Virulence and resistant genes detection of *Staphylococcus aureus* associated with arthritis in chickens

Ashraf A. Abd El- Tawab[1], Khalid I. Alekhnawy[2] and AsmaaT.Talaie[2]

[1]*Bacteriology, Immunology and Mycology Department, Faculty of Veterinary Medicine, Benha University, Egypt.*

[2]*Animal Health Research Institute, Tanta branch*

A B S T R A C T

The present study was planned for bacteriological examination of *S.aureus* associated with arthritis in chickens and characterization the molecular bases of virulence and antimicrobial resistance of isolated strains .In this study a total of 100 chickens (15 – 70 days) with arthritis symptoms, were collected from different chicken farms, swabs were taken from affected hock joints and cultivated on(Brain Heart Infusion broth, Baired Parker media and Manitol Salt agar media) for isolation of *Staphylococcus aureus* (*S.aureus*).The results revealed that 19 (19%) samples were positive for *S.aureus* isolation. Multiple antibiotic-resistant were observed in all *S.aureus* isolates, with the majority of isolates displaying resistance to erythromycin, amoxicillin and cefoxitin with a percent 52.6%,78.9% and 57.8% respectively. Detection of (*mph*C ,*bla*Z and *mec*A) resistant genes demonestrated that (20%) were positive for *mph*C gene ,(100%) positive for *bla*Z gene and (40%) positive for *mec*A gene. Amplification of (*clf*A, *spa*, *coa* and *ica*A) virulance genes of *Staphylococcus aureus* by using PCR showed (100%) detection of *clf*A, *spa* and *coa* genes in all tested strains, while (40%) only of tested strains were positive for *ica*A virulence gene. It could be concluded that S.aureus is one of the most important pathogenic organisms associated with chicken arthritis in Egypt, Studying of virulence and resistance genes is very important to know the danger and severity of the present microorganisms.

Keywords: *S.aureus, arthritis, virulence genes, resistant genes, chickens.*

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1. INTRODUCTION

Arthritis is one of the major problems facing the chickens resulting in decrease body weight, emaciation and may death because the affected birds became unable to get feed and water. Arthritis also cause great problem at marketing time as chickens with arthritis would be rejected by the consumer. Septic arthritis is infection of one or more joints that affected by microorganisms. Normally, the degree of infection somewhat depend on the microbe causing the infection and the predisposing risk factors (Huang *et al*., 2002).

The affected joints, usually the hocks, are hot, swollen and painful and the affected birds are usually depressed, lameness and reluctant to walk, the synovial membranes of tendon sheaths became thickened and edematous, with

fibrinous exudate within and around the tendon sheaths(Jordan *et al*., 2002).

Staphylococci have a high affinity for collagen-rich surfaces such as the articular surface of joints, and synovial sheaths located around joints and tendons (Jordan and Pattisson, 1996).

The development of antibiotic resistance in many bacterial organisms constitutes serious problems in the control of infectious diseases. Antibiotic misuse has been found to be the most important selecting force in bacterial antibiotic resistance (Okoli, 2006).

Antimicrobial resistance genes can be transmitted across species using mobile genetic elements (MGEs), which are DNA (deoxyribonucleic acid) fragments that carry both virulence and resistant determinants. Furthermore, they produce enzymes that allow them to be transferred and integrated into a new host's DNA. The transfer of MGEs among cells is known as horizontal gene transfer (HGT), and it can occur among prokaryotes and eukaryotes (Malachowa and DeLeo,2010). Assessment of antimicrobial resistance of bacteria at molecular level is a useful tool for understanding the contribution of genetic elements responsible for developing and dissemination of resistance in bacteria (Angulo *et al*., 2004).

PCR is a powerful molecular biology technique that was introduced to facilitate the detection virulence factors by using DNA probes that detect specific virulence factors (Nataro and Kaper, 1998).

S. aureus expresses many potential virulence factors: (1) Surface proteins that promote colonization of host tissues. (2) Invasions that promote bacterial spread in tissues *(*leukocidin, kinases, and hyaluronidase). (3) Surface factors that inhibit phagocytic engulfment (capsule, Protein A). (4) Biochemical properties that enhance their survival in phagocytes (carotenoid, catalase production). (5) Immunological disguises (Protein A, coagulase, clotting factor). (6) Membrane-damaging toxins that lyse eukaryotic cell membranes (hemolysins, leukotoxin, leukocidin). (7) Exotoxins that damage host tissues or otherwise provokesymptoms of disease (SEA-G, TSST, and ET). (8) Inherent and acquired resistance to antimicrobial agents (Toder, 2005).

Clumping factor A(*cif*A) is fibrinogen – binding surface protein of *S.aureus* which acts as virulence factor in certain infections by inhibiting phagocytosis, as well as promoting adhesion to fibrin and fibrinogen (Higgins *et al*., 2006).

The *spa* gene, which is mostly used for typing of *S.aureus*, encodes for protein A. The coagulase (*coa*) gene is another example of a *S.aureus* virulence gene that is regarded as important, because it plays an essential role in the alliance with other genes to survive inside host cells and to invade immune system cells in the host (Balaban and Rasooly,2000)

Therefore, the present study was planned for bacteriological examination of *S.aureus* associated with arthritis in chickens and characterization the virulence and antimicrobial resistance genes of isolated strains.

2. Materials and methods

2.1. Samples collection:

A total of 100 samples from chicken with arthritis symptoms (14-70 days old), were collected from different broilers chicken farms at EL-Gharbia Governorate were subjected to clinical examination as well as for isolation and identification of Staphylococcus aureus.

2.2. Bacteriological examination:

2.2.1. Bacterial isolation

By using sterile scalpels and cotton swabs ,swabs were taken from affected joints inoculated on Brain Heart Infusion broth (Oxoid) and incubated aerobically at 37ºC for

12-24 hours. A loopful from incubated brain heart broth was streaked on the surface of Baired-Parker medium and Mannitol Salt agar (Oxoid). The plates were incubated for 24-48 hours at37°c (Quinn *et al.,* 1994).

2.2.2. Morphological examination of Staphylococcus aureus

Films were prepared from the suspected pure isolates and stained with Gram's stain then examined microscopically (Cruichshank *et al*., 1975).

2.2.3. Biochemical identification

Each colony showed typical colonial appearance were subjected to biochemical identification according to Quinn *et al., (*1994).

2.3. Antimicrobial susceptibility testing:

Antimicrobial susceptibility testing was performed according to the Kirby- Bauer disk diffusion method (Finegold and Martin.,1982), using Muller Hinton broth, Muller Hinton agar and the following antibiotic discs: erythromycin (15 µg), gentamicin (10 µg), ciprofloxacin (5 µg), amoxicillin/clavulinic acid(30 µg), doxycycline (30 µg), vancomycin (15 µg), cefoxitin (30 µg), and amoxicillin (10 µg), (Oxoid) and the results were interpreted according to the criteria recommended by the Clinical and Laboratory Standards Institute for antimicrobial susceptibility testing CLSI (2011).

2.4. Genotypic characterization by PCR:

2.4.1. Extraction of bacterial DNA

DNA was purified using QIAamp DNA mini kit. PCR Master Mix and cycling conditions of the primers during PCR was prepared according to Emerald Amp GT PCR mastermix (Takara) Code No. RR310A kit. Oligonucleotide primers used in PCR have specific sequence and amplify a specific product as shown in table (1) and (2).DNA samples were amplified in a total of 25 µl as follows: 12.5 µl of Emerald Amp GT PCR Master Mix, 1 µl of each primer of 20 pmol concentrations, 4.5 µl of water, and 6 µl of template DNA. The reaction was performed in a Biometra thermal cycler. Temperature and time conditions of the primers during PCR were applied (table 3). Aliquots of amplified PCR products were electrophoresed in 1.5% agarose gel (ABgene) in 1x TBE buffer at room temperature (Sambrook *et al.,* 1989). For gel analysis, 15 µl of PCR products were loaded in each gel slot. A 100 bp DNA Ladder (QIAGEN Inc, Valencia, CA, USA) was used to determine the fragment sizes. The gel was photographed by a gel documentation system and the data was analyzed through computer software.

3. RESULTS

3.1. Incidence of S.aureus in chickens:

In this study out of 100 samples of hock joints with arthritis 19 (19%) were positive for *Staphylococcus aureus* isolation. The recovered isolates in this study showed black small 1mm colonies after 24 hours of incubation and large 2.5 mm colony after 48 hours incubation surrounded by an opalescent ring and a clear zone on Baired Parker medium and yellow color surrounded by yellow halozone with yellow colored medium on Mannitol salt agar medium. Morphological examination of *S. aureus* organisms appeared as Gram positive, grapes like cocci and arranged in clusters. The results of biochemical identification showed characteristic identical biochemical reaction to be *S.aureus*, coagulase positive, catalase positive and oxidase negative

3.2. Antimicrobial sensitivity test:

Antibiotic sensitivity test revealed that out of 19 isolates, 14/19 (73.6 %) were sensitive for vancomycin , 13/19 (68.4%) were sensitive for amoxycillin/clavulinic acid and 8/19 (42.1%) were sensitive for ciprofloxacin and 6/19 (31.5%) were sensitive for

gentamycin,while10/19 (52.6%) were resistant to erythromycin,15/19 (78.9%) were resistant to amoxicillin and 11/19 (57.8%) were resistant to cefoxitin as shown in table(4).All isolates were resistant to an antibiotic, but only 26.3% of isolates were resistant to 3 or more antibiotics which called multi-drug resistant strains.

3.3. Molecular characterization of resistant genes of S.aureus:

Five isolates of multidrug resistant *S. aureus* isolates were examined for detection of (*mph*C, blaZ and *mec*A) resistant genes. The results showed that 1 /5 (20%) was positive for *mph*C gene, 5/5 (100%) were positive for *bla*Z gene and 2/5 (40%) were positive for *mec*A gene in tested isolates, as shown in table (5) and figure (1, 2and 3).

3.4. Molecular characterization of virulence genes of S.aureus:

Five isolates of *S. aureus* isolates were examined for detection of (*clf*A, *spa*, *coa* and *ica*A) genes, 5/5 (100%) of isolates were positive for *clf*A, *spa* and *coa*, while only 2/5 (40%) were positive for *ica*A, as shown in table (5) and figure (4, 5, 6 and 7).

Table 1: Oligonucleotide primers sequences of resistant genes of *S.aureus*.

Gene	Primer sequence (5` - 3`)	Length of amplified product	Reference
*mph*C	**F:** GAGACTACCAAGAAGACCTGACG **R:** CATACGCCGATTCTCCTGAT	722 bp	Schlegelova*et al*.,(2008)
*mec*A	**F:** GTA GAA ATG ACT GAA CGT CCG ATA A **R:** CCA ATT CCA CAT TGT TTC GGT CTA A	310 bp	McClure *et al*.,(2006)
*bla*Z	**F:** ACTTCAACACCTGCTGCTTTC **R:** TGACCACTTTTATCAGCAACC	173 bp	Duran*et al*.,(2012)

Table 2: Oligonucleotide primers sequences of virulence genes of *S.aureus.*

Gene	Primer sequence (5` - 3`)	Length of amplified product	Reference
*ica*A	**F:** CCT AAC TAA CGA AAG GTA G **R:** AAG ATA TAG CGATAA GTG C	1315 bp	Ciftci*et al*.,(2009)
Coa	**F:** ATA GAG ATG CTG GTA CAG G **R:** GCT TCC GAT TGT TCG ATG C	630 bp	Iyer and Kumosani, (2011)
Spa	**F:** TCA ACA AAG AAC AAC AAA ATG C **R:** GCT TTC GGT GCT TGA GAT TC	226 bp	Wada *et al*.,(2010)
*clf*A	**F:** GCAAAATCCAGCACAACAGGAAACGA **R:** CTTGATCTCCAGCCATAATTGGTGG	638 bp	Mason *et al., (*2001)

Table 3: Temperature and time conditions of the primers during PCR cycles.

Gene	Primary denaturation	Secondary denaturation	Annealing	Extension	No. of cycles	Final extension
mecA	94˚C 5 min.	94˚C 30 sec.	50˚C 30 sec.	72˚C 30 sec.	35	72˚C 7 min.
blaZ	94˚C 5 min.	94˚C 30 sec.	54˚C 30 sec.	72˚C 30 sec.	35	72˚C 7 min.
icaA	94˚C 5 min.	94˚C 30 sec.	49˚C 40 sec.	72˚C 1 min.	35	72˚C 12 min.
mphC	94˚C 5 min.	94˚C 30 sec.	55˚C 40 sec.	72˚C 50 sec.	35	72˚C 10 min.
Coa	94˚C 5 min.	94˚C 30 sec.	55˚C 40 sec.	72˚C 45 sec.	35	72˚C 10 min.
Spa	94˚C 5 min.	94˚C 30 sec.	55˚C 30 sec.	72˚C 30 sec.	35	72˚C 7 min.
clfA	94˚C 5 min.	94˚C 30 sec.	55˚C 40 sec.	72˚C 45 sec.	35	72˚C 10 min.

Table 4: Antimicrobial sensitivity test of isolated S.aureus strains.

Antimicrobial	Concentration of disc	Sensitive strains		Intermediat strains		Resistant strains	
		No.	%	No.	%	No.	%
Amoxicillin/Clavulinic acid	30 µg	13	68.4	2	10.5	4	21
Gentamycin	10 µg	6	31.5	5	26.3	8	42.1
Ciprofloxacin	5 µg	8	42.1	2	10.5	9	43.7
Doxycycline	30 µg	2	10.5	11	57.8	6	31.5
Cefoxitin	30 µg	5	26.3	3	15.7	11	57.8
Erythromycin	15 µg	4	21	5	26.3	10	52.6
Amoxicillin	10 µg	3	15.7	1	5.2	15	78.9
Vancomycin	15 µg	14	73.6	3	15.7	2	10.5

Table 5: Result of PCR virulence and resistant genes of S.aureus strains.

S.aureus No.	Virulance genes				Resistant genes		
	*clf*A	*Spa*	*Coa*	*ica*A	*mph*C	*bla*Z	*mec*A
1	+	+	+	+	-	+	+
2	+	+	+	+	+	+	+
3	+	+	+	-	-	+	-
4	+	+	+	-	-	+	-
5	+	+	+	-	-	+	-

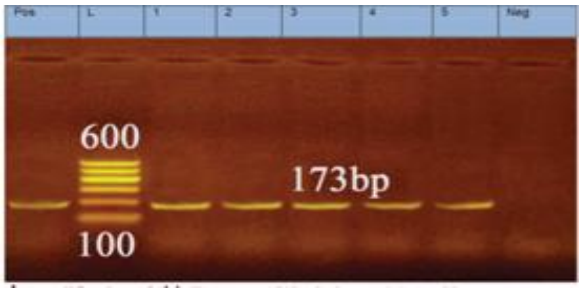

Fig.1. Agarose gel electrophoresis showed amplification of (*bla*Z) gene at 173bp in lane 1,2,3,4 and 5; L: ladder (100-600bp) Pos: positive control (ATCC25923); Neg: negative control.

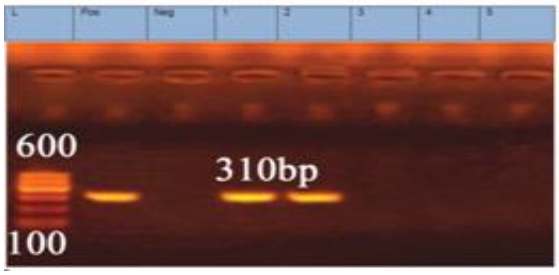

Fig.2. Agarose gel electrophoresis showed amplification of of *(mec*A) gene at 310 bp in lane 1, 3, 4 and lane (2 and 5) showed negative result; L: ladder (100-600bp) Pos: positive control (ATCC25923); Neg: negative control.

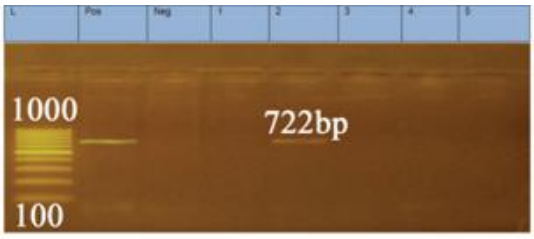

Fig.3. Agarose gel electrophoresis showed amplification of (*mph*C) gene at 722pp in lane (2) while lane (1, 3, 4 and 5) showed negative result; L: ladder (100-1000 bp) Pos: positive control TCC25923); Neg: negative control

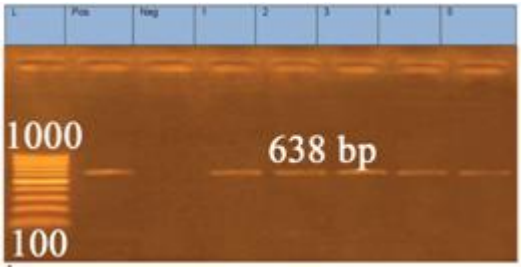

Fig.4. Agarose gel electrophoresis showed amplification of (*clf*A) gene at 638 bp in lane (1, 2, 3, 4 and 5); L: ladder (100-1000 bp) Pos: positive control (ATCC25923); Neg:negative control.

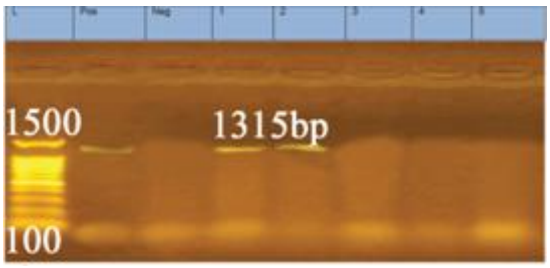

Fig.5. Agarose gel electrophoresis showed amplification of (ica) gene at 1315 bp in lane (1 and 2) while lane (3, 4 and 5) showed negative result; L: ladder (100-1500 bp) Pos: positive control (ATCC25923). Neg: negative control.

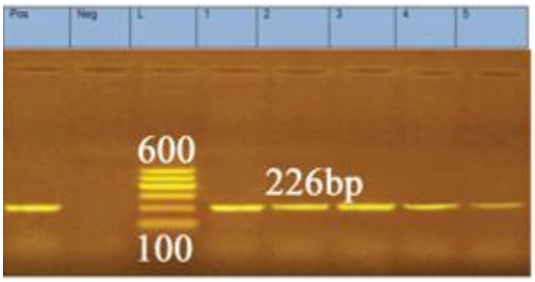

Fig.6. Agarose gel electrophoresis showed amplification of (*spa*) gene at 226 bp in lane (1, 2, 3, 4 and 5); L: ladder (100-600 bp) Pos: positive control (ATCC25923); Neg: negative control.

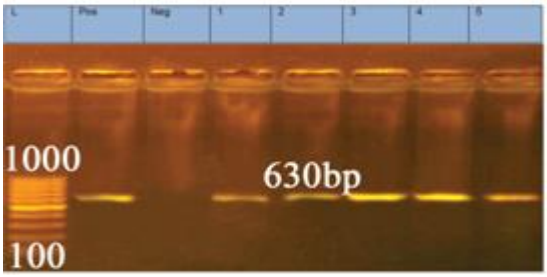

Fig.7. Agarose gel electrophoresis showed amplification of (coa) gene at 630 bp in lane (1, 2, 3, 4 and 5); L: ladder (100-1000 bp) Pos: positive control (ATCC25923); Neg: negative control.

4. DISCUSSION

Staphylococcus aureus is one of the most common causes of bacterial arthritis in broiler chickens .They recorded a high mortality in broilers with lesion of swollen joints, gaseous exudates, cartilage injury, and synovial membrane thickening with infiltration of inflammatory cells (Gu *et al*., 2013).

The incidence of *S.aureus* in this study was (19%).This result agreed with Joiner *et al.,(* 2005) who demonstrated that 20.4% of commercial flocks had typical bacterial skeletal lesions were primarily associated with *Staphylococcus aureus* , and also agreed with Wladyka *et al*., (2011) who stated that the incidence rate of *staphylococcosis* in chickens ranges from 0.5 to 20%.On the other hand ,the current result disagreed with Rasheed ,(2011) who isolated a higher percent 50.98% of *S.aureus* from broiler chickens suffering from arthritis. The difference in percentage of isolation could be attributed to many factors, some related to birds such as age, immune

status and medication during sample collection. Other factors might be related to difference in hygienic measure inside farms.

Extensive and misuse of antimicrobial drugs in poultry industry is the main cause of resistance to commonly used antibiotics and rising of multi-drug resistant strains Sharada *et al*.,(2009). In the present study *S.aureus* isolates showed high rates of resistance to erythromycin, amoxicillin and cefoxitin with a percent 52.6%, 78.9% and 57.8% respectively. This results were disagree with Aml et al., (2018) who demonstrated that 93.8% of *S.aureus* isolates were resistant to cefoxitin. Moreover, 90.1% and 87.6 % of isolates were resistant to penicillin and amoxicillin/clavulinic acid, respectively.

The differences in resistance patterns are widely due to factors which include differences in geographical locations, particular bacteria species involved, the animal production systems, the extent to which antibiotics are used, sampling techniques and period of sampling (Adzitey *et al*.,2015).

The molecular investigations of resistance mechanisms of multidrug resistant *S.aureus* isolates to amoxicillin, erythromycin and cefoxitin antimicrobials could be explained by the presence of *bla*Z , mphC and *mec*A genes in the tested isolates with a percent (100%),(20%)and (40%) respectively.

*bla*Z gene was positively amplified in all 5 tested isolates with apercent (100%),this result confirmed the result of antimicrobial sensitivity test in which all 5 tested isolates were resistant to amoxicillin. *mph*C gene was amplified in only 1isolate of five tested isolates with a percent (20%) .This result was dissimilar to the result of antimicrobial susceptibility test in which all 5 isolates were resistant to erythromycin. This difference between those results may be related to the different mechanisms (other than *mph*C) for the resistance of *S.aureus* to macrolides, or may due to presence of other mechanism of resistance such as efflux pump activity which responsible for a significant loss of antibiotic susceptibility (Davin-Regli *et al*., 2008). The *mec*A gene was positively amplified in 2 isolates out of 5 resistant *S.aureus* strains with a percent (40%), This result also was dissimilar to the result of antimicrobial susceptibility test.This was clarified by Mathews *et al*.,(2010) who reported two types of strains that show phenotypic resistance to oxacillin however they don't harbor the *mec*A gene. The 1st type of those two strains is called borderline oxacillin resistant *S.aureus* (BORSA) which hyper produces beta -lactamase and while they appeared as oxacillin resistant, do not possess the usual genetic mechanism for such resistance. They reported also that another type of strains known as modified *S. aureus* (MODSA) which possess a modification of existing penicillin binding proteins rather than the acquisition of a new PBP as is the mechanism for classical MRSA.

Staphyloccocal infections are usually associated with the presence of virulence genes.The bacterial adhesion thought to be an important step in the beginning of the infections. *can*, *fnb* A ,and *clf* Awere the most important *staphylococcal* adhesions(Arciola *et al*., 2005). In the present study the result of PCR of virulence genes revealed that presence of *clf*A, *spa* and *coa* in all tested samples with a percent (100%) but *ica*A gene could be detected in 40% only of tested samples ,this result agree with Kalorey *et al*., (2007) who found *clf*A gene in alltested isolates with percentage of 100%,and disagree with Tawfik *et al*.,(2016) who stated that the percentage of *clf*A gene was 37.5%, other result recorded by Momtaz *et al*,(2013) who reported that *clf*A,*spa* and *coa* detected with apercent 76.82%, 26.82% and 63.41%, respectively. In the present study *ica*A gene could be detected

in 40% only of tested samples ,this result disagree with Bnyan, (2013) who stated that *ica*A gene could be detected in 100% of all isolates.

5. Conclusion

Based on the present study, it could be concluded that Staphylococcus *aureus* is one of the most important pathogenic organisms associated with arthritis in Egypt.

The high incidence of antibiotic resistant *Staphylococcus aureus* among broilers chicken which were detected in the current study confirmed the misuse and abuse of antibiotics in broiler farms which will accelerate the development and spreading of antibiotics resistant bacteria not only between chickens but also in the environment or even to the human being. So the restriction of the antibiotics usage in poultry farms is needed. Moreover, this study reported the presence of at least three virulence genes detect the pathogenic importance of isolated strains.

6. REFERENCES

Aml, A. B.; Amen, O.; AL Habaty, H. S.and Darwish, F.S (2018): Prevalence of *Staphylococcus aureus*in broiler chickens with special reference to beta-lactam resistance genes in the isolated strains. AJVS. 57 (2): 25-33.

Adzitey, F.;Teye, G.A.; and Anachinaba, I.A. (2015): Microbial quality of fresh and smoked guinea fowl meat sold in the Bolgatanga Municipality, Ghana. Asian Journal of Poultry Science, 9: 165-171.

Angulo, J.Y.; Ezike, E. and Asmar, B.I. (2004): Antibacterial resistance, Indian Journal of Pediatrics. 71, 229-239.

Arciola, C.R.; Compoccia, D.; Gamberini, S. and Baldassarri, L. (2005): Prevalence of cna, fnb A and fnb B adhesion genes among *S. aureus* is dates from orthopedic infections associated to different types of implant. FEMS Microbial. Lett, 246:81 – 86.

Balaban, N. and Rasooly A., (2000): Staphylococcal enterotoxins, International Journal of Food Microbiology 61, 1–10.

Bnyan, I. (2013): Detection of Adhesion Genes and Slim Production among *Staphylococcus Aureus* and *Staphylococcus* epidermidis isolated from hemodialysis Patients. Advances in Life Science and Technology, 15:27-32.

Ciftci, A.; Findik, A.; Onuk, A. and Savasan, S. (2009): detection of methicillin resistance and slime factor production of *staphylococcus aureus* in bovine mastitis. Brazilian Journal of Microbiology, 40:254-261.

Clinical and Laboratory Standards Institute (CLSI), (2011): Performance standards for antimicrobial susceptibility testing; twenty-first informational supplement, CLSI document M100-S21, Wayne, PA, USA, 31, 42-46.

Cruickshank, R.; Duguiel, J.P.; Marmion, B.P. and Swain, R.H. (1975): Medical Microbiology. 12th ed. Vol. II Churchill Living stone, Edenburg, London and New York. Des; 5:895–913.

Davin-Regli, A.; Bolla, J.M. and James C.E. (2008): Membrane permeability and regulation of drug "influx and efflux" in enterobacterial pathogens.Curr. Drug Targets, 9: 750-759.

Duran, N.; Ozer, B.; Duran, G.G.; Onlen, Y. and Demir, C. (2012): Antibiotic resistance genes & susceptibility

patterns in *staphylococci*. Indian J Med Res., 135, 389-396.

Finegold, S.M. and Martin, W.T. (1982): Diagnostic Microbiology. 6th Ed. C.V. Mosby Co. St. Louis Toronto, Londan.

Gu,C.Q.; Hu, X.Y.; Xie, C. q.; Zhang, W. P.; Wang, D. H.; Zhou, Q.and Cheng, G.F.(2013): Observation on arthritis in broiler breeder chickens experimentally infected with *Staphylococcus aureus*. Mortality, 100: 10.

Higgins, J.; Loughman, A.; Van Kessel, P. M. and Foster J.T. (2006): Clumping factor A of *Staphylococcus aureus* in habits phagocytosis by human polymorph nuclear leucocytes. FEMS Microbial Litt –258: 290-296.

Huang, J.G.; Hu, X.Y.; Cheng, G.F.; Zhou, S.Q. and Song, N.H. (2002): The diagnosis of *Staphylococcus* arthritis in breeding broilers. J. Hubei Agri. Sci. 3(4): 78-79.

Iyer, A.P. and Kumosani, T.A. (2011): PCR based detection of nosocomial infection causing MRSA (Methicillin resistant *Staphylococcus aureus*). 2nd International Conference on Biotechnology and Food Science IPCBEE, 7 IACSIT Press, Singapore.

Joiner, K.S.;Hoerr, F.G.; van Santen, E. and Ewald, S.G. (2005): The avian major histocompatibility complex influences bacterial skeletal disease in broiler breeder chickens. Vet. Pathol., 42: 275-281.

Jordan, F.; Pattison, M.; Alexander, D.and Faragher, T. (2002): Poultry diseases. 5th ed. London:W.B.Saunders; 163p.

Jordan, F.T.; Pattisson, M. (1996): Poultry diseases. 4th ed. London: W.B. Saunders company Ltd; 66-67.

Kalorey, D. R.; Shanmugam, Y.; Kurkure, N.V.and Barbuddhe, S. B. (2007): PCR-baseddetection of genes encoding virulence determinants in *Staphylococcus aureus* from bovine subclinical mastitis cases. J Vet Sci., 8:151-154.

Malachowa, N. and DeLeo, F.R. (2010): Mobile genetic elements of Staphylococcus aureus, Cellular and Molecular Life Sciences 67(18), 3057–3071.

Mason, W.J.; blevins, J.S.; beenken, K.; wibowo, N.; ojha, N. and smeltzer, M.S. (2001): Multiplex PCR Protocol for the diagnosis of *Staphylococcal* infection. Journal of Clinical Microbiology, 39(9): 3332–3338.

Mathews, A.A.; Thomas, M.; Appalaraju, B., Jayalakshmi, J. (2010): Evaluation and comparison of tests to detect methicillin resistant *S. aureus*. Ind. J. Path. Microb., 53: 79-82.

McClure, J.A.; Conly, J.M.; Lau, V.; Elsayed, S.; Louie,T.; Hutchins,W. and Zhang, K. (2006): Novel multiplex PCR assay for detection of the *staphylococcal* virulence marker Panton-Valentine leukocidin genes and simultaneous discrimination of methicillin-susceptible from -resistant *staphylococci*. J Clin., Microbiol., 44: 1141-1147.

Momtaz, H.; Dehkordi, F.S.; Rahimi, E.; Asgarifar, A.and Momeni, M. (2013): Virulence genes and antimicrobial resistance profiles of *Staphylococcus aureus* isolated from chicken meat in

Isfahan province, Iran', Journal of Applied Poultry Research 22, 913–921.

Nataro, J. P. and Kaper, J. B. (1998): Diarrheagenic Escherichia coli. Clin.Microbiol. Rev., 11:142-201.

Okoli, I.C.(2006): Anti-Microbial Resistance Profile of *E. coli* isolated from Free Range Chicken in Urban Rural Environment of Imo state, Nigeria, http://www.ojhas?/issue 17/2006-1-3.htm.

Quinn, P.J.; Cater, M.E.; Markey,B.K. and Cater ,G.R.(1994): Clinical veterinary Microbiological Mosby- Year Book Europe Limited. *Staphylococcus* species: 118-127.

Rasheed B.Y. (2011): Isolation and identification of bacteria causing arthritis in chickens. Iraqi Journal of Veterinary Sciences. 25(2): 93-95.

Sambrook, J.; Fritscgh, E.; and Mentiates, E. (1989): Molecular coloning. A laboratory manual.Vol 1, Cold spring Harbor Laboratotry press, New York.

Schlegelova, J.; Vlkova, H.; Babak, V.; Holasova, M.; Jaglic, Z.; Stosova, T. and Sauer, P. (2008): Resistance to erythromycin of *Staphylococcus* spp. isolates from the food chain. Veterinarni Medicina, 53, (6): 307–314.

Sharada, R.; Ruban, S. W. and Thiyageeswaran, M. (2009): Antibiotic resistance pattern of E. coli isolated from poultry in Bangalore. Internet J. Microbiol., 7.

Tawfik, R. G.; Khalil, S.A.; Ellakany, H.F. and Torky, H.A. (2016): Mycoplasma Synoviae and other Associated Bacteria Causing Arthritis in Chicken. Alexandria Journal for Veterinary Sciences, 49, (2).

Toder, K. (2005): *Staphylococcus*:toder's online textbook of bacteriology, Veterinary Sciences; 11(1):51-55.

Wada, M.; Lkhagvadorj, E.; Bian, L.; Wang, C.; Chiba, Y.; Nagata, S.; Shimizu, T.; Yamashiro, Y.; Asahara, T. and Nomoto, K. (2010): Quantitative reverse transcription-PCR assay for the rapid detection of methicillin-resistant *Staphylococcus aureus*. Journal of Applied Microbiology, 108:779–788.

Wladyka, B.; Dubin, G. and Adam, D. (2011): Activation mechanismofthiol protease precursor from broiler chicken specific *Staphylococcus aureus*strain CH-91.Vet. Microbiol., 147:195-199.

Zmantar, T.; Chained, K.; Makni, H. and Bakhrof, A. (2008): Detection by PCR of adhesions genes and Slime production in clinical *S. aureus*.J. Basic Microbial 48: 308 – 314.