j.Egypt.vet.med.Assoc 78, no 4. 897- 919 (2018)

DETECTION OF SOME VIRULENCE AND ANTIBIOTIC RESISTANCE GENES OF *KLEBSIELLA PNEUMONIAE* ISOLATED FROM DISEASED BROILER CHICKENS

By

Aml M. Ragab*, Maha R. Basyoni* and Khaled E. EL-Ekhnawy**

* Bacteriology Department, Animal Health Research Institute, ARC Tanta Branch

** Chemistry Dept., Animal Health Research Institute, ARC Dokki

ABSTRACT

Respiratory diseases affected broiler chickens is a problem threat their production and cause agreat loses among them. This research was conducted to identify the extent of the spread of *Klebsiella pneumoniae* at different age's clinically diseased broiler chickens in Gharbia governorate. A total of 400 internal organs (heart, lung, liver, and spleen) were obtained from 100 sick chickens with respiratory symptoms were used to determine the prevalence of *Klebsiella pneumoniae* and the susceptibility patterns of isolates to antibiotics using disc diffusion assay. In addition, they were tested for some virulence genes such as, *rmp*A, *mag*A, *wca*G and antibiotic resistance genes (*bla*TEM and *bla*SHV) by using PCR. Bacteriological examination revealed that isolation of *Klebsiella Pneumonia* with a total prevalence rate reached (13%) as 13 out of 100 examined cases where (27) isolates of *K. pneumoniae* were detected. Lung (13%) had the largest percentage of *K. pneumoniae* isolates, liver (7%), spleen (5%), and heart (2%). All of the isolates were resistance to Ampicillin. 25 (92.6%) to Lincomycin, 24 (88.9%) to Ox tetracycline, 22 (81.5%) to cefotaxime and Doxycycline. 9 (33.3%) to ciprofloxacin, 8(29.6%) to Amoxicillin /clavulanic acid, 7 (25.9%) to Norfloxacin, 6 (22.2%) to gentamicin, andall of the isolates were sensitive toamikacin.

The presence of *gyr*A and *16S-23S* ITS genes in *Klebsiella Pneumonia* isolates were confirmed by PCR, all isolates tested were positive for (bla_{TEM} and bla_{SHV}), negative for *mag*A, *rmp*A and *wca*G genes.

INTRODUCTION

Klebsiella spp. is one of the normal flora of poultry's gut, but it can cause infections if the birds' immune systems are impaired. *Klebsiella pneumoniae* is an opportunistic pathogen linked to serious nosocomial infections includes septicemia, pneumonia, and urinary tract infections.

K. pneumonia subsp. pneumonia, K. pneumonia subsp. Ozaenae, and *K. pneumonia subsp. Rhinoscleromatis* are the three subspecies *of K. pneumonia.* **(Brisse and Verhoef, 2001).** *Klebsiella pneumoniae* is a lactose fermenting bacteria that is Gram negative, encapsulated, non-motile, and has a rod shape, they are facultative anaerobic bacteria that cause infections when a bird's immune system is weak**.(Janda and Abbott, 2006).** On the cell surface of *Klebsiella,* there are two types of antigens. Smooth lipopolysaccharide (O-antigen) is the first, while capsular polysaccharide is the second (K-antigen). These antigens play a role in its pathogenesis. Polysaccharide capsule is a major factor in the virulence of *Klebsiella* and is also responsible for the mucoid colony phenotype, **(Sikarwar and Batra, 2011).** *K. pneumoniae* can produce several virulence factors as smooth *LPS,* pilli for adhesion to host cells, capsules which are ant phagocytic, siderophores help this bacteriumin its competition with the host for iron uptake **(SAljanaby and Alhasani, 2016).** The *gyr*A and *gyr*B genes, these are made up of two A and two B subunits. For *Klebsiella* detection, genes encoding subunit A of DNA gyrase (*gyr*A) is employed **(Brisse and Verhoef, 2001).** This bacterium is found in regions of the environment causing embryonic death and high losses in turkeys and hens **(Orajaka and Mohan, 1985).**

It has been isolated from chickens where it was the predominant pathogen and was accompanied by respiratory symptoms, septicaemia, peritonitis, salpingitis, air sac disease, omphalitis,artheritis,endopthalmitis, disturbances in intestinal tract and egg production has decreased **(Sandra and Duarte, 1998); (Aly *et al.*, 2014) and (Saif *et al.*, 2003).**

On the genomic map of *K. pneumoniae* capsule, gene clusters can be found as following: *rmp*A (Regulator of mucoid phenotype A), *Wb* (O-specific polysaccharide), *cps* (Capsular polysaccharide synthesis), *mag*A (Mucoviscosity associated gene A) and *kfu* gene(Iron uptake system gene) **(Regue *et al.*, 2005 and Seidler *et al.*, 1975),** *rmp*A gene regulates the synthesis of the *Klebsiella* polysaccharide capsule, *mag*A is a member of *K. pneumoniae* serotype *K1 capsular* polysaccharide gene that boosts toits pathogenicity **(Fang *et al.*, 2004)**, *mag*A is involved in serious *Klebsiella* infections as pneumonia, septicemia and bacteremia **(Chan *et al.*, 2005 and Chung *et al.*, 2007),** *kfu* gene which codes for an iron uptake system, this gene is thought to be particularly significant iniron uptake from the host cell.**(Aher *et al.*, 2012)** which is a putative pathogenic gene, more prevalent in hypervirulent strains **(Ma *et al.*, 2005).**On the transferrable areas ofchromosome, *wca*G gene is important for

K. pneumoniae capsule biosynthesis, it is requiredto convert of mannose to fucose, and this helps bacteria to resist macrophage phagocytosis **(Shu *et al.*, 2009).** Resistance and virulence do not have mutually exclusive characteristics,and their interaction could be crucial in pathogenicity of *K. pneumoniae* **(Vila *et al.*, 2011**).Most of the criteria of virulence and resistance have been transferred across bacteria byhorizontal gene transfer, DNA transfer is likely most essential mechanism for virulence andresistance traits spread and co-selection **(Da Silva and Mendonc, 2012).**

Gram negative bacilli produce extended-spectrum beta-lactamases (ESBLs), they are plasmid-mediated enzymes, penicillin and cephalosporin resistance is conferred by extended- spectrum beta-lactamases. Also, these plasmids carry resistance genes to other bacteria (Multidrug resistant bacteria) **(Jacoby, 1997).**

Transmission of transmissible plasmids, which may also carry virulence genes, is typically linked to antimicrobial resistance. Gaining resistance and virulent features may make the microbe have the ability to endure and survive **(Da Silva and Mendonc, 2012).** By increasing capsular polysaccharide synthesis, *rmp*A gene provides a hypermucoviscous phenotypic to *K. pneumonia*. Also, the extensive and wrong use of these antimicrobials, it cause the bacteriato develop itself, so that it can overcome these antibiotics **(Wright *et al.*, 2005).** Therefore, Food contamination with multidrug resistant bacteria is a serious public health concern, transmission of antibiotic- resistant features to pathogenic bacteria could impair clinical treatment **(Van *et al.*, 2007).** This study sought to examine the virulence and resistance factors of *K. pneumonia.*

MATERIAL AND METHODS

Samples:

400 samples (heart, lung, liver and spleen) were collected from 100 varying age broiler chickens suffered from respiratory manifestation from some farms in Gharbia governorate, Egypt.

Isolation and identification:

Specimens were inoculated directly onto nutrient broth, incubated at 37°C for 24 h, and then streaked onto MacConkey agar and (XLD) agar and incubated at 37°C for 24hours,mucoid

lactose fermenting colonies were purified and confirmed by biochemical tests according to **(Harada *et al.,* 2013) and (Dashe *et al.*,2013).**

PCR Procedures:

a-DNA Extraction:

DNA extraction from pure isolates (isolate from each organ) were performed according to **(Qiagen, Germany, GmbH)** with some changes , 200 µl of sample and 10 µl of proteinase K and 200 µl of lysis buffer were incubated at 56°C for 10 minutes . Added 200 µl ethanol to the lysate. After that, washing and centrifugation to the sample. Using100 µl elution buffer to elute nucleic acid.

b-Oligonucleotide Primer:

The primers, which are listed as in (Table 1), **Metabion (Germany).** Amplification of genes tested by PCR: 12.5 µl Emerald Amp (Takara, Japan), 1 µl of primer at a concentration of twenty pmol 45 µl H2o, 6 µl of DNA template were added to a 25- µl reaction.

c-Analysis of the PCR Products:

By electrophoresis (Applichem, Germany, GmbH), at room temperature on 1.5% agarosegel in 1x TBE buffer using gradients of 5V/cm to separate PCR products . Each gel slot wasfilled with 20 µl of uniplex PCR products for gel analysis. The fragment sizes were determined using 100 base pair DNA ladders (Qiagen, Germany, GmbH). (AlphaInnotech, Biometra) was used to photograph the gel, data was processed using computer software.

Table (1): Gene sequence of gene primers and phases of cycling conditions.

Target gene	Primers sequences	Amplied segment (bp)	Primary denaturation	Amplification (35 cycles)			Final extension	Reference
				Secondary denaturation	Annealing	Extension		
Klebsiella gyrA	F.CGC GTA CTA TAC GCC ATG AAC GTA R. ACC GTT GAT CAC TTC GGT CAG G	441	94°C 5 min.	94°C 30 sec.	55°C 40 sec.	72°C 40 sec.	72° C 10 min.	Brisse and Verhoef, (2001)
magA	F. GGTGCTCTTTACATCAT TGC R. GCAATGGCCATTTGCGT TAG	1282	94°C5 min.	94°C 30 sec.	50°C 40 sec.	72°C 1.2 min.	72° C 12min.	Yeh *et al*, (2007)
rmpA	F. ACTGGGCTACCTCTGCT TCA R. CTTGCATGAGCCATCTT TCA	535	94°C 5 min.	94°C 30 sec.	50°C 40 sec.	72°C 40 sec.	72° C 10 min.	
K. pneumoniae 16S-23S ITS	F. ATTTGAAGAGGTTGCA ACGAT R. TTCACTCTGAAGTTTC TTGTGTTC	130	94°C 5 min.	94°C 30 sec.	55°C 30 sec.	72°C 30 sec.	72° C 10 min.	Turton *et al*, (2010)
WcaG	F. GGTTGGGTCAGCAC GTA R. ACTATTCCGCCAATT TGC	169	94°C 5 min.	94°C 30 sec.	58°C 30 sec.	72°C 30 sec.	72° C 10 min.	Derakhshan *et al*, (2016)
BlaTEM	F. ATCAGCAATAAACCAGC R. CCCCGAAGAACGTTTTC	516	94°C 5 min.	94°C 30 sec	54°C 30 sec.	72°C 30 sec	72° C 10 min.	Colom *et al*, (2003)
blaSHV	F. AGGATTGACTGCCT TTTTG R. ATTTGCTGATTTCGCTCG	392	94°C 5 min.	94°C 30 sec	54°C 30 sec.	72°C 30 sec	72° C 10 min.	

Antimicrobial susceptibity testing:

Bacterial resistance profile was identified by disc diffusion assay **(CLSI, 2007).**

The antibacterial agents which were tested: Ampicillin (Amp10 µg), Oxytetracycline (30 µg), Gentamicin (CN10 µg), Amoxicillin / Clavulinic acid (Amc 20 µg), Doxycycline (DO30 µg), Norfloxacin (NOR10 µg) Cefotaxime (CTX 30µg), Ciprofloxacin (CIP 5 µg), and Amikacin (Ak30µg) and Lincomycin (NY10µg), **(Janet and John, 2007).**

RESULTS AND DISCUSSION

Table (2): Frequency of *Klebsiella Pneumonia* in different organs.

Samples	No. of examined samples	*Klebsiella spp*		*Klebsiella* Pneumonia	
		No	%	No	%
Liver	100	16	16%*	7	7%*
Lung	100	29	29%*	13	13%*
Spleen	100	13	13%*	5	5%*
Heart	100	7	7%*	2	2%*
Total	400	65	16.25%**	27	6.75%**

*** % in relation to number of each organ.**

**** % in relation to total number of organs.**

Bacterial cultures and identification:

The morphological examination showed that suspected colonies were circular, large and mucoid colonies **(Dashe *et al.*, 2013).** The biochemical identification proved that, the isolated strains were negative to indole, oxidase, H2S production, methyl red, and coagulase, positive to lactose fermentation, urea hydrolysis, catalase, citrate, voges proskauer, lysine decarboxylase, so they were confirmed to be *K. pneumoniae*. **(Barbara *et al.*, 1994).**

400 organ samples were collected from 100 clinically diseased broiler chickens (Liver, lung, heart and spleen). Bacteriological examination revealed that isolation of *Klebsiella spp*. with the highest prevalence rate (29%) as 29 out of 100 examined cases and *K. pneumonia* with the highest prevalence rate (13%) as 13 out of 100 examined cases.

A low prevalence rate of *K. pneumonia* has been reported by **(Aher *et al.*, 2012)** at ratio of 5.6% and **(Yimer and Asseged, 2007)** revealed rate of *Klebsiella spp.* at ratio 1.3%. 65 isolates of *Klebsiella spp*. were isolated from 400 organs, 27 isolates of *K. pneumoniae* were recovered from 400 organs, (Four samples per each bird), the greatest isolation rate of *K. pneumonia* was from lung (13%) then liver (7%) then spleen (5%) and the least of them was heart (2%) (Table2). These findings were lower than that reported by **(Aya *et al.*, 2017)** and **(Türkyilmaz, 2006)** who recovered *Klebsiella* species with a prevalence rate 22.78%. 47.1% respectively.

While our findings were higher than the results reported by **(Hossain *et al.*, 2013); (Khalda**

***et al.*, 2013); (Aly *et al.*, 2014) and (Younis *et al.*, 2016)** with frequency rate (6%, 8.69%, 10.2%, 10% and 15%) respectively. *Klebsiella* was isolated from the lungs at a higher rate than from other organs. These results corroborate those of **(Younis *et al.*, 2016)** who said that *Klebsiella* was isolated with the highest percentage of lungs and disagrees with **(Aya *et al.*, 2017)** who said that *Klebsiella* species recovered from liver is the highest percentage. The presence of *K. pneumoniae* in the internal organs of infected birds may have the presence of concomitant extra intestinal illnesses **(Türkyilmaz, 2006). Dashe *et al.*, (2013)** recoverd *K. pneumoniae* from the lungs and liver of 400 seemingly healthy hens with an 8% success rate. **Younis *et al.*, (2016)** reported that 30 (15%) *Klebsiella* species isolates were yielded from 200 tissue samples which differentiated into *K. pneumoniae* 11 % (22/200) and *K. oxytoca* 4 % (8/200), these results are higher than our results. **Aya *et al.*, (2017)** isolated (7.78%) *K. pneumonia*, high isolation rate (64%, 73.33% and 18%) was reported by **(Ajayi and Egbebi, 2011), (Younis *et al.*, 2016) and (Kumbish *et al.*, 2006)** respectively. Lesser prevalence was noted by **(Dashe *et al.*, 2008) and (Yehia and Riyadh, 2013)** (1.5% and 3.33%) respectively.

Table (3): Antibacterial susceptibility testing of *K. pneumoniae* **(CLSI, 2007).**

Antimicrobial.	Resistance	Sensitive
Norfloxacin	**7 (25.9%)**	**20 (74.1%)**
Gentamicin	**6 (22.2%)**	**21(77.8%)**
Ciproflxacin	**9 (33.3%)**	**18 (66.7%)**
Amikacin	**- (0%)**	**27 (100%)**
Ampicillin	**27 (100%)**	**- (0%)**
Oxytetracycline	**24 (88.9%)**	**3 (11.1%)**
Cefotaxime	**22 (81.5%)**	**5 (18.5%)**
Lincomycin	**25 (92.6%)**	**2 (7.4%)**
Amoxicillin / clavulanic acid	**8 (29.6%)**	**21 (77.8%)**
Doxycycline	**22 (81.5%)**	**5 (18.5%)**

***%: calculated according to the No. of tested isolates (27).**

Disc diffusion assay was used to test 27 *K. pneumoniae* isolates against ten antibiotic drugs, all of these isolates were ampicillin resistant, 25 (92.6 %) to lincomycin, 24 (88.9%) to oxytetracycline, 22 (81.5%) to cefotaxime and doxycycline. 9 (33.3%) to ciprofloxacin, 8 (29.6%) to amoxicillin / clavulanic acid, 7 (25.9 %) to norfloxacin, 6 (22.2%) to gentamicin, and all of the isolates were sensitive to amikacin as shown in (Table 3). The results showed that 18.5% (5/27) of our strains had multiple resistances (more than three classes of antibiotics). β - Lactam antibiotics are commonly used to treat *K. pneumoniae* infections; however, one of the most often used resistance medications is beta-lactam antibiotics, which has been a major issue in medical clinics in recent years. **(Fang *et al.*, 2004 and Amin *et al.*, 2009).**

In our study, antibacterial sensitivity tests revealed that our strains were resistant to various antibiotic groups. The percentage of ampicillin resistance was very high (100%); due to a constitutively generated chromosomal class Ab-lactamase, the majority of *Klebsiella* isolates are spontaneously resistant to ampicillin **(Livermore 1995).** Amoxicillin/clavulanic acid resistance was found in 29.6% of the isolates that agrees with **(Beyene and Tsegaye, 2011) and (Behnam *et al.*, 2014). Younis *et al.*, (2016)** who reported that amoxicillin and amoxicillin/clavulanic acid resistance was found in all of their strains in contrast with our finding Veterinary bacterial isolates have been discovered to be resistant to amoxicillin **(Brisse and Van Dujjkeren, 2005).** About (60.5%) of *K. pneumoniae* isolates were resistant to amoxicillin/clavulanic acid **(Derakhshan *et al.*, 2016).** Gentamicin sensitive was found in 77.8% of *K. pneumoniae* isolates, these results *nearly* agree with **(Behnam *et al.*, 2014);** the sensitivity rate they recorded was 65.89%, a low prevalence of *K. pneumoniae* isolates were sensitive (48.5%) to gentamicin **(Derakhshan *et al.*, 2016).** As for antimicrobial resistance to cephalosporin, (Table 3) showed that, the resistance rate for cephalosporin (cefotaxime) was 81.5%, this was roughly in agreement with those reported by **(Singh and Goyal 2003) and (Younis *et al.*, 2016)**,resistance to cephalosporin were (86.67% for cefotaxime, 66.67% for ceftriaxone and 70 % for cefapime), other research revealed that low resistance to cephalosporins **(Ullah *et al.*,2009)and (Derakhshan *et al.*, 2016),** (60.0 %) to cefotaxime. Resistance of *K. pneumoniae* isolates to ciprofloxacin was 33.3% in our research which was almost similar to previous results recorded by **(Villegas *et al.*, 2004). Derakhshan *et al.*, (2016) and Younis *et al.*, (2016)** recorded a high level of resistance of ciprofloxacin to *Klebsiella pneumoniae*, (50% and 66.67 %) respectively, this agrees with the results reported by

Ullah *et al.*, (2009). Ciprofloxacin is fluoroquinolone antibiotic **(Periti *et al*., 1998),**mutation that occurred in the *gyr*A gene (It is code for the quinolone activity target)led to resistance to ciprofloxacin **(Bagel *et al.*, 1999).** Aminoglycosides are effective against gram-negative bacteria, which are significant in therapeutic practice **(Ramirez and Tolmasky, 2010).** Our isolates were sensitive to amikacin. *Klebsiella* isolates showing 10 % resistance to amikacin were reported by (**Younis *et al.*, 2016**). *K. pneumoniae* had a low incidence of amikacin resistance (7%) according to the study **(Gundogan *et al.*, 2011).**

As per**(Ullah *et al.*, 2009)** and **(Derakhshan *et al.*, 2016),** 63.04 percent and 68.5 percent of *Klebsiella* isolates, respectively were sensitive to amikacin. A high prevalence of *K. pneumoniae* isolates were resistance to Oxytetracycline and Doxycycline (88.9%) and (81.5%) respectively.

These findings support the findings of **(Kim *et al.*, 2005) and (Wu *et al.*, 2012),** they found substantial numbers of beta-lactam and tetracycline resistance, however, in Iran, the rate of antibiotic resistance in *K. pneumoniae* was also recorded to be 13%.25 %, 19.6% and 46.6% by **(Davies *et al.*, 2016; Bonnedahl *et al.*, 2014; Irajian *et al.*, 2009) and (Mohammadi-mehr and Feizabadi, 2011)** respectively. Other research results have found a link between virulence factors production and resistance phenotypes **(Da Silva and Mendonc, 2012) and (Mansouri *et al.*, 2011).** It's also been discovered that *K. pneumoniae* isolates that produce ESBL are more invasive and resistant to bactericidal action **(Sahly *et al.*, 2004 and Sahly *et al.*, 2008).** Virulence genes and class 1 integrons together increases the likelihood of horizontal gene transfer spreading antibiotic resistance and virulence determinants.

Furthermore, virulent strains' accumulation of resistance determinantsmay result in long-term microbe persistence in clinical settings **(Derakhshan *et al.*, 2016)**. Extended spectrum beta-lactamases hydrolyze the β- lactam ring, rendering cephalosporin and penicillin medicines inactive **(Beyene and Tsegaye, 2011).** Furthermore, some genes are migratory amongst isolates and diffuse all over the environment. It's possible that resistance genes propagate due to a different route of gene transfer, such as horizontal gene transfer between serotypes. **(Madhusudana and Surendran, 2010) and (Sharma and Navin 2006).** In our study, *K. pneumoniae* was multidrug- resistant to fluoroquinolones, aminoglycosides and trimethoprim; these results are similar to the results of **(Kumar *et al.*, 2011).** There are different reasons that lead

to the resistance of these bacteria to antibiotics, including large component of the genetic and phenotypic diversity of clinical isolates and multiple mechanism of fluoroquinolones. *Klebsiella* resistant to many antibiotics (multiple antibiotic resistance, MAR) is steadily increasing, and this is due to the indiscriminate use of antibiotics in many poultry farms. **Kilonzo-Nthenge *et al.*, (2008)** have approved administering antibiotics at low levels for a long time, some bacterial species become resistant**.**

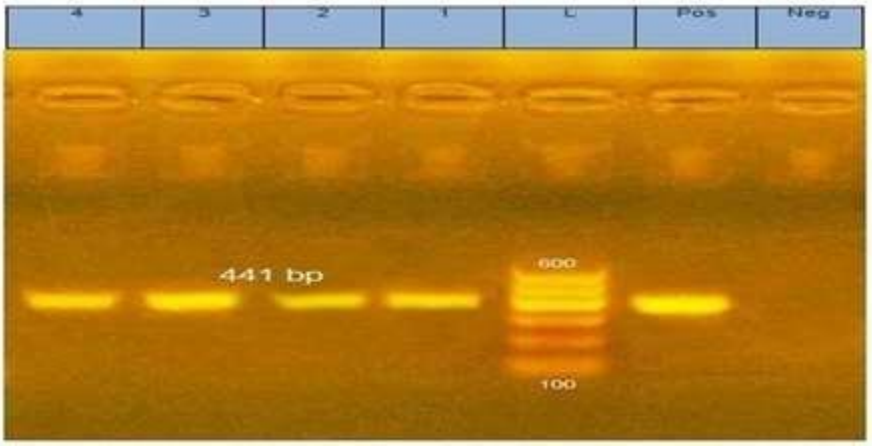

Fig. (1): PCR amplification for *gyr*A gene at 441 bp, lane (1, 2, 3, and 4) positive samples.

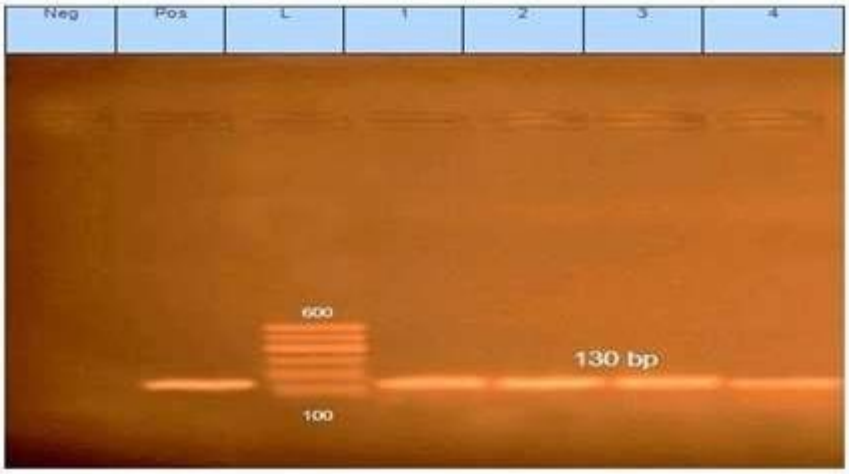

Fig. (2): PCR amplification for 16S-23S ITS at 130 bp, lane (1, 2, 3 and 4) positive for *K. pneumoniae*.

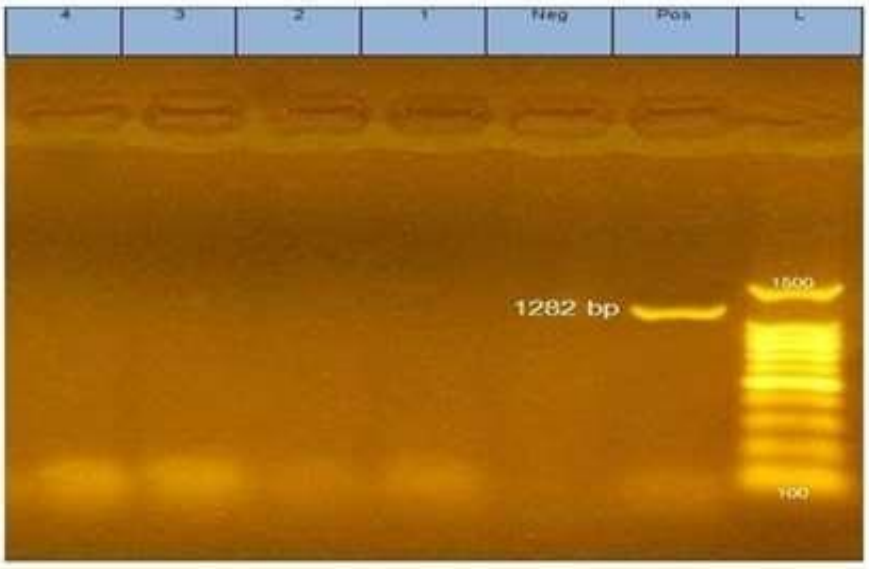

Fig. (3): PCR amplification for *mag*A gene at 1282 bp, (Lane 1, 2, 3 and 4) negative samples.

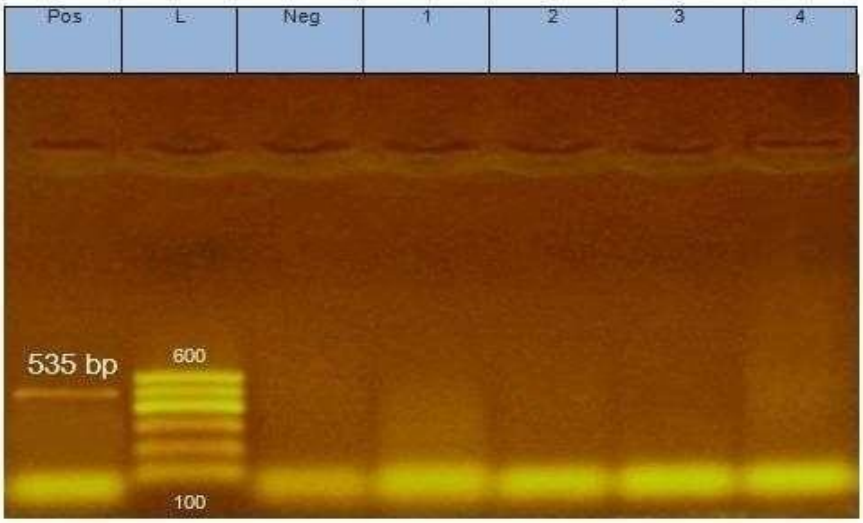

Fig. (4): PCR amplification of *rmp*A gene at 535 bp, (Lane 1, 2, 3 and 4) negative samples

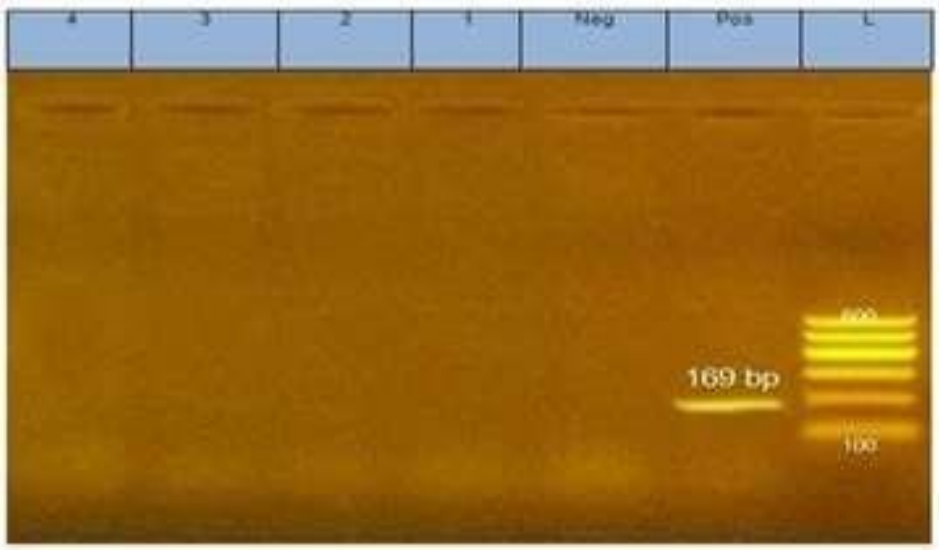

Fig. (5): PCR amplification of *wca*G at 169 bp, (Lane 1, 2, 3 and 4) negative samples.

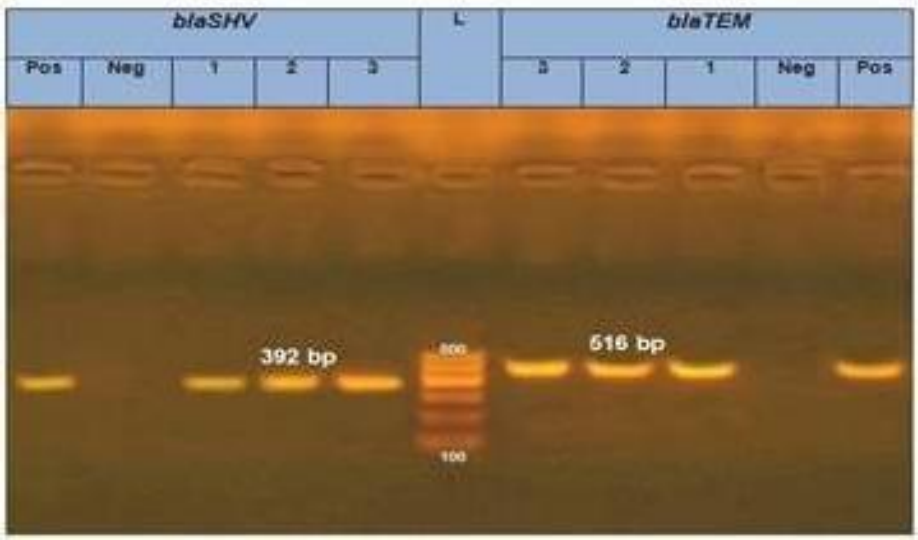

Fig. (6): PCR amplification of bla_{TEM} and bla_{SHV} at 516 bp and 392 bp respectively, (Lane 1, 2, and 3) positive samples for both genes.

GyrA primers were constructed using similarities between the *gyr*A sequence and other bacteria's sequences. They were amplifying a subset of the genes that were commonly amplified to identify mutations in the region that determined quinolone resistance, which was used for *Klebsiella* species. **(Brisse and Verhoef, 2001).** Using primers for the 16S-23S internal transcribed spacer region and genus specific primer sequences (*gyr*A) (Table 1), some strains were validated by polymerase chain reaction, which amplify at 130 bp and 441 bp, respectively Fig. (1, 2). This supported the identification of *K. pneumoniae*. Because related species frequently exhibit identical biochemical characteristics, conventional methods are frequently unreliable. **(Lopes *et al.,* 2007).**

The entire isolates were tested by PCR (using specified primer sequences), to determine the presence of *mag A* and *rmp A* genes that amplify at 1282 bp and 535 bp, respectively, *mag*A and *rmpA*, genes were not detected. Fig. (3, 4). Unexpectedly, genes of extracapsular polysaccharides and mucoviscosity, (*rmpA and magA*) were absent. These results similar to results of **(Davies *et al.,* 2016). El-Fertas-Aissani *et al.,* (2013)** noted a low incidence of *rmpA* and absence of the *mag*A from all tested samples. These genes are prevalent in human patients who present with invasive illnesses in some parts of the world and they play a significant role in the virulence of *K. pneumoniae* related to the invasiveness and resistance. Given the negative outcomes for *magA* and *rmpA*, more research is required to determine their exact functions and how they interact with the exopolysaccharides and capsular polysaccharides that make up bacterial surface envelopes **(Fang *et al.,* 2004).** Although *rmpA* was present in all serotype K1 and K2 strains and *mag*A was specific to serotype K1 strains, **(Yeh *et al.,* 2007)** divided all strains into 4 categories: K1 *(magA* and *rmpA* positive), K2 (*mag*A negative and *rmpA* positive), *rmpA*-positive non-K1/K2 (*magA* negative), and *rmpA*- negative non-K1/K2 (*magA* negative). The presence of these genes may be a sign of a *Klebsiella* isolate's potential for pathogenicity. Bacteria with the *magA* and *rmpA* genes in their capsular serotypes K1 and K2 are more invasive and phagocytosis-resistant. According to **Yu *et al.,*(2006),** *rmpA* and *mag*A prevalence were 48% and 17 %, respectively in Taiwan. According to **(Fang *et al.,* 2004; Ku *et al.,* 2008; Cheng *et al.,* 2010 and Yu *et al.,* 2007),** the *rmpA* gene is associated with the presence of phenotypic evidence of mucoidity. According to **Derakhshan *et al.,* (2016),** *rmpA* was found in 14 of the 200 *K. pneumoniae* isolates they studied (7.0 percent), *rmpA* controls extracapsular

polysaccharide production and promotes expression of hypermucoviscous phenotype, *rmpA* has been linked to a specific liver abscess syndrome **(Vila *et al.*, 2011).** According to **Fang *et al.*, (2005) and Struve *et al.*, (2005),** *magA* is a distinct virulence factor that contributes to some *K. pneumoniae* strains' higher pathogenicity.

Using PCR, **(Zamani *et al.*, 2013)** found *mag*A gene in 4 (3.8 percent) isolates from 101 strains of *K. pneumonia*, whereas **(Struve *et al.*, 2005)** demonstrated that *mag*A is confined to gene cluster of *K. pneumoniae* capsule serotype K1, according to **(Fang *et al.*, 2004),** invasive and non-invasive *K. pneumoniae* had 98% and 29% of *mag*A gene, respectively, **(Behnam *et al.*, 2014),** proved that, 4 (2.31%) of the 173 isolates positive for *mag*A gene, while 169 (97.68%) tested negative, these results explained the fact that, with the exception of liver abscesses, the *magA* gene is rarely seen in other illnesses caused by *K. pneumoniae.*

The low indicator of iron-uptake system (*kfu*), a unique mechanism on the chromosome of this bacterium to absorb iron, may explain why *mag*A was not detected in our isolates. This system is seen primarily in strains of positive *mag*A that caused hepatic abscesses. **(Fang *et al.*, 2005).** All strains that were examined displayed at least one gene related to iron absorption when grown in iron-deficient media. *Kfu* was the most widespread gene. *Kfu* also plays a significant part in sepsis and respiratory illnesses. **(El-Fertas-Aissani *et al.*, 2013).**

By *PCR*. All strains were negative for *wca*G gene Fig. (5), which is disagree with **(Derakhshan *et al.*, 2016),** of the 200 isolates, *wca*G was detected in 47 isolates (23.5%) and *rmpA* in 14 isolates (7.0%). The transferred chromosomal regions include the *wca*G gene **(Shu *et al.*, 2009).** In relation to beta-lactamases, bla_{TEM} and bla_{SHV} were detected by PCR, all strains were positive to the bla_{TEM} and bla_{SHV} genes Fig. (6), this resistance to ampicillin was high correlated to production of acquired beta-lactamases, more than 340 b-lactamases have been described the genes which encoded by ESBLs are located in plasmids, (gene bla $_{CTX-M}$), *TEM* (bla_{TEM}), *PER* (bla_{PER}), *VEB* (bla_{VER}) and *SHV* (bla_{SHV}) are the major subgroups **(Jemima and Verghese 2008), (Brinas *et al.*, 2002)** examined the occurrence of bla_{TEM} -,bla_{SHV} and bla_{OXA}-type beta-lactamases, suggesting either *TEM* hyperproduction **(Shannon *et al.*, 1990 and Wu *et al.*, 1995),** possible found of inhibitor-resistant *TEM* enzymes. The *Klebsiella* genus is where the broad-spectrum beta-lactamase *TEM*-1 with action against penicillins was originally discovered **(Heritage *et al.*, 1999).** ESBL-containing genes are linked to a number of distinct genomic architectures.

Transposons, insertion sequences, and integrons, among other mobile genetic elements, are key players in the spread of ESBL genes. *TEM*-type *ESBL* genes are acquired via mutation of plasmid-mediated, the primary generator of *TEM*-type *ESBLs*, *TEM*-1 and -2 genes, are found in the *Enterobacteriaceae* family and are found in the earliest known bacterial transposons. **(Chong *et al.*, 2011).**

The early diagnosis of the infection in vulnerable hosts is made possible by the molecular detection of these genes. Further research is required to contribute to the knowledge of physiological and molecular progression of disease in light of all the findings.

CONCLUSION

-*K. pneumoniae* is one of the main reasons of respiratory diseases in broiler chickens cause severe losses among them.

-In spite of the absence of some virulence genes of *K. pneumoniae* under study, the isolated strains' ability to cause respiratory tract illness in broiler chickens was validated, as was their link to clinical symptoms. There is proof that routine antibiotic usage in animal farming causes bacterial antibiotic resistance, which makes infection control and treatment more difficult.

The identification of virulence genes is essential for monitoring, treating and understanding the incidence of *K. pneumoniae* infection.

- These bacteria which are resistant to antibiotics, they spread among humans through direct contact and through animal-derived foods. So, reducing and elimination antibiotic use is essential. This can be accomplished through enhancing animal husbandry practices, curing animal diseases, and making the best use of already available vaccines. High sensitivity to some antibiotics still exists, despite rising resistance over time. The sensitivity of organisms to antibiotics has to be investigated in more geographically diverse investigations. Strict hygienic measures and disinfection programs in broiler chicken farms should be applied.

We should avoid miss use of antibiotics that would increase resistance of bacteria to antibiotics.

REFERENCE

Aher T.; Roy A. and Kumar P. (2012): Molecular Detection of Virulence Genes Associated with Pathogenicity of *Klebsiella* spp. Isolated from the Respiratory Tract of Apparently Healthy as well as Sick Goats Israel Journal of Veterinary Medicine Vol. 67 (4).

Ajayi A.O. and Egbebi A.O. (2011): Antibiotic susceptibility of *Salmonella typhi and Klebsiella pneumoniae* from poultry and local birds in Ado-Ekiti, Ekiti-State, Nigeria. Ann. Biol. Res., 2 (3): 431-437.

Aly M. M.; Khalil S. and Metwaly A. (2014): Isolation and Molecular Identification of *Klebsiella* Microbe Isolated from Chicks. Alexandria Journal of Veterinary Sciences, 43 (1): 97-103.

Amin A.; Ghumro P.B.; Hussain S. and Hameed A. (2009): Prevalence of antibiotic resistance among clinical isolates of *Klebsiella pneumoniae* isolated from a tertiary care Hospital in Pakistan. Malaysian J Microbiol; 5: 81-86.

Aya I. Younis; Amany I. Elbialy; Etab M. Abo Remila and Ahmed M. Ammar (2017): Molecular Detection of Genus *Klebsiella* and Genotypic Identification of *Klebsiella pneumoniae* and *Klebsiella oxytoca* by Duplex Polymerase Chain Reaction in Poultry Global Veterinaria 18 (3): 234 -241.

Bagel S.; Hullen V.; Wiedemann B. and Heising P. (1999): Impact of *gyr*A and *par*C mutations on quinolone resistance, doubling time, and supercoiling degree of *Escherichia coli*. Antimicrob. Agents Chemother. 43:868 - 875.

Barbara J.; F.K. Johan; Alices; F.S. Thomas and. C.T. Richard, (1994): Clinical and pathogenic microbiology, secones edition .Mosby. London. Vet. Med., 9 (2): 138 - 142.

Behnam Zamand; Hadis Amraie; Pegah Shakib; Samaneh Rouhi and Neda Bakhshandeh (2014): Prevalence assessment of *mag*A gene and antimicrobial susceptibility of *Klebsiella pneumoniae* isolated from clinical specimens in Shahrekord, Iran. J. Microbiol. Vol. 6, No 5, 311-316.

Beyene G and Tsegaye W. (2011): Bacterial uropathogens in urinary tract infection and antibiotic susceptibility pattern in jimma university specialized hospital, south west Ethiopia. J Health Sci 2011; 21: 141-146.

Bonnedahl J.; Hernandez J.; Stedt J.; Waldenstrom J.; Olsen B. and Drobni M. (2014): Extended-Spectrum β-lactamases in Escherichia coli and *Klebsiella pneumoniae* in gulls, Alaska, USA .Emerging Infectious Diseases, 20, 897-899.

Brinas L.; Zarazaga M. and Saenz Y. (2002): b-Lactamases in ampicillin resistant *Escherichia coli* isolates from foods, humans, and healthy animals. Antimicrob Agents Chemother, 46: 3156-63.

Brisse S. and Van Duijkeren E. (2005): Identification and antimicrobial susceptibility of 100 *Klebsiella* animal clinical isolates. Vet. Microbiol. 105: 307-312.

Brisse S. and Verhoef J. (2001): Phylogenetic diversity of *Klebsiella pneumoniae* and *Klebsiella oxytoca* clinical isolates revealed by randomly amplified polymorphic DNA, *gyr*A and *par*C genes sequencing and automated ribotyping. Int J Syst Evol Microbiol; 51: 915 - 924.

Chan K.S.; Chen C.M.; Cheng K.C.; Hou C.C.; Lin H.J. and Yu W.L. (2005): Pyogenic liver abscess: A retrospective analysis of 107 patients during a 3-year period. Infect Dis. 58: 366-368. Cheng H.Y.; Chen Y.S.; **Wu C.Y.; Chang H.Y.; Lai Y.C. and Peng H.L. (2010):** *rmp*A regulation of capsular polysaccharide biosynthesis in *Klebsiella pneumoniae* CG43. J. Bacteriol. 192: 3144 -3158.

Chong Yong; Yoshikiyo Ito and Tomohiko Kamimura (2011): Genetic evolution and clinical impact inextended-spectrum β - lactamase-producing *Escherichia* coli and *Klebsiella pneumoniae*. Evolution Volume, Pages 1499 -1504.

Chung D.R.; Lee S.S.; Lee H.R.; Kim H.B.; Choi H.J. and Eom J.S. (2007): Emerging invasive liver abscess caused by K1 serotype *Klebsiella pneumoniae* in Korea. J. Infect. 54: 578-583.

Clinical and Laboratory Standards Institute (2007): Performance Standards for Antimicrobial Disk and Dilution Susceptibility Tests for Bacteria Isolated From Animals. CLSI M31-A3, Wayne, PA, USA.

Colom K.; Perez J.; Alonso R.; Aranguiz, A.F.; Larino E. and Cisterna R. (2003): Simple and reliable multiplex PCR assay for detection of bla_{TEM}, bla_{SHV} and bla_{OXA}_1 genes in *Enterobacteriaceae*. FEMS Micro. Let. 223: 147-151

Dashe Y.G.; Raji M.A.; Abdu P.A. and Oladele B.S. (2013): Distribution of aerobic bacteria in visceral organs of sick and apparently health chickens in Jos, Nigeria. Int. Res. J. Microbiol (IRJM). 4 (3): 79-83.

Dashe Y.G.; H.M. Kazeem; P.A. Abdu; M. Bello and Odugbo M. (2008): *Klebsiella pneumoniae* isolated from birds affected by natural outbreaks of highly pathogenic avian influenza (H5N1) in Nigeria Sokoto J. Vet. Sci., 7 (2): 55-57.

Da Silva G.J. and Mendonc A. N. (2012): Association between antimicrobial resistance and virulence in *Escherichia coli*. 3:18-28.

Davies Y.M.; Cunha M.P.V.; Oliveira M.G.X.; Oliveira M.C.V.; Philadelpho N. Romero D.C.; MilaneloL; Guimaraes M.B.; Ferreira A.J.P.; Moreno A. M.; L. RM.S. and Knobl T. (2016): Virulence and antimicrobial resistance of *Klebsiella pneumoniae* isolated from passerine and psittacine birds. Avian Pathology 45 (2):1-28.

Derakhshan S.; Peerayeh S.N. and Bakhshi B. (2015): Genotyping and characterization of CTX-M-15- producing *Klebsiella pneumoniae* isolated from an Iranian hospital. J Chemo ther.28 (4): 289 - 96.

Derakhshan S.; Peerayeh S.N. and Bakhshi B. (2016): Association between Presence of Virulence Genes and Antibiotic Resistance in Clinical *Klebsiella Pneumoniae* IsolatesLab Medicine. 47 (4): 306 - 311.

El - Fertas-Aissani R.; Messai Y.; Alouache S. and Bakour R. (2013): Virulence profiles and antibiotic susceptibility patterns of *Klebsiella pneumoniae* strains isolated from different clinical specimens. Pathologie Biologie, 61, 209 - 216.

Ewers C.; Li G.; Wilking H.; Kiessling S.; Alt K.; Antáo E.M.; Laturnus C.; Diehl I.; Glodde S.; Homeier T.; Böhnke U.; Steinrück H.; Philipp H.C. and Wieler LH. (2007): Avian pathogenic, uropathogenic, and newborn meningitis-causing *Escherichia coli:* how closely related are they. Int J Med. Microbiol; 297 (3):16376.

Fang C.T.; Chuang Y.P.; Shun C.T.; Chang S.C. and Wang J.T. (2004): A novel virulence gene in *Klebsiella pneumoniae* strains causing primary liver abscess and septic metastatic complications. J. Exp. Med. 199: 697-705.

Fang F.C.; Sandler N. and Libby S.J. (2005): Liver abscess caused by *mag*A *Klebsiella pneumonia* in North America. J. Clin. Microbiol. 43: 991- 992.

Forbes B.A. and Sahm D.F. (2002): Bailey and Scott's diagnostic microbiology. 11 th ed. Mosby Publications. USA.

Gundogan N.; Citak S.andYalcin A. (2011): Virulence properties of extended spectrum b- Lactamase-producing *Klebsiella* species in meat samples. J. Food Protec. 74 (4): 559 - 564.

Harada Y.; Morinaga Y.; Yamada K.; Migiyama Y.; Nagaoka K. and Uno N. (2013): Clinical and molecular epidemiology of extended-spectrum β lactamase producing *Klebsiella pneumoniae and Escherichia coli* in a Japanese tertiary hospital. J. Med. Microbiol. Diagn. 2: 127.

Heritage John; Fatima H. M'Zali; Deborah Gascoyne-Binzi; Peter M. and Hawkey (1999): Evolution and spread of *SHV* extended-spectrum β-lactamases in Gram-negative bacteria. Journal of Antimicrobial Chemotherapy, Volume 44, Issue 3, Pages 309 -318.

Hossain M.S.; Akter S.; Ali M.; Das P.M. and Hossain M.M. (2013): Bacteriological and pathological Investigationof nasal passageinfectionsof chickens (Gallus gallus). The Agriculturists, 11 (1): 47- 55.

Irajian G.; Jazayeri-Moghadas A. and Beheshti A. (2009): Prevalence of extended- spectrum beta lactamase positive and multidrug resistance pattern of *Escherichia coli and Klebsiella pneumoniae* isolates, Semnan, Iran. Iran J Microbiol; 1: 49 - 53.

Jacoby G.A. (1997): Extended-spectrum β-lactamases and other enzymes providing resistance to oxyimino- β-lactams. Infect. Dis. Clin. N. Am. 11: 875 - 887.

Janda J.M. and Abbott S.L. (2006): The genera *Klebsiella* and *Raoultella*. In: The Enterobacteria (2nd ed.), ASM Press, Washington, USA. Pp. 115-129.

Janet F.; Hindler and John Stelling (2007): Analysis and Presentation of Cumulative Antibiograms: A New Consensus Guideline from the Clinical and Laboratory Standards Institute. Clinical Infectious Diseases, Volume 44, Issue 6, Pages 867-873.

Jemima S.A. and Verghese S. (2008): Multiplex PCR for *bla (CTX-M)* and *bla (SHV)* in the extended spectrum beta lactamase (ESBL) producing gram negative isolates. Indian J. Med.Res.128: 313 -317.

Khalda. A.K.; E.S. Abedlrahim; M.E. Iman; S.O. Ahmed A. Ballal and Elhaj J.I. (2013): A retrospective Study (2000-2005) of Poultry Diseases Diagnosed at Department of Avian diseases and Diagnosis, Veterinary Research Institute (VRI) - Khartoum, Sudan. Journal of American Science, 9 (7s).

Kim S.H.; Wei C.I.; Tzou Y.M. and An H. (2005): Multidrug resistant *Klebsiella pneumoniae* isolated from farm environments and retail products in Oklahoma. Journal of Food Protection, 68, 2022 - 2029.

Kilonzo-Nthenge A.; Nahashon S.N.; Chen F. and Adefope N. (2008): Prevalence and antimicrobial resistance of pathogenic bacteria in chicken and guinea fowl. Poult Sci. 87: 1841-8.

Kumar V.; Sun P.; Vamathevan J.; Li Y.; Ingraham K. and Palmer L. (2011): Comparative genomics of *Klebsiella pneumoniae* strains with different antibiotic resistance profiles. Antimicrob Agents Chemother; 55:4267- 4276.

Kumbish P.R.; Bello M.K., Jambalang A.R.; Hussaini, B.A.; Solomon P.; Ekong P.S. and Nanbol D. (2006): Bacterial and parasitic agents associated with Outbreaks of Highly Pathogenic Avian Influenza (H5N1) in Nigeria. Vom Journal of Veterinary Science, 3: 23-31.

Ku Y.H. Chuang Y.C. and Yu W.L. (2008): Clinical spectrum and molecular characteristics of *Klebsiella pneumoniae* causing community-acquired extrahepatic abscess.J.Microbiol. Immunol. Infect. 41: 311-317.

Livermore D.M. (1995): Beta-lactamases in laboratory and clinical resistance. Clin. Microbiol. Rev 8: 557-584.

Lopes A.C.S.; J.F. Rodrigues; M. Clementino; C.A. Miranda; A.P.A. Nascimento and Morais Júnior M.A.D. (2007): Application of PCR ribotyping and tDNA-PCR for *Klebsiella pneumoniae* identification. Memórias do Instituto Oswaldo Cruz, 102 (7): 827-832.

Ma L. C.; Fang C. T.; Lee C. Z.; Shun C. T. and Wang J. T. (2005): Genomic heterogeneity in *K. pneumoniae* strains is associated with primary pyogenic liver abscess and metastatic infection. J. Infect. Dis. 192:117-128.

Madhusudana Rao B. and Surendran P.K. (2010): Genetic heterogeneity of non-O1 and non-O139 Vibrio cholerae isolates from shrimp aquaculture system: a comparison of RS-, REP- and ERIC-PCR fingerprinting approaches. Lett Appl Microbiol; 51: 65 - 74.

Mansouri S.; Norouzi F.; Moradi M. and Nakhaee N. (2011): Comparison of virulence factors among clinical isolates of *Pseudomonas aeruginosa* producing and non- producing extended spectrum b-lactamases. Curr Res Bacteriol; 4:85-93.

Mohammadi-mehr M. and Feizabadi M.M.(2011): Antimicrobial resistance pattern of gram- negative bacilli isolated from patients at ICUs of Army hospitals in Iran. Iran J Microbiol; 3: 26-30.

Orajaka L. J.E. and Mohan K. (1985): Aerobic bacterial flora from dead in shell chicken embryo from Nigeria. Avian Disease, 29:583-589.

Periti P.; Mazzei T. and Curti M.E. (1998): Efficacy and safety of high dose intravenous ciprofloxacin in the treatment of bacterial pneumonia. Int. J. Antimicrob. Agents. 10: 215-222.

Ramirez M.S. and Tolmasky M.E. (2010): Aminoglycoside modifying enzymes. Drug Resist Update.13 (6):151-71.

Regue M.; Lzquierdo L.; Fresno S.; Pique N.; Corsaro M.M. and Naldi T. (2005): A second outer-core region in *Klebsiella pneumonia* lipopolysaccharide. J. Bacteriol. 187: 4198 - 4206.

Sahly H.; Navon-Venezia S. and Roesler L. (2008): Extended-spectrum b-lactamase production is associated with an increase in cell invasion and expression of fimbrial adhesins in *Klebsiella pneumoniae*. Antimicrob Agents Chemother. 52:3029 -3034.

Sahly H.; Aucken H. and Bebedi V.J. (2004): Increased serum resistance in *Klebsiella pneumoniae* strains producing extended-spectrum b-lactamases. Antimicrob AgentsChemother.48:3477-3482.

Saif Y.M.; Barnes H.J.; Glisson J.R.; Fadly A.M.; McDougald L.R. and Swayne D.E. (2003): Diseases of Poultry (11 th edn), Iowa State University Press, Blackwell Publishing Company, London, United Kingdom. Pp. 849.

SAljanaby A.A.J. and Alhasani A.H.A. (2016): Virulence factors and antibiotic susceptibility *pneumoniae* isolated from different clinical infections. African Journal of Microbiology Research, 10 (22): 829 - 843.

Sandra O. J. and Duarte C. J. H. (1998): Potential pathogens recovered from the upper respiratory tract of psittacin birds. File// E: *Klebsiella*.htm. 10 (1): 3438 -3446.

Seidler R.J.; Knittel M.D. and Brown C. (1975): Potential pathogens in the environment: cultural reactions and nucleic acid studies on *Klebsiella pneumoniae* from clinical and environmental sources. Appl. Microbiol. 29: 819-825.

Sharma A. and Navin Chaturvedi A. (2006): Prevalence of virulence genes (*ctxA, stn, OmpW* and *tcpA*) among *non-O1 Vibrio cholerae* isolated from fresh water environment. Int J Hyg Environ Health; 209: 521-526.

Shannon K.; Williams H.; King A. and Philipps I. (1990): Hyperproduction of TEM-1 β- lactamase in clinical isolates of *Escherichia* coli serotype O15. FEMS Microbiol Lett.; 67: 319-324.

Shu H.Y.; Fung C.P. and Liu Y.M. (2009): Genetic diversity of capsular polysaccharide biosynthesis in *Klebsiella pneumoniae* clinical isolates. Microbiology; 155 (12):4170 - 4183.

Sikarwar A.S. and Batra H.V. (2011): Identification of *Klebsiella pneumoniae* by capsular polysaccharide polyclonal antibodies. International Journal of Chemical Engineering and Applications, 2 (2): 130.

Singh N.P. and Goyal R. (2003): Changing trends in bacteriology of burns in the burns unit, Delhi, India. Burns. 29 (2): 129 -132.

Struve C.; Bojer M.; Nielsen F.M..; Hansen D.S. and Krogfelt K.A. (2005): Investigation of the putative virulence gene magA in a worldwide collection of 495 *Klebsiella* isolates: *mag*A is restricted to the gene cluster of *Klebsiella pneumoniae* capsule serotype K1. J. Med. Microbiol. 54: 1111-1113.

Tumbarello M.; Spanu T.; Sanguinetti M.; Citton R.; Montuori E. and Leone F. (2006): Bloodstream infections caused by extended-spectrum-beta-lactamase-producing *Klebsiella pneumoniae*: risk factors, molecular epidemiology, and clinical outcome. Antimicrob Agents Chemother Feb; 50 (2):498 -504.

Türkyilmaz S. (2006): Isolation and serotyping of Ornithobacterium rhinotracheale from poultry. Turkish Journal of Veterinary and Animal Sciences, 29 (6): 1299 - 1304.

Turton J.F.; Perry P.; Elgohari S. and Hampton C.V. (2010): PCR characterization and typing of *Klebsiella pneumoniae* using capsular type-specific, variable number tandem repeat and virulence gene targets. Journal of Medical Microbiology, 59, 541-547.

Ullah F.; Malik S.A. and Ahmed J. (2009): Antimicrobial susceptibility pattern and ESBL prevalence in *Klebsiella pneumoniae* from urinary tract infections in the North-West of Pakistan. Afr. J. Microbiol. Res. 3: 676 - 680.

Van T.T.; Moutafis G.; Istivan T.; Tran L.T. and Coloe P.J. (2007): Detection of *Salmonella* spp. in retail raw food samples from Vietnam and characterization of their antibiotic resistance. Appl Environ Microbiol; 73: 6885 - 90.

Van den Bogaard A.E. and Stobberingh E.E. (2000): Epidemiology of resistance to antibiotics. Links between animals and humans. Int. J. Antimicrob. Agents. 14: 327- 335.

Vila A.; Cassata A. and Pagella H. (2011): Appearance of *Klebsiella pneumoniae* liver abscess syndrome in Argentina: case report and review of molecular mechanisms of pathogenesis. Open Microbiol J. 5:107-113.

VillegasV.M; Correa A.; Federico P.;Miranda M.C.; Zuluaga T.and Quinn J.P. (2004): Prevalence and characterization of extended spectrum b-lactamases in *Klebsiella pneumoniae* and *Escherichia coli* isolates from Colombian hospitals. Diagn. Microbiol. Infect. Dis. 49:217-222.

Wright J.G.; Tengelsen L.A. and Smith K.E. (2005): Multidrug resistant *Salmonella typhimurium* in four animal facilities. Emerg Infect Dis; 11: 1235- 41

Wu H.; Liu B. G. and Pan Y. S. (2012): Phenotypic and molecular characterization of CTX-M-14 extended-spectrum B-lactamase and plasmid- mediated ACT-likeAmpC Blactamase produced by *Klebsiella pneumoniae* isolates from chickens in Henan Province, China. Genetics and Molecular Research, 11, 3357-3364.

Wu P.J.; Shannon K. and Phillips I. (1995): Mechanisms of hyperproduction of TEM-1 β- lactamase by clinical isolates of *Escherichia coli.* J Antimicrob Chemother.; 36: 927- 939.

Yeh K.; Kurup A.; Siu L.K.; Koh Y.L.; Fung C.; Lin J.; Chen, T.; Chang F. and Koh T. (2007): Capsular Serotype K1 or K2, Rather than *mag*A and *rmp*A, Is a Major Virulence Determinant for *Klebsiella pneumonia* Liver Abscess in Singapore and Taiwan. Journal of Clinical Microbiology, p. 466 - 471.

YehiaH.M. and Riyadh K.S.A. [(2013): Antimicrobial resistance patterns of *Enterobacteriaceae* and *non-Enterobacteriaceae* isolated from poultry intestinal. Life Science Journal, 10 (1).

Yimer N. and Asseged B. (2007): Aerobic bacterial flora of the respiratory tract of healthy sheep slaughtered in Dessie municipal abattoir, north-eastern Ethiopia. Revue Med. Vet. 158:473- 478.

Younis G.; Awad A.; El-Gamal A. and Hosni R. (2016): Virulence properties and antimicrobial susceptibility profiles of *Klebsiella species* recovered from clinically diseased broiler chicken. Adv. Anim. Vet. Sci. 4 (10): 536 -542.

Yu V.L.; Hansen D.S.; Ko W.C.; Sagnimeni A.; Klugman K.P.; and von Gottberg A. (2007): Virulence characteristics of *Klebsiella* and clinical manifestations of *K. pneumoniae* blood stream infections. Emerg. Infect. Dis. 13: 986 - 993.

Yu W.; Ko W.; Cheng, K.; Lee H.; Ke D.; Lee C.; Fung C. and Chuang Y. (2006): Association between *rmp*A and *mag*A Genes and Clinical Syndromes Caused by *Klebsiella pneumoniae* in Taiwan. Clin. Infect. Dis. 42:1351-1358,

Zamani A.; Yousefi Mashouf R.; Ebrahimzadeh Namvar A.M. and AlikhaniM.Y. (2013): Detection of *mag*A Gene in *Klebsiella spp*. isolated from clinical samples. Iran J Basic Med Sci; 16:173-176.