Journal homepage:
http://www.bsu.edu.eg/bsujournals/JVMR.aspx

Online ISSN: 2357-0520 Print ISSN: 2357-0512

Original Research Article

Bacteriological studies on bacterial pathogens isolated from broiler chickens with swollen head syndrome

Ismail Abd El-Hafeez Radwan[1], Mohamed Fathy Mohamed[1] and Aya Kamal Eldin Ahmed[1].

Department of Bacteriology, Mycology and Immunology , Faculty of Veterinary Medicine, Beni-Suef University, Beni Suef 62511, Egypt.

ABSTRACT

This work was planned to investigate the bacteria isolated from broiler chickens head suffered from naked eye pathological lesions. Out of 200 examined head lesions, the result revealed that the major pathogens associated with swollen head syndrome (SHS) were *Escherichia coli*, *Streptococcus dysgalactiae* and *Pseudomonas aeruginosa*. Antimicrobial sensitivity pattern against 11 different antimicrobials proved that isolates were resistant to most of the tested antimicrobial agents. PCR was applied on 4 MDR *E. coli*, 4 *S. dysgalactiae* and 2 *P. aeruginosa* for detection of some resistance and virulence genes. The results of *E. coli* isolates revealed that *blaTEM* gene was the most prevalent in all isolates (100%) followed by *tetA (A), aada1, aada2* and *aacC* genes. Meanwhile *tetA (B)* gene was found in 3 (75%), while *aadB* gene was not detected in any isolates. All *S. dysgalactiae* proved to harbour 16srRNAgene also all *S. dysgalactiae* were 100% positive for *tuf* gene followed by *speF* gene which found in 2 isolate (50%). The results of PCR of *P. aeruginosa* isolates revealed that *toxA* gene was the most prevalent gene found in all isolates (100%) followed by *lasI*. Then, *phzM* gene was found in one isolate (50%).

ARTICLE INFO

Article history:

Received

11 /2018

Accepted

12/2018

Available Online

12/2018

Keywords:

bacterial pathogens, broiler chickens

*Corresponding author. *Department of Bacteriology, Mycology and Immunology*, Faculty of Veterinary Medicine, Beni-Suef University, Beni Suef 62511, Egypt. Email: microbiologist111@yahoo.com

1. Introduction

Swollen head syndrome (SHS), one of the localized forms of colibacillosis that has been described in most intense poultry-producing areas, is characterized by an acute to subacute cellulitis involving the periorbital and adjacent subcutaneous tissues of the head of poultry **(Barnes and Gross, 1997).** SHS is characterized also by swelling of the peri- and infraorbital sinuses, torticollis and neurological disorders **(Pattison *et al.* 1989)**. It initially began as snicking, conjunctivitis, swelling of lacrimal gland and around eyes, over the head into the sub-mandibular region, and then development of sub-cutansous oedema of the head **(Tanaka *et al*.1995).** It was first reported in South Africa and called SHS since then, the syndrome has been recognized in England and sporadic areas of other countries (**Wyrth *et al.* 1987)**. *E. coli* strains isolated from chickens with SHS, these strains were isolated in the region of Campinas, SP (Brazil). The bacteria were recovered originally from a variety of tissues, with most isolates being collected from air sac, trachea and infraorbital sinus **(Parreira *et al.* 1998). Hafez and LoÈhren, (1990)** proved that SHS is caused by *a pneumovirus* in which the initial viral infection causes acute rhinitis, which is often followed by invasion of the facial subcutaneous tissues by *E. coli*. SHS lasts from 2 to 3 weeks, during which time there is a 2±3% reduction in egg production and a 3±4% rate of mortality **(Morley and Thomson, 1984).** The disease gradually spread on the farm and the signs became most apparent during the following 4 to 5 days and mortality ranged from 3 to7% among **flocks (Nunoya *et al.* 1991). Pattison *et al*., 1989** considered that spread of localized infection from either the nasal or tympanic cavity might have caused inflammation of air spaces, which were in communication with the nose and middle ear. **(Morris 1994)** reported that *E.coli* is the predominant microorganism isolated from cellulitis lesions in previous studies, although other agents, such as *Pasteurella multocida, P. aeruginosa, Enterobacter agglomerans, Proteus vulgaris* and *S. dysgalactiae,* have also been isolated. No clinical signs have been associated with cellulitis in live birds, but the presence of the lesion results in condemnation of part of or the entire carcass at processing. **Vaillancourt *et al.* 1992** revealed that *S. dysgalactiae* has been isolated from broilers with cellulitis. **Facklam, 2002** described that the Genus *Streptococcus* consists of numerous Gram-positive, non-motile, chain-forming cocci commonly found in the normal oral and bowel flora of warm-blooded animals. The Genus is comprised of 49 species and 8 subspecies of which 35 have been isolated as a source of infection in humans, *S. dysgalactiae* subsp. *dysgalactiae* is the only species listed that is not beta-hemolytic, large-colony-forming species (diameter, 0.5 mm) presenting either the Lancefield group C or the Lancefield group G antigen **(Facklam, 2002*)***. *Streptococcus* species have been associated with infections causing growth depression and increased mortality without obvious clinical signs **(Chadfield *et al*. 2004).** (**Collins *et al*. 2002)** studied that the *Streptococcus* species were identified using both classical phenotypic methods and 16S rRNA sequencing as each technique alone is not a reliable diagnostic tool. A universal primer-multiplex PCR method (UP-MPCR) method can detect the presence of five *P. aeruginosa* enterotoxin genes *(toxA, phzM, lasBe, xoU, and exoS)* in a single assay more rapidly and sensitively than conventional methods. In 214 drinking water and environmental isolates, the *exoU, exoS, phzM, toxA,* and *lasB* genes were detected in 20 (9 %), 180 (84 %), 179 (84 %), 196 (92 %), and 171 (80 %) isolates, respectively **(Shi H *et al* . 2012*)***. *E. coli* isolates harboring resistance gene responsible for tetracycline *(tetA),* beta lactams *(blaCMY)* and sulphanamide *(sulI)* antibiotics were found in 65.1, 65.1 and 54.0%, respectively. Twenty-five out of the 63 (39.7% %) *E. coli* isolates have got antimicrobial resistance gene to three or more classes of drugs **(Messele *et al.* 2017).** The present study was proposed to perform isolation and complete identification of bacterial pathogens recovered from broiler chickens head suffered from SHS. Moreover, to perform antimicrobial sensitivity tests of the isolates and to perform molecular identification of some resistance and virulence genes.

2. Material and Methods

2.1. Samples:

Two hundred head samples of broiler chickens [Hubbard and Ross] ranged in age from three to five weeks were selected when suffered at postmortem examination from naked eye swollen head syndrome. All samples were collected under complete aseptic conditions and cultivated on laboratory media according to (**Quinn *et al*., 2002).**

2.2. Isolation and identification:

Under complete aseptic condition, the collected broiler heads were incised and swaps were collected

followed by inoculation of these swaps on tryptone soya broth. All of the inoculated tubes were incubated aerobically at 37 °C for 24 hr, then plated on tryptone soya agar, MacConkey's agar and Eosin methylene blue (Oxoid). The plates were incubated aerobically at 37 °C for 24 hr. The recovered isolates primary identified using Gram's stain followed by biochemical identification using Microbact 24E.

2.3. Antimicrobial susceptibility test:

The disk diffusion method was used to determine the susceptibility of *E. coli, S. dysgalactiae and P. aeruginosa* isolates to 11 different antibiotic disks including amoxicillin (10µg), rifampicin (5µg), oxacillin (1µg), kanamycin (30µg), gentamicin (10 µg), chloramphenicol (30µg), vancomycin (30µg), clindamycin (2µg), cefeprime (3oµg), cefeprrazone (75µg), sulphamethoxazole-trimethoprime (25µg) (Oxoid) of veterinary significance according to the standards and interpretative criteria described by CLSI (Clinical and Laboratory Standards Institute, 2015).The results were recorded based on CLSI guidelines (CLSI, 2015).

2.4. Molecular Identification:

DNA was extracted by using bacterial DNA extraction kits (Qiagen) *according* to the manufacturer instructions. Primers sequences and the amplicons size are listed in Table 1**.** PCR was employed as published previously **(Tartor YH and El-Naenaeey EY, 2016).**

Table (1): Oligonucleotide primers sequences and size of the PCR-targeted products PCR for E. coli, S. dysgalactia and P. aearuginosa.

Target M.O.	Primer	Sequence	Amplified product	Reference
E. coli	***aadA1***	TATCAGAGGTAGTTGGCGTCAT GTTCCATAGCGTTAAGGTTTCATT	484 bp	Randall *et al.* 2004
	tetA (A)	GGTTCACTCGAACGACGTCA CTGTCCGACAAGTTGCATGA	576 bp	
	tetA (B)	CCTCAGCTTCTCAACGCGTG GCACCTTGCTCATGACTCTT	633 bp	
	aada2	TGTTGGTTACTGTGGCCGTA GATCTCGCCTTTCACAAAGC	622 bp	Walker *et al*., 2001
	blaTEM	ATCAGCAATAAACCAGC CCCCGAAGAACGTTTTC	516 bp	Colom *et al.,* 2003
	aacC	GGCGCGATCAACGAATTTATCCGA CCATTCGATGCCGAAGGAAACGAT	448 bp	Lynne *et al.,* 2008
	aadB	GAGCGAAATCTGCCGCTCTGG CTGTTACAACGGACTGGCCGC	319 bp	Frana *et al.,* 2001
P. aeruginosa	***toxA***	GACAACGCCCTCAGCATCACCAGC CGCTGGCCCATTCGCTCCAGCGCT	396 bp	Matar *et al*., 2002
	phzM	ATGGAGAGCGGGATCGACAG ATGCGGGTTTCCATCGGCAG	875 bp	Finnan *et al.,* 2004
	lasI	ATGATCGTACAAATTGGTCGGC GTCATGAAACCGCCAGTCG	606 bp	Bratu *et al.,* 2006
S. dysgalactiae	***tuf***	GTACAGTTGCTTCAGGACGTATC ACGTTCGATTTCATCACGTTG	196 bp	Picard *et al.,* 2004
	16S rRNA	GGAGTGGAAAATCCACCAT CGGTCAGGAGGATGTCAAGAC	549 bp	Prabhu *et al.,* 2013
	speF	TACTTGGATCAAGACG GTAATTAATGGTGTAGCC	782 bp	Rato *et al.,* 2011

3. Results

3.1. Bacterial isolates recovered from examined head lesions in broiler chickens:

The result showed that out of 200 samples, the most prevalent bacterial isolates were *E. coli* (50 isolates), *S. dysgalactiae* spp. (40 isolates) and *P. aeruginosa* (25 isolates) with incidences of 43%, 34.7%, and 21.7%; respectively.

3.2. Results of antimicrobial sensitivity of some examined isolates:

A strong resistance of tested antimicrobials has been observed as all isolates showed complete resistance to amoxycillin, rifampicin, gentamycin, cefepime, oxacillin, clindamycin, vancomycin, chloramohenicol, kanamycin, cefoperazone and sulphamethaxzole/trimethoprim with an incidence rate of 100%.

3.3. Results of molecular detection of resistance and virulence genes of some MDR isolates:

PCR was applied on 4 MDR *E. coli*, 4 *S. dysgalactiae* and 2 *P. aeruginosa* for detection of some resistance and virulence genes. The results of *E. coli* isolates genes revealed that *blaTEM* gene was the most prevalent found in all isolates (100%) followed by *tetA (A), aadA1, aadA2* and *aacC* genes. Meanwhile *tetA (B)* gene was found in 3 (75%), while *aadB* gene was not detected in any isolates. All *S. dysgalactiae* proved to harbour 16srRNAgene also all *S. dysgalactiae* were 100% positive for *tuf* gene followed by *speF* gene which found in 2 isolate (50%). The results of PCR of *P. aeruginosa* isolates revealed that *toxA* gene was the most prevalent found in all isolates (100%) followed by *lasI.*On the other hand, *phzM* gene was found in one isolate only (50%).

Table (2): Prevalence of resistance associated genes among some examined MDR E. coli isolates.

Tested gene	No. of *E. coli* isolates	Positive		Negative	
		No.	%	No.	%
***tetA*(*A*)**	4	4	100	0	0
***tetA*(*B*)**		3	75	1	25
***bla*$_{TEM}$**		4	100	0	0
aadA1		4	100	0	0
aadA2		4	100	0	0
aacC		4	100	0	0
aadB		0	0	4	100

% was calculated according to number of examined isolates. (NO. = 4)

Table (3): Prevalence of virulence-associated genes among some examined *S. dysgalactiae*.

Tested gene	No. of *S. dysgalactiae* isolates	Positive		Negative	
		No.	%	No.	%
16srRNA	4	4	100	0	0
tuf		4	100	0	0
speF		2	50	2	50

% was calculated according to number of examined isolates. (NO. = 4)

Table (4): Prevalence of virulence-associated genes among two examined *P. aeruginosa*.

Tested gene	No. of *P. aeruginosa* isolates	Positive		Negative	
		No.	%	No.	%
toxA	2	2	100	0	0
phzM		1	50	1	50
lasI		2	100	0	0

% was calculated according to number of examined isolates (NO. = 2)

4. Discussion

In Egypt, there are several problems facing the poultry industry. Colibacillosis is a serious and economically devastating disease of chickens, the Gram-negative bacterium *E. coli* is an important cause of diseases resulting in serious economic losses to the poultry industry. The strains are designated avian pathogenic *E. coli* (APEC) the most frequently reported disease and may be either localized or systemic infection caused entirely or partly by APEC including colisepticemia, coli granuloma, chronic respiratory disease (CRD), cellulitis, swollen-head syndrome, peritonitis, salpingitis, synovitis, pan-ophthalmitis, omphalitis and colisepticemia is the most common form of colibacillosis (**Salehi and Bonab, 2006)**. Our survey on major pathogens associated with head lesions in broilers which revealed that *E. coli* (50 isolates), *S. dysgalactiae* (40 isolates) and *P. aeruginosa* (25 isolates) with incidences of 43%, 34.7% and 21.7%; respectively. The bacterial isolates recovered from chickens were identified using oxidase, catalase and the microbact system. Previous studies showed that the incidence of *E.coli* in apparently healthy broiler chickens was 15.7%, diseased broiler chickens 37.1% and in freshly dead ones 55% in winter season while in summer season was 15.8% in apparently healthy, 17.5% in diseased broiler chickens and 18.7% in freshly dead one (**Abd El Tawab *et al.* 2015)**. Also, the prevalence of pathogenic *E. coli* in broiler house was independent of the prevalence of other commensal or environmental *E. coli* (**Jeffrey *et al.* 2004)**. The risk for SHS increases with increasing infection pressure in the environment. A good housing hygiene and avoiding overcrowding are very important. Other principal risk factors are the duration of exposure, virulence of the strain, breed and the immune status of the bird. Every damage to the respiratory system favors the infection with APEC. Bacteriological examinations of the infraorbital sinuses of the affected birds resulted in the isolation of *E. coli* (seven cases, 87.5%) and *Staphylococcus spp*. (one case, 12.5%) (**Georgiades *et al*. 2001)**. *E. coli* is the most frequently isolated bacterium from cellulitis lesions, other bacteria such as *S. dysgalactiae*, *P. multocida*, *P. vulgaris*, *E. agglomerans*, and *P. aeruginosa* have been isolated from cellulitis lesions (**Norton, 1997)**. Resistance to antimicrobials was obvious in our results including amoxycillin, gentamycin, cefepime, oxacillin, rifampicin, kanamycin, chloramphenicol, vancomycin, clindamycin, cefoperazone and sulphamethaxzole/trimethoprim. The presence of several resistance and virulence genes has been positively linked to the pathogenicity and antimicrobial resistance. The isolated *E. coli* were resistant to cloxacillin, nalidaxic acid and

erythromycin.(**Dec *et al*. 2017**) revealed that high prevalence of resistance in *E. coli* isolates of chicken origin to tiamulin (90% resistant isolates), tetracyclines (74%), and lincosamides (70%), and moderately high frequency of resistance to enrofloxacin (48%), macrolides (42%), aminoglycosides (12.5–31%), ampicillin (26% resistant isolates) and chloramphenicol (23%). Multi drug resistant *P. aeruginosa* had become a serious problem in hospital, especially in patients on ventilators (**Aoki *et al.* 2009)**. 10 bacterial isolates that recovered from broiler chickens with SHS including 4 *E. coli*, 4 *S. dysgalactiae* and 2 *P. aeruginosa* were subjected for PCR for detection of antimicrobial resistance and virulence genes (*tetA (B)* and *tetA (A*) of tetracycilin, *blaTEM* of Ampicillin, *aadA1*and *aadA2* of streptomycin, *aacC* and *aadB* of aminoglycoside). The establishment of PCR assays was to facilitate determination of the frequency with which the various virulence-associated genes occur. The results of *E. coli* isolates genes revealed that *blaTEM* gene was the most prevalent found in all isolates (100%) followed by *tetA(A),aadA1,aadA2* and *aacC* genes. Meanwhile *tetA (B)* gene were found in 3 (75%), while *aadB* gene was not detected in any isolates and the results of PCR of *S. dysgalactiae* isolates revealed that 16srRNA gene and *tuf* gene were the most prevalent found in all isolates (100%). Then, *speF* gene was found in 2 isolate (50%). The results of PCR of *P. aeruginosa* isolates revealed that t*oxA* gene was the most prevalent found in all isolates (100%) followed by *lasI*. Then, *phzM* gene was found in one isolate (50%). **Tartor YH and El-Naenaeey EY (2016)** analyzed antimicrobial susceptibility of *P. aeruginosa* isolated from broiler chickens and cattle as well as expression of five significant exotoxin genes (*exoU, exoS, toxA, lasB, and phzM).* The highest resistance was found to ampicillin, erythromycin, followed by chloramphenicol, trimethoprim/sulfamethoxazole and tetracycline, intermediately sensitive to ceftazidime, cefoperazone, and highly sensitive to gentamicin, levofloxacin, imipenem, ciprofloxacin and colistin. In conclusion, the increased antibiotic resistance and virulence of bacteria isolated from broiler chicken suffered from swollen head syndrome complicate treatment decisions and increase public health hazard.

References

Abd El - Tawab, A. A.; Ammar, A. M.; Nasef, S. A. and. Reda, R. M. (2015). Antibacterial resistance and resistance gene detriments of *E. coli* isolated from chicken. Benha Veterinary Medical Journal. 28(2): 231-240.

Aoki, N. ; Tateda, K.; Kilkuchi, Y. ; Kimura, S. ; Miyazaki, C. ; Ishii, Y. ; Tanabe, Y. ; Gejyo, F. and Yamaguchi, K. (2009). Efficacy of colistin combination therapy in a mouse model of pneumonia caused by multidrug-resistant *P. aeruginosa*. J. Antimicrob Chemother., 63 (3): 534-542.

Barnes, H. J. and Gross, W. B. (1997). Colibacillosis. In B.W. Calnek, H.J. Barnes, C.W. Beard, L.R. McDougald and Y.M. Saif (Eds), Diseases of Poultry 10th Ed. (pp. 131-141). Ames, IA: Iowa state Univ. press.

Bratu, S.; Gupta, J. and Quale, J. (2006). Expression of the las and rhl quorum-sensing systems in clinical isolates of Pseudomonas aeruginosa does not correlate with efflux pump expression or antimicrobial resistance. Journal of Antimicrobial Chemotherapy (2006) 58, 1250–1253.

Chadfield M.S., Christensen J. P., Christensen H. and BisgaardM. (2004). Characterization of streptococci and enterococci associated with septicaemia in broiler parents with a high

prevalence of endocarditis. P. 610-617 | Avian Pathology (December 2004) 33(6), 610 /617.

CLSI (Clinical and Laboratory Standards Institute) (2015). Performance standards for antimicrobial disk and dilution susceptibility tests for bacteria isolated from animals M100-S25.

Collins, M.D., Hutson, R.A., Falsen, E., Ingana¨s, E. and Bisgaard, M. (2002). *Streptococcus gallinaceus sp*. nov., from chickens. International J. of Systematic and Evolutionary Microbiology, 52,1161 /1164.

Colom K, PèrezJ, Alonso R, Fernández-Aranguiz A,LariñoE, Cisterna R. (2003). Simple and reliable multiplex PCR assay for detection of bla_{TEM}, bla_{SHV} and bla_{OXA-1} genes in Enterobacteriaceae. FEMS Microbiology Letters 223 (2003) 147-151.

Dec M, Urban-Chmiel R, Stępień-Pyśniak D and Wernicki A .(2017). Assessment of antibiotic susceptibility in Lactobacillus isolates from chickens. Gut Pathog. 2017 Sep 19;9:54.

Droual, R. and Woolcock, P.R. (1994). Swollen head syndrome associated with *E. coli* and infectious bronchitis virus in the Central Valley of California. Avian Pathology, 23, 733–742.

Facklam, R. (2002). What happened to the streptococci: overview of taxonomic and nomenclature changes. Clin. Microbiol. Rev. 2002,15, 613–630.

Frana, T. S., Carlson, S. A. & Griffith, R. W. (2001). Relative distribution and conservation of genes encoding aminoglycoside-modifying enzymes in *Salmonella enterica* serotype Typhimurium phage type DT104. Applied and Environmental Microbiology 67, 445–8.

Finnan, S.; Morrissey, J.P.; Gara, F.O and Boyd, E.F. (2004). Genome Diversity of *Pseudomonas aeruginosa* Isolates from Cystic Fibrosis Patients and the Hospital Environment. JOURNAL OF CLINICAL MICROBIOLOGY, Dec. 2004, p. 5783–5792.

Georgiades G1, Iordanidis P, Koumbati M. (2001). Cases of swollen head syndrome in broiler chickens in Greece.Avian Dis. 2001 Jul-Sep;45 (3):745-50.

Hafez, H.M., LoÈhren, V., (1990). Swollen head syndrome: Clinical observations and serology in West Germany. Dtsch Tieraerztl Wochenschr 97, 322±324.

Jeffrey. J. S.; Singer, R. S.; O'Connor, R., and Atwill, E. R. (2004). Prevalence of Pathogenic *Escherichia coli* in the broiler house environment. Avian Diseases 48(1):189-195.

Lynne, A. M., Rhodes-Clark B S., Kimberly Bliven, Shaohua Zhao, and Foley, S. L. (2008). Antimicrobial Resistance Genes Associated with *Salmonella enteric* Serovar Newport Isolates from Food Animals. .ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, . 353–356 Vol. 52, No. 1

Matar, G.M.; Ramlawi, F.; Hijazi, N.; Khneisser, I. Abdelnoor, A.M. (2002). Transcription Levels of *Pseudomonas aeruginosa* Exotoxin A Gene and Severity of Symptoms in Patients with Otitis Externa. CURRENT MICROBIOLOGY Vol. 45 (2002), pp. 350–354.

Messele YE, Abdi RD, Yalew ST, Tegegne DT, Emeru BA and Werid GM.(2017) Molecular determination of antimicrobial resistance in *Escherichia coli* isolated from raw meat in Addis Ababa and Bishoftu, Ethiopia.Ann Clin Microbiol Antimicrob. 2017 Aug 15;16(1):55.

Morris, M.P., (1994). Broiler cellulitis update. Broiler Industry, March, 36^39.

Morley, A.J. & Thomson, D.K. (1984). Swollen-head syndrome in broiler chickens. Avian Diseases, 28,238-243.

Norton, R.A. (1997). Avian cellulitis. World's Poultry Science Journal, 53, 337– 349.

Nunoya T, Tajima M, Izuchi T, Takahashi K, Otaki Y, Nagasawa Y, Hakogi E. (1991). Pathology of a broiler disease characterized

by the swollen head. J Vet Med Sci. 1991 Apr;53(2):347-9.

Parreira V.R and Yano T (1998). Cytotoxin produced by *Escherichia coli* isolated from chickens with swollen head syndrome (SHS) /Veterinary Microbiology 62 (1998) 111±119.

Pattison, M., Chettle, N., Randall, C.J. and Wyeth, P.J. (1989). Observations on swollen head syndrome in broiler and broiler breeder chickens. Veterinary Record, 125, 229-231.

Picard, F.J.; Ke, D.; Boudreau, D.K.; Boissinot, M.; Huletsky, A.; Richard, D.; Ouellette, M.; Roy, P.H. and Bergeron, M.G. (2004). Use of *tuf* Sequences for Genus-Specific PCR Detection and Phylogenetic Analysis of 28 Streptococcal Species. JOURNAL OF CLINICAL MICROBIOLOGY, Aug. 2004, p. 3686–3695.

Prabhu, K.N.; Isloor, S.; Hegde, R.; Rathnamma, D.; Veeregowda, B.M.; Murthy, H.N.N.; Shome, R.; Suryanarayana, V.V.S. (2013). Development of polymerase chain reaction for detection of predominant streptococcal isolates causing subclinical bovine mastitis. Indian Journal of biotechnology. Vol. 12, pp208-212.

Quinn, P.J.; Markey, B.K.; Carter, M.E.: Donnelly, W.J. and Leonard, F.C. (2002). Veterinary microbiology and microbial disease. Text book MPG books Ltd, comwell

Randall, L.P.; Cooles, S.W.; Osborn, M.K.; Piddock, L.J.V. and Woodward, M.J. (2004). Antibiotic resistance genes, integrons and multiple antibiotic resistance in thirty-five serotypes of *Salmonella enterica* isolated from humans and animals in the UK. *Journal of Antimicrobial Chemotherapy.* 53, 208–216.

Rato, M.G.; Nerlich, A.; Bergmann, R.; Bexiga, R.; Nunes, S.F.; Vilela, C.L.; Santos-Sanches ,I and Chhatwal, G.S. (2011). Virulence gene pool detected in bovine group C Streptococcus dysgalactiae subsp. dysgalactiae isolates by use of a group A S. pyogenes virulence microarray. J Clin Microbiol; 49(7):2470-9.

Salehi, T. Z.; and Bonab, S. F. (2006). Antibiotics susceptibility pattern of *Escherichia coli* strains isolated from chickens with colisepticemia in Tabriz Province, Iran. International Journal of Poultry Science 5 (7): 677-684.

Shi H, Trinh Q, Xu W, Zhai B, Luo Y and Huang K. (2012). A universal primer multiplex PCR method for typing of toxinogenic *Pseudomonas aeruginosa*. Appl Microbiol Biotechnol.2012 Sep; 95 (6):1579-87).

Tartor YH and El-Naenaeey EY. (2016). RT-PCR detection of exotoxin genes expression in multidrug resistant *Pseudomonas aeruginosa*. Cell Mol Biol (Noisy-le-grand). 2016 Jan 22;62 (1):56-62).

Tanaka M., Takuma H., Kokunai N., Oishi E., Obi T., Hiramtsu K. and Shimizu Y. (1995). Turkey Rhinotracheitis virus isolated from broiler chicken with swollen head syndrome in Japan J.Vet. Med .Sci .57(5);939- 941 ,1995.

Vaillancourt, J.P., Elfadil, A. and Bisaillon, J.R. (1992). Cellulitis in the broiler fowl. Medicine Veterinaire Quebec, 22, 168 /172.

Walker, R. A., Lindsay, E., Woodward, M. J. et al. (2001). Variation in clonality and antibiotic-resistance genes among multi-resistant Salmonella enterica serotype Typhimurium phage-type U302 (MR U302) from humans, animals, and foods. Microbiological Research 7, 13–21.

Wyeth, P.H., Chettle,N. J.,Cough, R.E.,Collins ,M.S.(1987). Vet.Rec .120 :286_287.