fermentas, Thermo Scientific,
Germany) was used to determine the fragment sizes. The gel was photographed by a gel documentation system (Alpha Innotech, Biometra) and the data was analyzed through computer software **(Monstein *et al*. 2007).**

**Table (1).** Primers sequences, target genes, amplicon sizes and cycling conditions for conventional PCR.

| Target Gene | Primers sequences | Amplified segment (bp) | Primary denaturation | Amplification (35 cycles) | | | Final extension | Reference |
|---|---|---|---|---|---|---|---|---|
| | | | | Secondary denaturation | Annealing | Extension | | |
| ***Sul1*** | CGGCGTGGGCTACCTGAACG<br>GCCGATCGCGTGAAGTTCCG | 433 bp | 94˚C 5 min. | 94˚C 30 sec. | 60˚C 40 sec. | 72˚C 45 sec. | 72˚C 10 min. | **Ibekwe *et al*., (2011)** |
| ***TetA(A)*** | GGTTCACTCGAACGACGTCA<br>CTGTCCGACAAGTTGCATGA | 576 bp | 94˚C 5 min. | 94˚C 30 sec. | 50˚C 40 sec. | 72˚C 45 sec. | 72˚C 10 min. | **Randall *et al.* (2004)** |
| ***blaTEM*** | ATCAGCAATAAACCAGC<br>CCCCGAAGAACGTTTTC | 516bp | 94˚C 5 min. | 94˚C 30 sec. | 54˚C 40 sec. | 72˚C 45 sec. | 72˚C 10 min. | **Colom *et al*., (2003)** |
| ***blaSHV*** | AGGATTGACTGCCTTTTTG<br>ATTTGCTGATTTCGCTCG | 392bp | 94˚C 5 min. | 94˚C 30 sec. | 54˚C 40 sec. | 72˚C 40 sec. | 72˚C 10 min. | |
| ***blaCTX*** | ATG TGC AGY ACC AGT AAR GTK ATG GC<br>TGG GTR AAR TAR GTS ACC AGA AYC AGC GG | 593bp | 94˚C 5 min. | 94˚C 30 sec. | 54˚C 40 sec. | 72˚C 45 sec. | 72˚C 10 min. | **Archambault *et al*, (2006)** |

## Results

Table (2) illustrated that *K. pneumoniae* (36%), *K. oxytoca* (16%), *S. aureus (*26%), *Proteus* spp. (20%), *E. coli* (18%) and *P. aeruginosa* (15%) were isolated from nasal swabs and lung samples.

**Table (2).** Prevalence rate of bacterial isolates among examines samples

| Samples / Bacterial isolates | Nasal swabs | | | | Lung samples | | | | Total (100) | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Sheep (No.25) | | Goats(No.25) | | Sheep (No.25) | | Goats (No.25) | | | |
| | No. | % | No. | % | No. | % | No. | % | No. | % |
| ***K. pneumoniae*** | 9 | 36% | 8 | 32% | 10 | 40% | 9 | 36% | 36 | 36% |
| ***K. oxytoca*** | 4 | 16% | 3 | 12% | 5 | 20% | 4 | 16% | 16 | 16% |
| ***S. aureus*** | 7 | 28% | 6 | 24% | 7 | 28% | 6 | 24% | 26 | 26% |
| ***Proteus* spp.** | 5 | 20% | 4 | 16% | 6 | 24% | 5 | 20% | 20 | 20% |
| ***E. coli*** | 4 | 16% | 3 | 12% | 6 | 24% | 5 | 20% | 18 | 18% |
| ***P. aeruginosa*** | 3 | 12% | 3 | 12% | 5 | 20% | 4 | 16% | 15 | 15% |

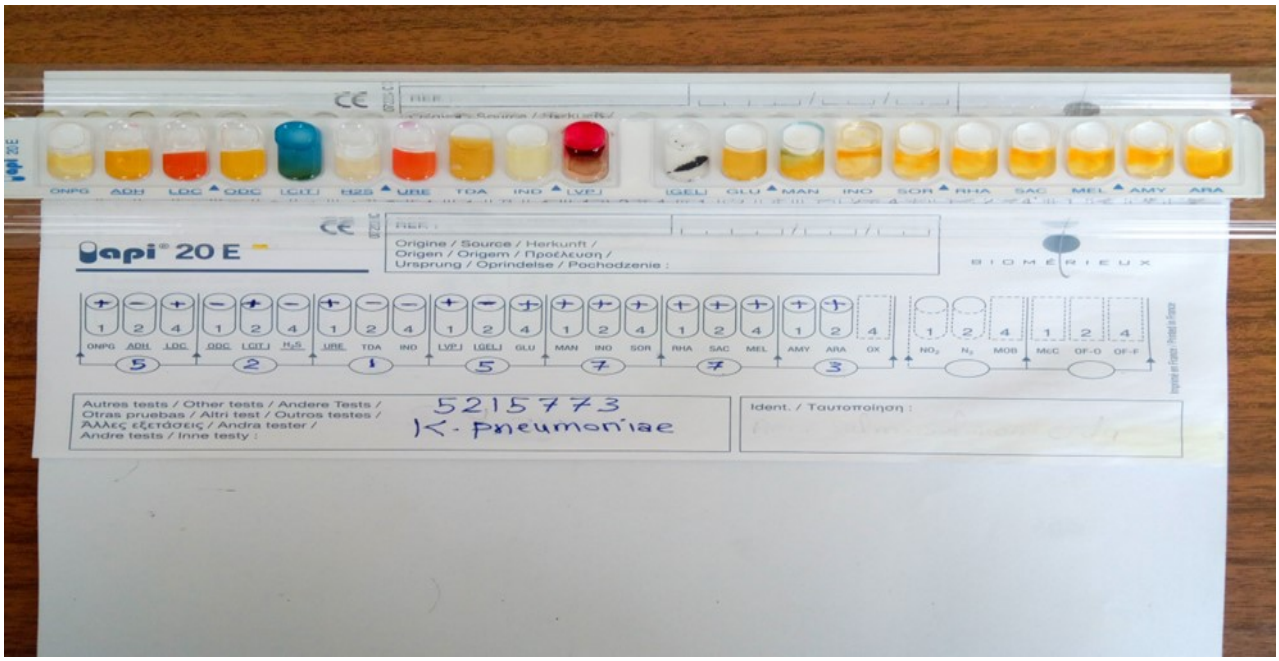


**Photo. (1):** API 20 E of *K. pneumoniae* isolated from nasal swabs and lung samples of sheep and goats.

## Antibiogram pattern among the isolates

As shown in Table (3) antibiotic sensitivity pattern of *K. pneumoniae* against eleven commonly used antibacterial agents showed that the isolates were highly sensitive to ceftriaxone, enrofloxacin, norfloxacin (100% of each) and sensitive to cefotaxone and ciprofloxacin (66.7% of each) while resistant sulphamethaxozle/trimethoprim (88.9%), oxytetracycline (86.1%), ampicillin (83.3%), amoxicillin (83.3%), chloramphincol (66.7%) and nalidexic acid (66,7%).

**Table (3).** Antibiogram pattern results of *K. pnumoniae* (36) isolated from nasal swabs and lung samples of the examined sheep and goats:

| Chemotherapeutic agents | Sensitive | | Resistance | |
|---|---|---|---|---|
| **Antibiotic discs (Symbol - Conc.)** | **N0.** | **%** | **N0.** | **%** |
| **Ampicillin (AM -10ug )** | 6 | 16.7% | 30 | 83.3% |
| **Amoxicillin (AMK -25ug )** | 6 | 16.7% | 30 | 83.3% |
| **Cefotaxime (CTX -30ug )** | 24 | 66.7% | 12 | 33.3% |
| **Ceftriaxone (CRO – 30ug)** | 36 | 100% | 0 | 0% |
| **Chloramphincol( C - 30 )** | 12 | 33.3% | 24 | 66.7% |
| **Ciprofloxacin ( CIP – 5ug )** | 24 | 66.7% | 12 | 33.3% |
| **Enerofloxacin( ENR – 30ug )** | 36 | 100% | 0 | 0% |
| **Nalidexic acid ( NA – 30ug )** | 12 | 33.3% | 24 | 66.6% |
| **Norfloxicin(NOR-10ug )** | 36 | 100% | 0 | 0% |
| **Oxytetracycline (OXT-25ug)** | 5 | 13.9% | 31 | 86.1% |
| **Sulphamethaxozle/Trimethoprim ( SXT – 25ug )** | 4 | 11.1% | 32 | 88.9% |

**Results of PCR:**

Table (**4**) and photos (2-4) recorded that the antimicrobial resistance genes *blaCTX* (40%), *Sul1* (100%), *blaTEM* (60%), *tetA* (80%) and *blaSHV* (80%) were detected among *K. pneumoniae* strains.

**Table (4).** Results of antibiotic resistance genes of *K. pneumoniae* isolated from sheeps and goats using PCR.

| Samples | Results | | | | |
|---|---|---|---|---|---|
| | ***blaCTX*** | ***Sul1*** | ***blaTEM*** | ***tetA*** | ***blaSHV*** |
| **1** | - | + | + | + | + |
| **2** | - | + | + | + | - |
| **3** | + | + | - | - | + |
| **4** | + | + | + | + | + |
| **5** | - | + | - | + | + |
| **Percentage (%)** | 40% | 100% | 60% | 80% | 80% |

Percentage (%) calculated for (5) tested samples *of K. pneumoniae*.

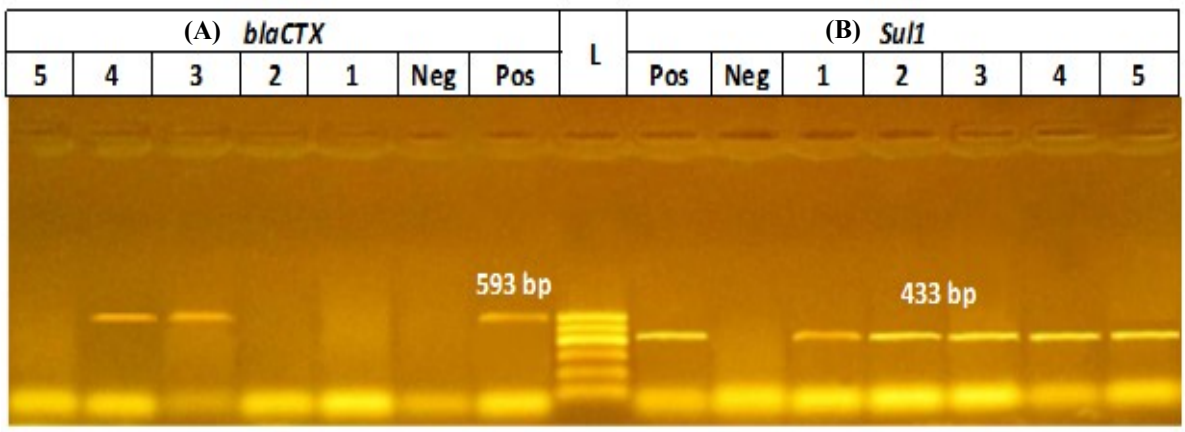


**Photo. (2): PCR results of antibiotic resistance genes *blaCTX* and *Sul1* among *K. pneumoniae***

**(A) *blaCTX* at range 433bp**
**Lane Pos.:** Positive control at rang 593 bp
**Lane Neg.:** Negative control
**Lanes:** Molecular weight marker
**Negative** *K. pneumoniae*: Samples no.1, 2, 5
**Positive** *K. pneumoniae*: Samples no. 3, 4

**(B) *blaSul1* at range 433bp**
**Lane Pos.:** Positive control at rang 392 bp
**Lane Neg.:** Negative control
**Lanes:** Molecular weight marker
**Negative** *K. pneumoniae*: 0
**Positive** *K. pneumoniae*: ALL samples no. 1, 2, 3, 4, 5

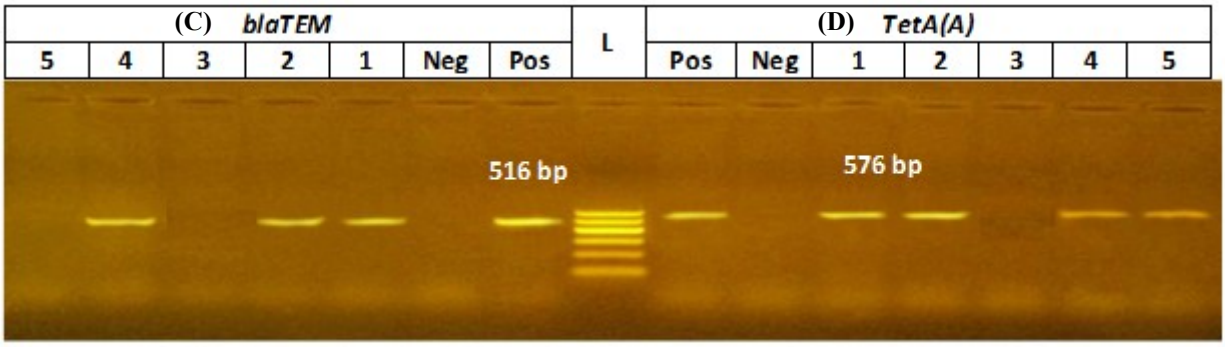


**Photo. (3): PCR results of antibiotic resistance genes *blaTEM* and *tetA* among *K. pneumoniae***

**(C) *blaTEM*** at range **516bp**
**Lane Pos**.: Positive control at rang 516 bp
**Lane Neg**.: Negative control
**Lanes**: Molecular weight marker
**Negative** *K. pneumoniae*: Samples no. 3, 5
**Positive** *K. pneumoniae*: Samples no. 1, 2, 4

**(D) *tetA*** at range **576bp**
**Lane Pos**.: Positive control at rang 576 bp
**Lane Neg**.: Negative control
**Lanes**: Molecular weight marker
**Negative** *K. pneumoniae*: Sample no.3
**Positive** *K. pneumoniae*: Samples no. 1, 2, 4, 5

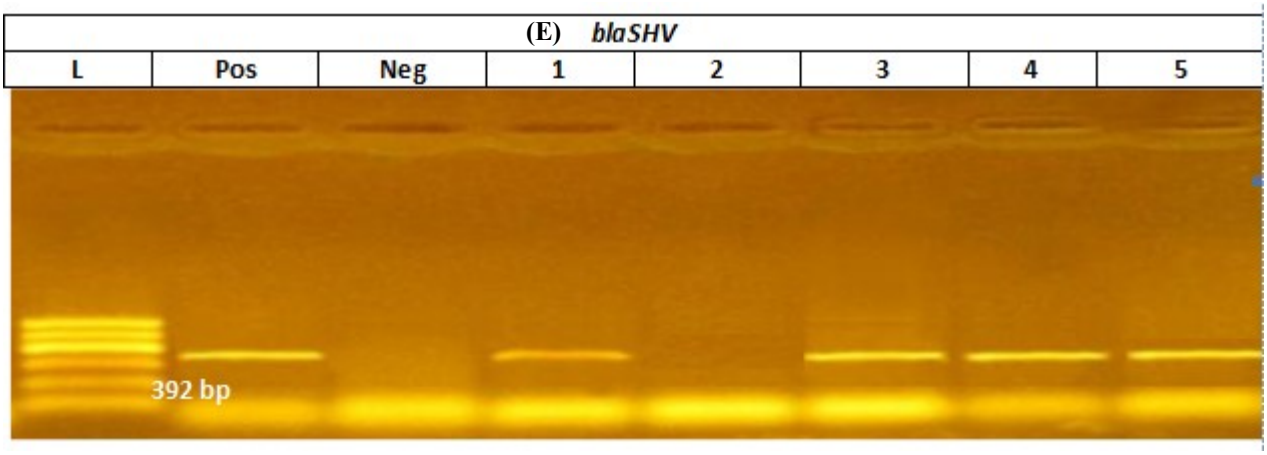


**Photo. (4): PCR results of antibiotic resistance gene (*blaSHV*) among *K. pneumonia***

**(E) *blaSHV*** at range **392bp**
**Lane Pos.:** Positive control at rang 392 bp
**Lane Neg.:** Negative control
**Lans:** Molecular weight marker
**Negative** *K. pneumoniae*: Sample no. 2
**Positive** *K. pneumoniae*: Samples no. 1, 3, 4, 5

## Discussion

Respiratory disorders are still serious problem facing sheep and goats raring **(Hatem *et al.*. 2003**), The importance of respiratory diseases of animals depends on their prevalence, their effect on productivity, the value of the animal and for some diseases, their international spread **(Ali *et al*. 2009**).

As shown in **Table (2) 6** different types of bacteria were recovered from nasal swabs and lung samples from the examined sheep and goats, which were *K. pneumoniae* (36%), *K. oxytoca* (**16%**) *S. aureus* (**26**%), *Proteus* spp. (**20**%), *E. coli* (**18**%) and *P. aeruginosa* (**15**%). The results cleared that *K. peumoniae* is the most predominant isolate in this study. These finding are **nearly agree** with those obtained by **El-Shabrawy, (2005**) and **Sayed and Zaitoun, (2009**) who isolate *S. aureus* (**22.43%)** and *E. coli* (**18.22%)** meanwhile less frequently among *Proteus* spp. (**7.01%)** and *K. pneumoniae* (**3.27%)** isolation. **Hafez (2002)** recorded low incidence of *S. aureus* **(1.5%)** and *E. coli* (**7%).** These isolated organisms are common in pneumonic lung tissues as widely documented by several authors, (**Ozbey and Muz, 2004**, **Ozyildiz *et.al*., 2013**

and **Ghanem *et al*., (2015).**
While our results are in accordance with **Mahmoud *et al*.(2005)** and **Saleh and Allam (2014)** who concluded that *K. pneumonae* were the most predominant bacteria isolated (48%), followed by *S. aureus* (**44%),** *Proteus* spp (**20%)** and recorded low percentage of *P. aeroginosa* and *E. coli* were (**10%** and **8%** respectively).

**Table (2)** showed that *K. pneumoniae* were recovered (36%) from nasal swabs (17%) and lung samples (19%) of sheep and goats. These results nearly agree with that obtained by **Elshehedi *et al*. (2017)** who isolated *K. pneumoniae* form apparently healthy and diseased lungs (26.7%) and (60%). While from nasal swabs (10%) and (20%) respectively.

On the other hand, our study was higher than that obtained by **Al-Tarazi (2001)** who isolated *K. pneumoniae* (14.66%) from pneumonic lungs in Jordan. **Hafez (2002)** isolated *K. pneumoniae* in an incidence (9.5%) from lung infection. Furthermore, (**Abdelmonem *et al*, 2009)** mentioned that *K. pneumoniae* was the major cause of nosocomial infection and gave rise to urinary and respiratory tract infections next to *E. coli*.

These bacteria are common in pulmonary mixed infections since the respiratory pathways act as a reservoir for potentially pathogenic micro- organisms which develop into pneumonia following stress, decline of hygiene measures or climatic conditions **(Moustafa, 2004).** The different sites of respiratory tract had been studied for presence of different bacterial isolates as shown that the bacterial isolates from nasal swabs, and lungs samples (**Jakleen, 2000**).

The variation in isolation percentage may be attributed to change in hygienic measure, stress factors, change in management and immune status of infected animals **(Sedeek and Thabet, 2001).**
As shown in Table (3) antibiotic sensitivity pattern of *K. pneumoniae* against eleven commonly used antibacterial agents showed that the isolates were highly sensitive to ceftriaxone, enrofloxacin,

norfloxacin (100% of each) and sensitive to cefotaxone and ciprofloxacin (66.7% of each) while resistant sulphamethaxozle/trimethoprim (88.9%), oxytetracycline (86.1%), ampicillin (83.3%), amoxicillin (83.3%), chloramphincol (66.7%) and nalidexic acid (66,7% ).

Our results agree with**, Moustafa (2004**) **and Sukanata *et al.* (2018**) who mentioned that the most isolates of *K. pneumoniae* were highly sensitive to ceftriaxone, enerofloxacin, and norfloacin and sensitive to while resistant to ampicillin, and sulphmethoxazol/trimethprime. **Cheng *et al*. (2018*)*** who recorded that *K. pneumoniae* isolates were resistance to ampicillin and amoxicillin.
Our data agree with **Brisse and Duijkeren (2005**) who found that *Klebsiella* animal clinical isolates were resistant against ampicillin (99%) but not against ceftazidime and sulfmethoxazole/trimethoprim. While **Mobasherizadeh *et al*. (2012**) recorded that *K. pneumoniae,* isolates were more resistant to first line drugs including ampicillin, sulphamethazole and oxytetracycline.
The variation of antibiotic sensitivity pattern of the respiratory bacterial isolates might be due to the presence of resistance genes. Indiscriminate use of antibiotics for treating the infected animals might also be responsible for acquiring antibiotic resistance **( Sukanta *et al*., 2018).**
*K. pneumonia*e may be naturally resistant to ampicillin and amoxicillin but not to Extended-Spectrum $\beta$-Lactam antibiotics due to a constitutively expressed chromosomal class A $\beta$-lactamse **(Mendonca** and **Ferreeira 2009**).
The most effective antimicrobial agents against all the tested isolates ciprofloxacin, oxytetracycline and ceftriaxone are the drugs of choice in the treatment of pneumonia in sheep **(AL-Doughaym *et al.* 1999**).
This result which is comparable with our studies in developing countries is due to the wide spread use of these drugs because of their low cost. Long term of carrying infection and antibiotic pressure select resistant strains **(Biedenbach *et al.,* 2004).**
Our study revealed that majority of isolates are resistant to, oxytetracycline, sulphamethazole,

and ampicillin which were coincided with **Azizian *et al.* (2014)** who recorded that 80% of *K. pneumoniae* having *sul1*gene**.** Due to complexity, it's important before therapy to know the drug sensitivities of the pathogen, but so many drug resistant patterns have been observed the resistance of bacterial isolates to antibiotics may be attributed to usage of wrong dose of antibiotic duration of treatment and route of administration. This result is comparable with our studies in developing countries is due to the wide spread use of these drugs because of their low cost **El-Shehedi *et al*. (2017).**
**Table (4)** illustrated that the prevalence of antibiotic resistance gene using specific PCR amplification showing variable results with tested strains of *K. pneumoniae* isolated from sheep and goats as follow: *blaCTX* (40%), *sul1* (100%), *blaTEM* (60%), *tetA* (80%), *blaSHV* (80%). These results nearly agree with that obtained by **De-Garcia *et al*. (2008)** who recorded that the antibiotic resistance genes which had *blaCTX* (40%), *blaSHV* (100%) and *blaTEM* (80%). **Li *et al*. (2013)** mentioned that *K. pneumoniae* infections were frequently reported as multidrug resistant producing ESBLs mainly including *blaTEM, blaSHV* and *blaCTX-M* types.

**Azizian *et al.* (2014)** and **Hamesipour and Tajbakhsh (2016)** found that 80% of *K. pneumoniae* strains possessed *sul1* gene. **Soge *et al*. (2011)** recorded that *β*-lactamases resistance is common among *K. pneumoniae* isolates due to production of *blaTEM-1*, *blaSHV*, *blaCTX-M* and *blaOKP* genes. In addition **Krishnamuthy *et al*. (2013)** mentioned that resistance genes seems to have 100% specificity and sensitivity in detection of extended spectrum lactamase producing *K. pneumoniae* when compared to phenotypic methods which lacks the constant sensitivity.

Determination of *blaTEM* and *blaSHV* genes by molecular techniques in ESBL producing bacteria and their pattern of antimicrobial resistance can supply useful data about their epidemiology **(Jain and Mondal, 2008).**
**Hiroi *et al.* (2012)** study the prevalence of extended spectrum β-lactamases (ESBLs) producing *K. pneumoniae* that harboring *blaCTX-M2* gene and *blaSHV-108* gene were detected. Also, *blaCTX-M* producing strains should be control due to the critical importance of cephalosporins and the zoonotic potential of ESBL-producing bacteria **(Hiroi *et al*, 2012)**.
PCR detection of proper MDR (multidrug resistance gene) isomers has been suggested, most MDR gene is *β*-lactamase gene (*bla*) which hydrolyses lactam ring of penicillin, other MDR genes include *tet*A gene isomers which encode membrane bound protein which kicks out tetracycline from bacterial cell cytoplasm, *sul*1 gene has been implicated in sulphamethoxazole resistance **(Kumar, 2016).**

## Conclusion

*K. pneumoniae* is recognized as an important opportunistic pathogen causing many diseases as respiratory tract infection, also *K. pneumoniae* naturally resistant to ampicillin, amoxicillin, this lead to β-lactam resistance genes.
The use of antibiotics as a control measures should be adopted to limit the spread of the multidrug resistant bacteriaand antibiotics should be given after making sensitivity test to the isolated organisms and must be given in recommended dose, routeof administration and duration of usage.

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