

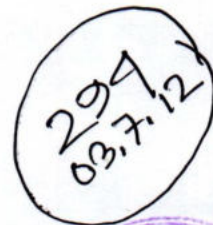
**CHARACTERIZATION OF *STAPHYLOCOCCUS SP* ISOLATED FROM
ARTHRITIC BROILER BREEDER FLOCK WITH ANTIBIOGRAM
STUDY OF IDENTIFIED FIELD ISOLATES**

A THESIS

BY

MD. SHAFIQUIL ISLAM

**REGISTRATION NO.1005018
SEMESTER: MARCH –AUGUST, 2011**



MASTERS OF SCIENCE (M.S.)

IN

MICROBIOLOGY



**DEPARTMENT OF MICROBIOLOGY
HAJEE MOHAMMAD DANESH SCIENCE AND TECHNOLOGY
UNIVERSITY DINAJPUR- 5200**

AUGUST, 2011

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SUBMITTED TO THE

DEPARTMENT OF MICROBIOLOGY

FACULTY OF VETERINARY AND ANIMAL SCIENCE

HAJEE MOHAMMAD DANESH SCIENCE AND TECHNOLOGY

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IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF

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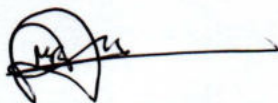
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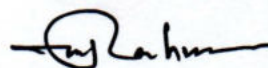
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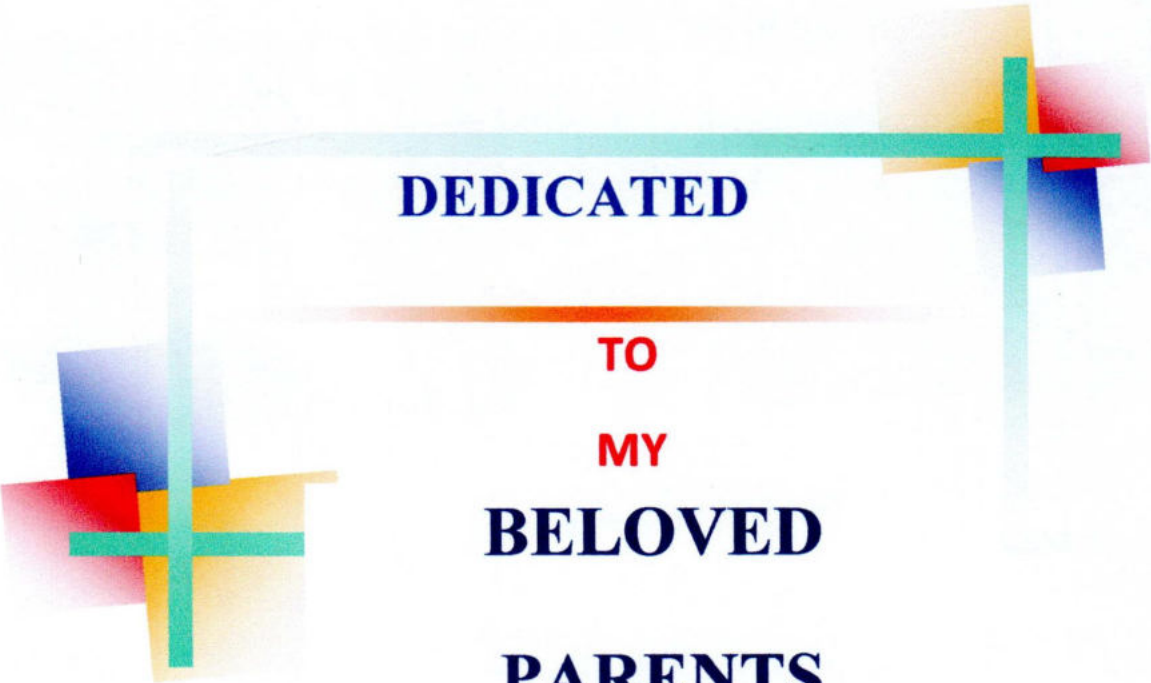
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AUGUST, 2011



DEDICATED

TO

MY

BELOVED

PARENTS

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*The author
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ABSTRACT

The study was conducted for characterization of *Staphylococcus sp* isolated from arthritic broiler breeder flock with antibiogram study of identified field isolates. The study was performed in a breeder flock. The flock consists of 7000 birds of different age groups, name of the breed cobb 500 selected for the study. The sample were collected from the affected birds and shifted aseptically to the Bacteriology laboratory of the department of Microbiology Hajee Mohammed Danesh Science and Technology University. The samples were subjected to clinical, postmortem and microbiological (morphological, cultural and biochemical) examination. The study shows that infection is very high in starter and grower (58.2%) than laying period (16.6%). The *staphylococcus sp* highly sensitive to different antimicrobial agents such as 100% sensitive to ciprofloxacin, doxycycline, penicillin, gentamicin and 70% sensitive nitrofurantoin 60% azithromicin and resistance to cephradine, ampicillin, amoxicillin sulfomithoxazole X Trimethoprim.

CONTENTS (CONTD)

Chapter	List of Contents	Page No
	ACKNOWLEDGEMENT	IV
	ABSTRACT	V
	CONTENTS	VI-VIII
	LIST OF PLATES	IX
	LIST OF TABLES	X
	LIST OF FIGURES	X
	LIST OF ABBREVIATIONS	XI-XII
1	INTRODUCTION	1-3
2	REVIEW OF LITERATURE	4-31
2.1	Isolation of <i>Staphylococcus sp.</i>	4-22
2.4.	Antibiotic sensitivity and resistance pattern	22-31
3	MATERIALS AND METHODS	32-44
3.1.	Materials	32
3.1.1	Selection of Study area.	32
3.1.2	Farm Selection and observations	32
3.1.3	Experimental birds	32
3.1.4	Collection of Sample	33
3.1.5	Bacteriological Media	33
3.1.5.1	Nutrient agar	33
3.1.5.2	Nutrient broth	33
3.1.5.3	Blood Agar (BA) medium	34
3.1.5.4	Mannitol Salt Agar	34
3.1.5.5	Staphylococcus agar no 110	34
3.1.6	Biochemical media	35
3.1.6.1	Triple Sugar Iron (TSI) agar slant	35
3.1.6.2	Methyl-Red Voges-Proskauer (MR-VP) broth	35
3.1.6.3	MIU (Motility, Indole, urea) medium	36
3.1.6.4	Indole test	36
3.1.6.5	Methyl red solution	36
3.1.7.1	Appliances	36
3.1.7.2	Reagents and Chemicals	37
3.1.7.3	Antibiotic Discs	37
3.2	Method of Isolation	38
3.2.1	The experimental design of the present study	38-39

CONTENTS (CONTD)

Chapter	List of Contents	Page No
3.2.2	Methods of cultural characterization of <i>Staphylococcus sp</i>	40
3.2.2.1	Nutrient agar (NA)	40
3.2.2.2	Manitol salt base agar	40
3.2.2.3	Staphylococcus agar no.110	40
3.2.2.4	Blood agar (BA)	40
3.2.3	Morphological characterization of organism by Gram staining method	41
3.2.4	Method of biochemical characterization	41
3.2.4.1	Methyl –Red test (MR)	41
3.2.4.2	Voges proskauer (VP) test	42
3.2.4.3	Indole test	42
3.2.4.4	Triple sugar Iron (TSI) agar	43
3.2.4.5	MIU (Motility, Indole, Urease) Test	43
3.2.5	coagulage test	43
3.2.6	Maintance of stock culture	43
3.2.7	Hemolytic activity	43
3.2.8	Isolation of bacteria in pure culture	43
3.2.8	Comparative antimicrobial sensitivity pattern of <i>staphylococcus sp.</i>	44
4	Results	45-64
4.1	Results of isolation and identification of pathogenic <i>Staphylococcus sp.</i>	46
4.1.1	Results of cultural examination	46
4.1.1.1	Culture on nutrient broth	46
4.1.1.2	Culture on nutrient agar	46
4.1.1.3	Culture on Manitol salt base agar	46
4.1.1.4	Culture on Staphylococcus Agar no 110	46
4.1.1.5	Culture on blood agar media	47
4.1.1.6	Result of Gram stain	47
4.1.2	Results of biochemical test	47
4.1.2.1	Results in TSI agar media	47
4.1.2.2	Results in MIU medium	47
4.1.2.3	Results in Manitol salt base agar	47
4.1.2.4	Results in MR-VP medium and Coagulage test	48

CONTENTS (CONTD)

Chapter	List of Contents	Page No
4.1.3	Results of antibiotic sensitivity pattern of isolated bacteria	50
4.1.3.1	Antibiotic sensitivity pattern of <i>Staphylococcus aureus</i>	50
5	Discussion	65-68
6	Summary and Conclusion	69-70
	References	71-81
	LIST OF APPENDICES	82-85

CONTENTS (CONTD)

Plates no.	Topics	Page no.
Plates.1-4	The organism from the sample produced circular, small smooth, convex, and yellowish colonies on nutrient agar.	54
Plates.5-8	Manitol salt base agar after incubation produced golden yellow mannitol fermenting colony with yellowish media (yellow) is <i>S. aureus</i> .	55
Plates.9	Presence of uniform turbidity on nutrient broth	56
Plates. 10-11	Staphylococcus Agar no110 media after incubation produced golden yellow color colony.	56
Plates-12	Microscopic feature of Gram staining of the <i>Staphylococci</i> sp isolates showing Gram positive, cocci shape, grape-like cluster.	57
Plates-13-14	Blood agar media after incubation produced smooth, shiny, round and convex colony with haemolysis.	57
Plates.15	Biochemical test showing Triple sugar iron test positive (TSI test, +ve).	58
Plates.16	Biochemical test showing MIU test negative (MIU test, -ve).	58
Plates-17	Biochemical test in Manitol salt base agar showing media becomes yellow due to manitol fermentation.	58
Plates-18	Biochemical test showing indole negative, no pink color ring at the adjacent	59
Plates-19	Biochemical test showing MR test positive (red color indicate MR test positive)	60
Plates-20	Biochemical test showing VP test negative,(no color change after adding reagent)	60
Plates -21	The test showing coagulage test positive	61
Plates -22-26	Antibiotic sensitivity test	61-63

LIST OF FIGURES

Figure no.	Topics	Page no.
Figure-1	Breeder flock. Name of the breed 'Hubbard classic'.	53
Figure-2	Affected leg, foot pad affected with <i>Staphylococcus</i> infection.	53
Figure-3	Joint affected with <i>Staphylococcus</i> Infection	53
Figure-4	Graphical presentation of antibiotic sensitivity pattern of isolated <i>Staphylococcus sp</i>	51

LIST OF TABLES

Table no.	Topics	Page no.
Table-1	Isolation and identification of <i>Staphylococcus sp</i> from live affected birds on the basis of inoculation of sample in bacteriological media and normal biochemical media	46
Table-2	General cultural media and colony characters	48
Table-3	Biochemical characteristics of <i>Staphylococcus sp</i>	48
Table-4	Frequency response of staphylococcus isolates to microscopic examination and specific biochemical test.	49
Table 5	Results of antibiotic sensitivity test of the isolates bacteria obtained from arthritis and bumble foot infection of broiler breeder	50
Table.6	Antibiotic sensitivity test of the isolated <i>Staphylococcus sp</i>	52

LIST OF ABBREVIATION AND SYMBOLS (CONTD)

ml	=	milliliter
mm	=	millimeter
MR	=	Methyl red
NA	=	Nutrient agar
NB	=	Nutrient broth
NF	=	Nitrofurantion
No	=	Number
N	=	Neomycin
P	=	Penicillin,
R	=	Resistance
Rpm	=	round per second
RT	=	room temperature
SL	=	Serial
Sq	=	Squire
SxT	=	Sulphamethoxazole and Trimethoprim
TE	=	Tetracycline
UV	=	Ultra-violet
VP	=	Voges Proskauer
µg	=	Microgram
µl	=	Microliter
+	=	Positive
-	=	Negative
%	=	Percentage

CHAPTER 1

INTRODUCTION

CHAPTER- 1

INTRODUCTION

The importance of Staphylococcosis in poultry sector has increased to be a growing concern day by day throughout the world during the last decades. *Staphylococcus aureus* is a normal inhabitant of the skin and upper respiratory tract of diseased and healthy chickens (Shiozawa *et al.*, 1980; Zhu *et al.*, 1999). Several species of *Staphylococcus* cause infection in poultry. *Staphylococcus aureus* is an important opportunist that can cause superficial to life-threatening illnesses in a variety of animal species. In poultry, this organism has been implicated in osteomyelitis, synovitis, and cellulites (White DG, 2003). The *Staphylococcus sp* cause a number of infection in poultry such as arthritis, osteomyelitis, dermatitis, endocarditis, septicemia, wound infection, and ophthalmitis. Staphylococcus infections tend to occur more frequently during the following four periods of a breeder's life: 0 - 2 weeks femoral head necrosis (or bacterial chondronecrosis) are often related to egg or hatchery contamination and minor surgeries at, 4 - 6 weeks infection hock and stifle joints secondarily related to other infection, at 10 - 20 weeks Infection hock and stifle joints is due to the stress of vaccination, feed restriction and sexual maturation. Overcrowding, poor feed distribution and insufficient feeder space exacerbate these problems. When the birds reaches at 24 - 30 weeks of age than there might be hock and stifle joints infection associated with bumble foot. Male aggression and injuries associated with feed equipment, nest boxes and slats also contribute to the development of staphylococcal infections. The *Staphylococcus aureus* infection has become an increasingly great problem in industrialised poultry farming (Bergmann, 1980).

Poultry rearing is now a rising sector in Bangladesh, because an almost assured and quick good return could be achieved in relatively short period of time by a moderate amount of investment. But staphylococcosis causes constant losses to poultry industry economically and thus makes difficult in the establishing this industry. Most of the poultry farms in Bangladesh are not organized and scientifically planned. Many of the farmers here are not well aware of poultry farm management or ignorant about maintenance of Bio-security measure and conditions attributed to farming system.

Poultry is essential to the national economy of Bangladesh and the welfare of human beings as well. Several constraints like the diseases, poor husbandry, low productivity and shortage of food affect the optimal performance of this industry in Bangladesh (Haque *et al*, 1991) with the great expansion of poultry rearing and farming, Staphylococcosis caused by *staphylococcus sp* is the most devastating diseases in Bangladesh causing continuous infection and high economic loss in broiler breeder. The diseases can be controlled by decreasing the stress by reducing overcrowding, insufficient feed and water, feed restriction, vaccination and other handling, faulty debeaking, poor litter management, onset of sexual maturity and egg production, inadequate clean out and biosecurity procedures, high temperature and poor ventilation.

Studied that food poisoning by *Staphylococcus aureus* affects thousands of people each year. *Staphylococcus aureus* also causes invasive diseases such as arthritis (in poultry) and septicemia in poultry and humans. (Hazariwala A *et al.*, 2002). High economic loss occurs due to high morbidity and mortality. With the great expansion of the poultry rearing

and farming, Staphylococcosis have become wide spread problem of breeder farm in Bangladesh and other areas of the world.

In view of the above consideration, the present study was under taken with the following objectives:

- I. To isolate and identify the etiological agent of arthritis in broiler breeder flock
- II. To characterize the isolated organism by using cultural and biochemical tests
- III. To evaluate the antibiotic sensitivity of the identified field isolates.

CHAPTER 2

REVIEW OF LITERATURE

CHAPTER -2

REVIEW OF LITERATURE

2.1. Isolation of *Staphylococcus sp.*

B. Y. Rashed. (2011) studied that sixty chickens 30-55days of age with arthritis symptoms, were collected from different broiler chickens farm, all sample were examined clinically, post mortem and bacterial isolation were done. The result revealed isolation of 26(50.98%) of *staphylococcus aureus*, which were found highly sensitive to amoxicillin. The experimental infection of 10 chickens was carried out on 35 days old by intravenous inoculated with 10 cfu/ml of isolated *staphylococcus aureus*. Arthritis occurred in 8(80%) chickens. Clinical sign and postmortem findings confined to depression, swollen joints and inability to stand.

Wladyka B et al. (2011) reported that *Staphylococcus aureus* strain CH-91 isolated from chicken arthritic lesions produces large quantities of thiol protease implicated in disease formation. Observed overproduction requires efficient activation of the protease precursor which mechanism is studied here in detail. Wild type and mutant precursor forms are expressed in *E. coli* to test different hypotheses on the activation process. It is demonstrated that wild type precursor undergoes rapid autocatalytic processing whereas proteolytically inactive catalytic triad cysteine mutant (C(249)A) of the precursor is stable, but can be processed by minute quantities of active protease. It is concluded that limited intramolecular proteolysis is mainly responsible for efficient activation but, a positive feedback loop also contributes to the process. Both activation pathways allow efficient production of mature extracellular thiol protease, a putative virulence factor specific for avian strains of *S. aureus*.

Mamza SA *et al.* (2010) reported that the occurrence of beta-lactamase-producing *Escherichia coli* and *Staphylococcus aureus* in chickens was investigated. Specimens (n = 1,300) were collected from 400 chickens and were streaked on MacConkey agar plates. From each plate, presumptive growths of organisms were picked and streaked on eosin methylene blue and Baird-Parker agars, respectively. Typical colonies of *E. coli* and *S. aureus* with similar morphologies were identified by biochemical tests. Isolates were tested for beta-lactamase production and antimicrobial susceptibilities. Results indicated that 805 *E. coli* isolates from which 89 (11%) were beta-lactamase-positive and 660 *S. aureus* from which 58 (8.8%) were beta-lactamase-positive. The implications of this for humans when handling and/or consuming chickens and chicken products contaminated with strains of such isolates, is a risk of transferrable multi-drug resistance and a failure of treatment. The results of our study indicated that beta-lactamase-producing *E. coli* and *S. aureus* are prevalent in chickens in Nigeria.

A. M. Shareef *et al.* (2009) Reported that four flocks of layer breeders consist of 28000(7000 birds each) at 25 weeks of age, hatchery and one day old chicks were subjected to swab sampling in order to isolate *Staphylococcus aureus* during the period from may till june 2008. Result revealed that more than half of the overall swab samples were positive (52.04%). Different isolation were recorded between four flocks, ranging from 62.5 to 79.16%. Hatchery samples revealed that working surfaces were heavily contaminated with *s. aureus* (75%) compared with the relatively low contaminated eggs flat and egg shells. One day old chick sample show relatively low percentage of *S. aureus* isolation (29.1%). Antimicrobial sensitivity of 20 *S. aureus* isolates were surveyed for susceptibilities to panel of 16 antimicrobial agents. *S. aureus* were 100%

sensitive to five antimicrobials, namely ; enrofloxacin ; methicillin; trimethoprim with sulphamethoxazol and vancomycin, while in the opposite direction, 100% antimicrobials include: gentamycin , chloramphenicol, penicillin, erythromycin, ciprofloxacin, colistin, lincomycin lincomycin cephaloxin and doxycillin the importance of the organism in breeder layers health was discussed.

Nemati M *et al.* (2009) *Staphylococcus aureus* isolates recovered from poultry between 1970 and 1972 (90 old isolates) and in 2006 (81 recent isolates) were screened for the presence of *bap*, *icaA* and *icaD* genes associated with biofilm formation, and for *bbp*, *cna*, *ebpS*, *eno*, *fib*, *fnbA*, *fnbB*, *clfA* and *clfB* genes that encode microbial surface components recognizing adhesive matrix molecules (MSCRAMMs). Most of the old isolates were collected from broiler breeders affected by staphylococcal tenosynovitis and arthritis, whilst nearly all recent isolates were derived from the nose and cloaca of healthy broilers. From this study, there is no indication that the presence of these genes has changed over time and no specific association could be found between the presence of certain MSCRAMM or biofilm genes in poultry *S. aureus* isolates and the isolate's capacity to cause disease.

Stepień-Pyśniak D *et al.* (2009) presented that the degree of contamination of table eggs with bacteria of the genus *Staphylococcus*, taking into account the source of the eggs. The results of the study indicate a relatively high degree of contamination of table eggs with *Staphylococcus* bacteria. In 1125 bacteriological tests conducted on whites, yolks and shells of eggs from three sources, staphylococci were found in 514 cases. Thirteen strains were isolated from the whites, but *Staphylococcus* bacteria were found more often in yolks--199 strains. The highest percentage of staphylococci were found on the surface of the egg

shell--302 strains. Twelve species of staphylococci were isolated from the eggs tested, including both coagulase-positive strains (*Staphylococcus aureus*, *S. hyicus*) and coagulase-negative strains, particularly *Staphylococcus lentus*, *S. warneri*, *S. epidermidis* and *S. xylosus*. This study determined that regardless of the source of the eggs, egg yolks were more often contaminated with *Staphylococcus aureus* than with coagulase-negative Staphylococci. It was also demonstrated that *S. aureus* dominated in the yolks and on the shells of eggs from the small-scale poultry farm.

Zhong Z et al. (2009) reported that *staphylococcus aureus* was used as an indicator to study the origin and spread of microbial aerosol in and around chicken houses. Air samples indoor, upwind (10 and 50 m), and downwind (10, 50, 100, 200, and 400 m) of four chicken houses were collected using Andersen-6 stages sampler. The genetic relationship among the isolates was determined by profiles of PCR-amplified repetitive extragenic palindromic (REP-PCR) elements. The results showed that the concentrations of *S. aureus* indoor of four chicken houses were higher than those upwind and downwind sites ($P < 0.05$ or $P < 0.01$), but there were no significant concentration differences among downwind sites ($P > 0.05$). The fingerprints and the phylogenetic tree indicated that a part of the *S. aureus* (55.6%, 10/18) isolates from indoor air had the same REP-PCR fingerprints as feces isolates. Thus, the use of *S. aureus* as an indicator to study the origin and spread of airborne pathogens from chicken houses is potentially useful for enhancing public health and understanding the airborne epidemiology of this pathogen. Meanwhile it can provide evidence for studying the spreading model of airborne pathogens.

Lowder BV et al. (2009) reported that the impact of globalization on the emergence and spread of pathogens is an important veterinary and public health issue. *Staphylococcus aureus* is a notorious human pathogen associated with serious nosocomial and community-acquired infections. In addition, *S. aureus* is a major cause of animal diseases including skeletal infections of poultry, which are a large economic burden on the global broiler chicken industry. Here, we provide evidence that the majority of *S. aureus* isolates from broiler chickens are the descendants of a single human-to-poultry host jump that occurred approximately 38 years ago (range, 30 to 63 years ago) by a subtype of the worldwide human ST5 clonal lineage unique to Poland. These genetic events have resulted in enhanced resistance to killing by chicken heterophils, reflecting avian host-adaptive evolution. Taken together, we have determined the evolutionary history of a major new animal pathogen that has undergone rapid avian host adaptation and intercontinental dissemination. These data provide a new paradigm for the impact of human activities on the emergence of animal pathogens.

Lin J et al. (2009) studied that *Staphylococcus aureus* is a cause of many diseases in both humans and animals. This pathogen is also a major target in the screening of slaughterhouse carcasses to monitor hygienic conditions during slaughter. During 2004 to 2006, *S. aureus* was recovered from 8.8% (38 of 430), 11.3% (77 of 680), and 4.3% (13 of 300) of pork carcass samples, respectively, collected at 53 slaughterhouses in Taiwan. During 2003 to 2005, it was recovered from 0.3% (1 of 305), 0.4% (1 of 260), and 7.8% (31 of 395) of rinse fluids from chicken carcasses, respectively, collected at 17 meat processing plants. The percentages resistant to methicillin (oxacillin), chloramphenicol, erythromycin, and tylosin were 19.4% (40 of 207), 18.8% (39 of 207), 23.2% (48 of 207), and 20.8% (43 of 207) with

MIC90s of 8, 64, ≥ 64 , and ≥ 128 microg/ml, respectively. The methicillin-resistant *S. aureus* (MRSA) strains exhibited resistance to more antibiotics than did the methicillin-susceptible strains, and 87.5% (35 of 40) of the MRSA strains carried the *mecA* gene sequence. Since MRSA infections have become a public health concern in both communities and hospitals, testing for the presence of MRSA in animal carcasses during slaughtering operations is warranted.

Zhou Q et al. (2007) reported that evaluate the role of interleukin-6 (IL-6) in arthritis induced by *Staphylococcus aureus*, a chicken model was developed for study. A total of 120 healthy broilers (8 wk old) were randomly divided into 4 groups. Two groups were injected with 0.35 mL of *Staph aureus* (7.1×10^9 cfu/mL) into the right hock joints and the other 2 were injected with 0.35 mL of sterile saline into the same joints. One group of each of the 2 treatment groups was fed levofloxacin at a dose of 5 mg/kg of BW on the third day postinoculation for 4 successive days. There was a strong correlation ($r=0.91$) between serum chIL-6 activities by the B9 bioassay and serum IL-6 concentrations determined by the human IL-6 ELISA. We concluded that chIL-6 is involved in the progression of chicken arthritis induced by *Staph aureus*, and that it contributes to disease incidence and mortality.

Pepe O et al. (2006) studied that we examined the presence of *Staphylococcus aureus* and staphylococcal enterotoxin A (SEA) in 20 industrial breaded chicken products obtained from different retail butchers and supermarket stores in Italy. The levels of contamination in the products analyzed were quite low, although the pH values and water activities a_w in the samples considered were in ranges favorable for *S. aureus* growth. As demonstrated by phenotypic and molecular characterization, in spite of the high percentage of coagulase-positive

Staphylococcus strains, only three strains could be referred to the species *S. aureus*. Moreover, all the strains were negative in PCR assays targeting staphylococcal enterotoxin genes (seA to seE, seG to seJ, and seM to seO), as well as the toxic shock syndrome toxin 1 gene, and no SEA was detected in the retail breaded chicken samples analyzed by a reversed passive latex agglutination assay or by Western blotting. Hence, we evaluated the thermal resistance of two strains of SEA-producing *S. aureus* in a laboratory-scale preparation of precooked breaded chicken to see if the thermal process used during their manufacture can limit staphylococcal contamination but cannot eliminate preformed toxins.

Rodgers JD et al. (2006) this study aimed to develop an enzyme-linked immunosorbent assay to detect antibody associated with *Staphylococcus aureus* that is produced during the chicken immune response to this organism. The protein nuclease was tested for suitability as an antigen to detect antibody in sera from broiler chickens that had been exposed to aerosolized *S. aureus* on day 1 post hatch during experiments to reproduce bacterial chondronecrosis and osteomyelitis. The influence of CAV and IBDV on the number of chickens with positive levels was most prominent in chickens aged up to 42 days. The study showed that nuclease-specific antibodies form part of the humoral immune response in broiler chickens that have been exposed to *S. aureus*. Co-infection with CAV and IBDV appeared to promote development of antibody in birds younger than 42 days; however, the presence of antibody did not necessarily prevent systemic infection.

Lowder BV et al. (2006) studied that *Staphylococcus aureus* is a major human pathogen associated with nosocomial and community-acquired infections, and is also responsible for several economically important infections of livestock. However, the evolutionary origin of animal strains and the potential for cross-species transmission has not been well

examined. We recently traced the origin of a common *S. aureus* clone which is a significant cause of morbidity in the global broiler poultry industry. We provided evidence that it evolved from a single human to poultry host jump which was followed by extensive genetic diversification including acquisition of novel mobile genetic elements and loss of virulence gene function. The clone has since been disseminated widely to several different continents presumably through globalization of the poultry industry. In the current article, we summarise the findings of the paper, discuss their implications and speculate on the potential for other *S. aureus* cross-species transfer events.

Vadari Y *et al.* (2006) reported that Poultry litter extracts were plated onto brain-heart infusion medium (BHI) containing an additional 0.75 mol l⁻¹ phosphate (BHI-P). Colonies were screened for the presence of inclusion granules with five being selected for further study. All strains displayed identical biochemical characteristics consistent with *Staphylococcus spp.* and grouped with *Staphylococcus spp.* by comparative 16S rDNA analysis. Thus all five strains were identified as such. All strains displayed elevated intracellular phosphate levels when cultured in BHI-P broth (0.417-0.600 microg phosphate mg⁻¹ protein) vs BHI broth (0.075-0.093 microg phosphate mg⁻¹ protein). Poultry litter contains novel *Staphylococcus spp.* capable of robust growth when exposed to phosphate levels comparable with that typically found in poultry litter. Data suggest intracellular phosphate levels in these strains increase in response to increasing phosphate in the medium or austere medium conditions. Intracellular phosphate did not reach levels comparable with known hyper-accumulating micro-organisms.

El-Sayed A *et al.* (2005) reported that the present study was designed to comparatively investigate 19 *Staphylococcus aureus* strains isolated from

specimens of 19 different birds during routine microbiological diagnostics. The *S. aureus* strains were characterized genotypically by polymerase chain reaction (PCR) amplification using 62 different oligonucleotide primers amplifying genes encoding staphylococcal cell surface proteins, exoproteins and two classes of the accessory gene regulator agr. In addition, all tested strains were positive for the genes hla and fnbA and negative for the genes seb, sec, sed, see, sej, tst, eta and etb. The remaining genes, including sbi, hlb, fnbB, ebpS, cna (domains A and B), cap5, cap8, set1, agr class I, agr class II, sea, seg, seh and sei were detected in a variable number of isolates. The presented data give an overview on the distribution of virulence determinants of *S. aureus* strains isolated from birds. This might be useful to understand the role of these virulence determinants in bird infections.

Kitai S *et al.* (2005) reported that a total of 444 samples of raw chicken meat (thighs, breasts, wings, livers, gizzards, hearts and ovaries) that retailed at 145 different supermarkets in 47 prefectures in Japan were examined for contamination with *Staphylococcus aureus* in association with its enterotoxigenicity. *S. aureus* was isolated from 292 (65.8%) of the samples, and from 131 of the 145 supermarkets Staphylococcal enterotoxins (SEs) produced were SEB (50 isolates), SEA (14), SEC (8), SED (2), SEA+SEB (2), and SEA+SEC (2). Most of the enterotoxigenic isolates belonged to the human and poultry biotypes, coagulase type VII, VIII or IV, and were lysed by phages of group III. The three patterns were found in isolates of samples that retailed at 17 supermarkets in 11 prefectures, indicating that they may be disseminated among raw chicken meat in Japan.

Kishida N Takeuchi S *et al.* (2004) H9N2 influenza viruses are frequently isolated from chicken meat and bone marrow imported from

China to Japan since 2001. These isolates were experimentally inoculated into specific pathogen-free chickens intranasally. Viruses were recovered from the meat and bone marrow of birds showing no overt signs. On the other hand, chickens co-infected with H9N2 virus and either *Staphylococcus aureus* or *Haemophilus paragallinarum* showed clinical signs severer than those shown by birds infected only with the virus alone or each of the bacteria alone. In addition, H9N2 viruses were more efficiently recovered from the chickens co-infected with *S. aureus* or *H. paragallinarum* than those from the birds infected with only the virus. The present results indicate that co-infection of H9N2 influenza virus with *S. aureus* or *H. paragallinarum* enhances the replication of the virus in chickens, resulting in exacerbation of the H9N2 virus infection. The xylanase dose of 1.8 U g⁻¹ moisture free pulp, exhibited bleach boosting of kraft pulps optimally at pH 9.5-10.0 and 50 degrees C after 4 h of reaction time. Pretreatment of pulp with xylanase and its subsequent treatment with 8% hypochlorite, reduced the kappa number by 30%, enhanced the brightness and viscosity by 11% and 1.8%, respectively, and improved the paper properties such as tensile strength and burst factor up to 10% and 17%, respectively.

Capita R et al. (2002) studied that ninety-six *Staphylococcus aureus* isolates from retail chicken carcasses in Spain were characterized using cultural and biochemical tests. The strains were phage typed with the international bacteriophage set for typing *S. aureus* of human origin. Eighty-eight (91.7%) strains were of the poultry ecovar. Strains of human ecovar were not found. These facts are congruent with findings of other authors. Ninety (93.7%) strains were phage type able. Lyses by phages of Group III were the most frequent with 66 (68.7%) sensitive strains. More than one *S. aureus* phage type was detected in 14 (35%) carcasses, which emphasizes the convenience of subtyping several *S. aureus* isolates from the same food sample in epidemiological studies. The *S. aureus* isolates

were closely related, as 78 strains showed the most common or indistinguishable (<2 phage reaction differences) phage patterns.

Eremin VI et al. (2002) studied that the data on the biological properties of the culture fluid of *Streptococcus* strain sp. TOM-1606 are presented. The native preparation has been shown to possess the capacity for stimulating the rate of the clearance of the peritoneal cavity of mice from *Staphylococcus aureus* cells, strain MT-1, rif. r., found to be insensitive to the action of the above-mentioned preparation in vitro. The crude preparation produces a transitory bacteriostatic effect on the streptococcal and staphylococcal strains under study. The preparation produces a prolonged bacteriostatic effect only on *Micrococcus luteus* test strain. All these data suggest that the crude preparation contains at least two active principles.

Takeuchi S et al. (2002) reported that recently whole genome sequencing of *Staphylococcus aureus* has revealed the genes encoding cysteine proteases such as staphopain and SspB. In this study, we cloned and sequenced the structural gene (ScpA) encoding a cysteine (thiol) protease of *S. aureus* strain CH-91 from a chicken with dermatitis using polymerase chain reaction (PCR) and inverse PCR methods. Which is not found in the published whole genome sequences of *S. aureus*. In immunoblot, PCR, and Southern blot assays, the ScpA or its gene was detected in high protease-producing strains from chickens, but was not recognized in bovine and porcine strains or low protease-producing avian strains. These results indicate that the ScpA of CH-91 type may be specific to the high protease-producing strains of *S. aureus* from chickens, namely, there is a strain specificity of the ScpA.

Hazariwala A et al. (2002) studied that food poisoning by *Staphylococcus aureus* affects hundreds of thousands of people each year. *Staphylococcus aureus* also causes invasive diseases such as arthritis (in poultry) and septicemia (in poultry and humans). Food borne disease is caused by the ingestion of a staphylococcal enterotoxin (SE). Enterotoxin has also been associated with other *S. aureus* illnesses in humans and domestic animals. In this study, polymerase chain reaction was used to detect the staphylococcal enterotoxin genes, SEA, SEB, SEC, SED, and SEE, in *S. aureus* isolates associated with invasive disease in poultry and humans. In the 34 poultry isolates, only one isolate was found to contain a SE gene, sec. In the 41 human isolates, over 51% tested positive for an SE gene with 12.2% positive for the gene for SEA, 2.4% for SEB, 22% for SEC, 24.4% for SED, and 0 for SEE. The disparity between the rates for SE gene(s) in poultry and human isolates suggests a lesser role for the enterotoxins in invasive poultry disease than in human disease.

Stedman NL et al. (2001) Avian leukosis virus subgroup J has a high tropism for myeloid lineage cells and frequently induces neoplastic transformation of myelocytes. The impact of congenital avian leukosis virus subgroup J infection on the function of circulating heterophils and susceptibility to staphylococcal infection was investigated. Six-week-old broiler chickens negative for exogenous avian leukosis viruses or congenitally infected with avian leukosis virus subgroup J were inoculated intravenously with 10(6) colony-forming units of *Staphylococcus aureus*, and pre- and postinoculation heterophil function was assessed. Osteomyelitis in the tibiotarsus or tarsometatarsus developed in 5/10 (50%) of the chickens in each group. *S. aureus* was recovered from the hock joint of 6/10 (60%) of the chickens in each group. Heterophils isolated from broiler chickens congenitally infected with avian leukosis virus subgroup J exhibit no significant functional

deficits, and infected and uninfected chickens exhibit similar susceptibility to staphylococcal infection.

Mutalib A et al. (2001) studied that groups of 6-week-old broiler chickens were exposed to *Staphylococcus aureus* by aerosol, intratracheal or intravenous (control) inoculation. No lesions were produced by aerosol. A single dose of up to 5×10^{10} organisms given intratracheally failed to produce any lesions. Single or repeated doses of 5×10^{11} organisms given intratracheally resulted in osteomyelitis in a low proportion of chickens.

X. Y. Zhu et al. (2001) conducted an experiment with broilers to determine if prior exposure to *Staphylococcus aureus* would facilitate the systemic infiltration of this pathogen following intradermal footpad challenge with live *S. aureus*. Litter-raised broilers were sensitized at 3 and 4 wk of age with s.c. injections in the neck with heat-killed *S. aureus* diluted in polyethylene glycol (PEG). Equal numbers of control birds were injected at the same times with PEG. At 7 wk of age, chicks previously sensitized to killed *S. aureus* or injected with PEG were injected intradermally in the right footpad with PBS or live *S. aureus*. The left footpads of all birds were injected with PBS. The difference in thickness between the right and left footpads was determined at 0, 24, and 48 h postchallenge. Blood, liver, spleen, lung, and synovial fluid were collected six times between 1 and 48 h postchallenge to *Staphylococcus aureus*, systemic infiltration, footpads, broilers, chickens.

Butterworth A et al. (2001) reported that a study was conducted to investigate the relationship between the presence of localized bacterial infection and lameness in broiler fowl (*Gallus gallus domesticus*). Isolation of bacteria from the proximal femur, proximal tibia, and

tibiotarsus from broilers with lameness revealed a probable association between lameness and the presence of *Staphylococcus aureus*. Other potential pathogens, including *Escherichia coli* and DNase-negative staphylococci, were also isolated from sound and lame birds, and their association with pathologies causing lameness was less well defined. After trials with a set of twenty 10-base oligonucleotide primers, a pair of primers giving optimal performance was selected. The *S. aureus* isolates were typed by random amplification of polymorphic DNA (RAPD) by using the pair of 10-mer primers, and groupings were defined by banding patterns after agarose gel electrophoresis. The putative RAPD groupings may provide a basis for epidemiological studies of *S. aureus* in broiler production systems.

McNamee PT *et al.* (2000) reported that bacterial chondronecrosis with osteomyelitis (BCO) in chickens was first reported in 1972 and is now recognized as an important cause of lameness in broiler chickens. Recent systematic studies of causes of lameness in birds reared in Northern Ireland have shown that it was the most common cause of lameness, being present in 17.3% of lame birds. Furthermore, it was also detected in birds presented as "found dead". Overall losses in male birds due to BCO were estimated to be 0.75% of all birds placed, which, in addition to welfare concerns, represents considerable economic loss. The disease has been seen in birds ranging from 14 to 70 days of age, but most cases occurred around 35 days old. It is most commonly caused by *Staphylococcus aureus*, but *Escherichia coli*, coagulase-negative staphylococci and *Enterococcus spp.* Furthermore, more than one type of bacterium is capable of causing the disease. The recent development of a disease model in which *S. aureus* is given by a natural route allows the potential for further investigation of the role of predisposing factors, and intervention strategies, including vaccination and bacterial interference, for the prevention of BCO.

Nawaz MS *et al.* (2000) The *ermA*, *ermB*, *ermC* and *msrA/msrB* genes were detected in multidrug-resistant *Staphylococcus spp.* strains by PCR. Among 25 human clinical staphylococcal isolates the *ermA*, *ermB*, *ermC* and the *msrA/msrB* genes were detected in 88, 72, 4 and 100% of the strains, respectively. Among 24 poultry isolates the *ermA*, *ermB*, *ermC* and the *msrA/msrB* genes were detected in 100, 16.6, 50 and 12.5% of the strains, respectively. The *ermA* gene was found exclusively on the chromosome, whereas the *ermC* gene was found on 2.4-4.2 kb plasmids. Restriction fragment length polymorphism (RFLP) analysis of the *ermA* gene with *Eco* RI revealed five patterns (25.0, 21.0, 10.5, 6.2 and 4.8 kb) for the clinical strains and two (8.0 and 6.2 kb) for the poultry strains. The 6.2 kb RFLP pattern, in both the poultry and human clinical isolates, indicates a common lineage for the *ermA* gene.

Awan MA *et al.* (1998) reported that in broiler operations, various health problems develop during the final 2 wk of the growing period, resulting in increased mortality and condemnation losses. At this stage, sickly birds were found to be systemically infected by various bacteria regardless of varied clinical signs, and the purpose of this study was to carry out thorough microbiological investigations on this problem. Thirty-one 6-wk-old broilers showing signs of illness were obtained from three farms, and bacterial isolations were carried out from the blood, liver, and hock joint. Bacteria were isolated from 87, 90, and 71% of the blood, liver, and hock joint samples, respectively. Mean bacterial counts in log₁₀ of the blood (per milliliter) and liver (per gram) were 2.15 and 2.93, respectively. These results indicate that systemic infections in addition, six species of gram-positive and five species of gram-negative organisms were isolated. These results indicate that systemic infections in market age broilers are caused by mixed bacterial species and suggest that they are caused by suppressed host antibacterial systems rather than pathogenic factors of microorganisms.

Andreasen JR Jr et al. (1993) reported that heterophil chemotaxis, in response to chemotactic factors generated by three different strains of staphylococcal bacteria, was measured using the modified Boyden-chamber technique. Heterophils were obtained from healthy 6-to-8-week-old broiler chickens. Each bacterial strain generated factors that were chemotactic for chicken heterophils. Factors generated by two pathogenic isolates of *Staphylococcus aureus*, however, induced significantly greater chemotaxis in chicken heterophils than those generated by a nonpathogenic *Staphylococcus* isolate.

Daum RS Stedman NL et al. (1990) studied that the occurrence, magnitude, and kinetics of bacteremia and the resultant osteomyelitis and septic arthritis in an avian model of *Staphylococcus aureus* infection. Thirty-day-old male broiler chicks were inoculated i.v. with 10(5), 10(6), or 10(7) cfu of strain Duntravis, a beta-hemolytic, coagulase-producing, capsular type 8 isolate from the synovial fluid of a 2-year-old black boy. Bacteremia occurred in 80%, 90%, and 100% of animals inoculated with 10(5), 10(6), or 10(7) cfu, respectively. The occurrence of osteomyelitis was uniform among continuously bacteremic animals and developed 1 to 23 hours after inoculation. Chickens are susceptible to systemic infections with *S. aureus*. Bacteremia, osteomyelitis, and septic arthritis may be induced in healthy chickens without prior manipulations that depress their resistance. **Hill JE et al., (1989)** reported that Experimental inoculation of 1-day-old male broiler chickens with avian reo-virus or *Staphylococcus aureus* caused tenosynovitis of the gastrocnemius and digital flexor tendons. Reovirus inoculation by either the oral or footpad route initiated a diffuse lymphocytic infiltration in the peritendineum, synovial membrane, and epitenon from 1 to 5 weeks postinoculation (PI). Heterophils were not a predominant feature of the inflammatory response, but when present they were localized with fibrin in and around synovial

spaces. The prevalence of microscopic tendon lesions was less common with staphylococcal infection than with reovirus infection. These results indicate that tenosynovitis due to inoculation with reovirus or staphylococcus may be differentiated histologically from 1 through 5 weeks PI. After 10 weeks, this may not be possible, because diffuse fibrosis was the major lesion with both. Perhaps fibrosis predisposes older, heavier broilers to tendon failure and rupture.

Kibenge FS et al. (1982) stated that Bacteriophage typing of *Staphylococcus aureus* from different outbreaks of tenosynovitis in broiler breeder replacement chickens showed that, although a mixture of phage types was present on affected farms, there was a predominant phage type isolated from lesions of affected chickens. The predominant phage type isolated from chickens in different outbreaks was variable. The source of *S. aureus* associated with tenosynovitis appeared to be a resident population present on the skin.

Kibenge FS et al. (1982) reported that the pathology and infectious agents associated with several outbreaks of clinical tenosynovitis in poultry were investigated. *Staphylococcus aureus* were recovered from tendon tissue of from 51.9% to 97.8% of affected chickens in different outbreaks of the disease. *S. aureus* were also present in the liver and heart blood and were considered to contribute to the high mortality observed following occurrence of clinical tenosynovitis in individual chickens. Bacteria other than *S. aureus* were also recovered but in a much lower proportion of chickens than was *S. aureus*. Adenoviruses and reoviruses were also recovered from tendon tissue of chickens with a concurrent bacterial infection. Although the pathology observed in clinically affected chickens was suggestive of a bacterial a etiology it is hypothesised that the tenosynovitis outbreaks investigated were due to an early viral infection which normally remained subclinical and that secondary

infection with bacteria, particularly *S. aureus*, was responsible for the development of clinical signs.

Köhler B et al. (1980) studied that day-old chicks were subcutaneously infected with *Staphylococcus aureus* strains of site variety gallinae and crystalviolet Type A. The lysis patterns involved were with phages 84 as well as with phages 53, 77, and 84 of the international base scheme for human staphylococci. In fowl with synovitis and arthritis, osteomyelitis, dermatitis, and septicaemia, 52 of 57 tested strains (91.2 per cent) produced on the site of injection pathognomic oedematous-haemorrhagic and necrotising changes of the skin and hypodermis (hemorrhagic dermatitis) in a way which is characteristic also of natural diseases. were tested subcutaneously, on day-old chicks, among them 21 strains of site varieties of bovis, canis, ovis, and hominis. However, only four of those strains, isolated from miscarried pig foetuses and cattle with mastitis, caused, oedematisation and exudation (7.02 per cent), but no haemorrhagia and only minor lysis and necrosis of skin. The need for delimitation of such strains has been confirmed by the above experimental infections. Against the background of the above results, guidelines are proposed for the control of *Staphylococcus aureus* infection of fowl.

Bergmann V et al. (1980) reported that *Staphylococcus aureus* infection has become an increasingly grave problem in industrialized poultry farming. This first communication in a series of publications on highly involved studies and experiments describes manifestations of the most common staphylococcal infections in fowl, including synovitis with arthritis, osteomyelitis, dermatitis, endocarditis, septicaemia, wound infection, and ophthalmitis. Reference in greater detail is made to manifestations with more important economic consequences, such as synovitis with arthritis, osteomyelitis, and dermatitis. Their frequency,

dependence on age, clinical patterns, pathomorphology, pathogenesis are expounded together with peculiarities of differential diagnosis, with reference being made to findings obtained by the authors from their own studies on three industrialised poultry units

Terzolo HR *et al.* (1979) reported that during 1976-78, 33 *S. aureus* strains were isolated from chickens suffering from dermatitis and septicemia, omphalitis, pneumonia, arthritis and tenosynovitis; and from a commercial balanced chicken food. All the strains were classified into the biotype B of Hájek and Marsálek. Twenty-nine (87.8%) strains could be classified into Sato *et al* biological type 4 although they differed from it in its ability to grow at 45 degrees C. The remaining 4 strains differed from the others because they failed in producing hemolysis in bovine and ovine blood agar, egg yolk reaction and splitting of Tween 80. Thirty (90.9%) strains were lysed by avian phages: 28 strains at 1 x RTD and 2 at 100 x RTD. The biological characters and bacteriophage typing of the *S. aureus* showed that almost all the strains belong to chicken types. The strains isolated from food belong to the same chicken biotype and phagetype as the strains isolated from dermatitis, septicemia, omphalitis and pneumonia. The strains isolated from arthritis and tenosynovitis was different from the ones isolated from other pathologies.

2.2. Antibiotic sensitivity and resistance pattern.

Graham JP *et al.* (2009) stated that the use of antimicrobials in commercial broiler poultry production results in the presence of drug-resistant bacteria shed in the excreta of these birds. Because these wastes are largely land-disposed these pathogens can affect the surrounding environment and population. In this analysis, we characterized the survival of antimicrobial-resistant enterococci and staphylococci and resistance genes in poultry litter. . persisted throughout the 120-day study

period. Resistance genes identified in the study include: erm(A), erm(B), erm (C), msr(A/B), msr(C), and vat(E). This study indicates that typical storage practices of poultry litter are insufficient for eliminating drug-resistant enterococci and staphylococci, which may then be released into the environment through land disposal.

Graham JP et al. (2009) studied that use of antibiotics as feed additives in poultry production has been linked to the presence of antibiotic resistant bacteria in farm workers, consumer poultry products and the environs of confined poultry operations. There are concerns that these resistant bacteria may be transferred to communities near these operations; however, environmental pathways of exposure are not well documented. We assessed the prevalence of antibiotic resistant enterococci and staphylococci in stored poultry litter and flies collected near broiler chicken houses were found in isolates recovered from both poultry litter and flies. Erm(B) was the most common resistance gene in enterococci, while erm(A) was the most common in staphylococci. We report that flies collected near broiler poultry operations may be involved in the spread of drug resistant bacteria from these operations and may increase the potential for human exposure to drug resistant bacteria.

Persoons D et al. (2009) Methicillin-resistant *Staphylococcus aureus* (MRSA) has been detected in several species and animal-derived products. To determine whether MRSA is present in poultry, we sampled 50 laying hens and 75 broiler chickens. MRSA was found in some broiler chickens but no laying hens. In all samples, spa type t1456 was found.

Miranda JM et al. (2008) the presence of *Escherichia coli*, *Staphylococcus aureus*, and *Listeria monocytogenes* was determined in 55 samples of organic poultry meat and in 61 samples of conventional

poultry meat. A total of 220 *E. coli*, 192 *S. aureus*, and 71 *L. monocytogenes* strains were analyzed by an agar disk diffusion assay for their resistance to ampicillin, cephalothin, chloramphenicol, ciprofloxacin, doxycycline, fosfomycin, gentamicin, nitrofurantoin, streptomycin, and sulfisoxazole (*E. coli*); chloramphenicol, ciprofloxacin, clindamycin, doxycycline, erythromycin, gentamicin, nitrofurantoin, oxacillin, and sulfisoxazole (*S. aureus*); and chloramphenicol, doxycycline, erythromycin, gentamicin, sulfisoxazole, and vancomycin (*L. monocytogenes*). The results indicated a significantly higher ($P < 0.0001$) prevalence of *E. coli* but not of *S. aureus* and *L. monocytogenes* in organic poultry meat as compared with conventional poultry meat. In the case of *E. coli*, the presence of multiresistant strains was significantly higher ($P < 0.0001$) in conventional poultry meat as compared with organic poultry meat. Organically farmed poultry samples showed significantly lower development of antimicrobial resistance in intestinal bacteria such as *E. coli*.

Nemati M, et al. (2008) reported that the susceptibilities of 12 antimicrobial agents for two collections of *Staphylococcus aureus*, isolated in the 1970s and in 2006 from poultry, were determined. For eight antibiotics, the percentage of resistance was significantly higher in the recent isolates. Ten recent isolates were methicillin resistant and had spa types t011 and t567, belonging to multilocus sequence type 398. This is the first report of "livestock-associated" methicillin resistant *S. aureus* from healthy poultry.

Lee JH et al. (2006) studied from 2001 to 2005, various specimens from cattle, pigs, and chickens were collected and examined for the presence of methicillin (oxacillin)-resistant *Staphylococcus aureus* (MRSA). The isolates from 19 specimens were tested for the presence of the *mecA* gene. Methicillin resistance was confirmed by determining the MICs for these isolates. Among these 19 *mecA*-positive isolates, 16 were

consistently found to be resistant to methicillin. The *mecR1* gene was found in all 19 *mecA*-positive *S. aureus*, and *mecI* was also detected in 15 of the *mecA*-positive *S. aureus*. In all 15 *mecI*-positive MRSA, the sequence of the *mec* promoter/operator region was identical to the reference sequence. This suggests other mechanisms for overcoming the repression of resistance caused by *mecI*, beyond the simple product interaction between the *mecA*, *mecRI*, and *mecI* genes.

White DG et al. 2003) stated that *Staphylococcus aureus* is an important opportunist that can cause superficial to life-threatening illnesses in a variety of animal species. In poultry, this organism has been implicated in osteomyelitis, synovitis, and cellulitis. Whereas most infections can be treated with antibiotics, because of the organism's propensity to acquire antimicrobial resistance, it is important to continually monitor antibiotic susceptibilities of clinical isolates. We surveyed 77 clinical poultry *S. aureus* isolates, collected from 1998 to 2000, for susceptibilities to a panel of 18 antimicrobial agents *Staphylococcus aureus* isolates were commonly resistant to tetracycline (40%; minimal inhibitory concentration [MIC]₉₀ > 32 microg/ml), lincomycin (19%; MIC₉₀ > 32 microg/ml), erythromycin (12%; MIC₉₀ > 8 microg/ml), and kanamycin (8%; MIC₉₀ < 128 microg/ml). A periodic assessment of antimicrobial susceptibilities of important avian pathogens like *S. aureus* will be important in helping the clinician's choice of antibiotic to control infection.

Khan SA White DG et al. (2003) reported that the transfer of *erm A* and *ermC* genes, the two most common resistance determinants of erythromycin resistance, was studied with Luria-Bertani broth in the absence of additional Ca(2+) or Mg(2+) ions. Since both the donors (Amp(s)-Tet(r)) and recipients (Amp(r)-Tet(s)) were resistant to

erythromycin, the transconjugants were initially picked up as ampicillin- and tetracycline-resistant colonies. A high frequency of transfer (4.5×10^{-3}) was observed in all of the 23 transconjugants obtained, and the direction of tetracycline and erythromycin resistance marker transfer was determined to be from poultry to clinical isolates. The transfers of the *ermA* and *ermC* genes were via transposition and transformation, respectively.

Zhu XY et al. (2001) conducted an experiment with broilers to determine if prior exposure to *Staphylococcus aureus* would facilitate the systemic infiltration of this pathogen following intradermal footpad challenge with live *S. aureus*. Litter-raised broilers were sensitized at 3 and 4 wk of age with s.c. injections in the neck with heat-killed *S. aureus* diluted in polyethylene glycol (PEG). Equal numbers of control birds were injected at the same times with PEG. At 7 wk of age, chicks previously sensitized to killed *S. aureus* or injected with PEG were injected intradermally in the right footpad with PBS or live *S. aureus*. The left footpads of all birds were injected with PBS. The difference in thickness between the right and left footpads was determined at 0, 24, and 48 h postchallenge. It was concluded that the intradermal challenge of the footpad with *S. aureus* resulted in systemic infiltration of *S. aureus* into the spleen, liver, and blood. Prior exposures to killed *S. aureus* as compared to PEG controls did not affect the systemic distribution of *S. aureus*.

Müller MG et al. (2000) studied that the 1990s, bumblefoot posed a major health problem to the falcons in the United Arab Emirates. This retrospective study based on statistical field research showed that in captivity wild falcons need a training frequency of twice a day to reduce the bumblefoot morbidity rate. In this context, they responded very well to free flight in aviaries during the molting season. In contrast, captive-bred falcons did not show a highly significant difference regarding

training frequencies. Wild falcons tested fed with pigeons, bustards, and ducks suffered significantly less from bumblefoot compared with those fed a diet of quail. Apart from the disease-reducing impact of beef and mice, captive-bred falcons tested did not show any significant difference regarding the influence of diet on the bumble foot occurrence.

Nawaz MS *et al.* (1999) studied that the epidemiology of the two common erythromycin-resistant methylase (*erm*) genes *ermC* and *ermA* was analyzed in 12 coagulase-negative *Staphylococcus spp.* and 34 coagulase-positive *Staphylococcus spp.* isolated from chicken. Southern hybridization indicated that only 2 of the 12 coagulase-negative *Staphylococcus spp.* strains contained the *ermC* gene on the plasmid; 1 strain of *Staphylococcus xylosus* harbored the *ermC* gene on a 2.5-kb plasmid, and 1 strain of *Staphylococcus cohnii* harbored the gene on a 4.0-kb plasmid. Our results indicate that either the *ermC* or *ermA* gene, homologous to those described in human isolates, was present in all avian *Staphylococcus spp.* and that *ermA* was the predominant gene in coagulase-negative and coagulase-positive avian *Staphylococcus spp.* The size and copy numbers of the *ermA* gene were different from its human counterpart.

Rodgers JD *et al.* (1999) studied that personnel from one broiler hatchery, and workers on 18 separate broiler parent farms which supply the hatchery, were tested for hand and nasal carriage of *Staphylococcus aureus*. In both locations, nasal carriage of *S. aureus* was more common than hand carriage. A total of 63 *S. aureus* strains were characterised by biotyping, protein A analysis and pulsed field gel electrophoresis (PFGE) typing. Of these, 36 were recovered from broiler hatchery personnel, 14 from broiler parent farm personnel and 13 from cases of skeletal disease in commercial broilers which is the predominant *S. aureus* strain type

associated with clinical disease in N. Ireland broiler flocks. The present study found no evidence of nasal carriage of *S. aureus* strains of poultry biotype by humans. The finding of hand carriage by broiler parent farm personnel, suggests that handling by personnel may contribute to the dissemination of I. 24 or other *S. aureus* strains associated with skeletal disease in broilers.

Takeuchi S et al. (1999) stated that protease produced by *Staphylococcus aureus*, isolated from a chicken suffering from dermatitis, was purified by successive precipitation with ammonium sulfate, ion-exchange chromatography on Q-Sepharose FF, Sp-Sepharose FF and Mono-Q columns. By Mono-Q column chromatography, two proteases (protease 1 and 2) were obtained. The molecular weights of protease 1 and 2 were estimated at 23.1 and 22.7 kDa, respectively, by SDS-polyacrylamide gel electrophoresis. Their isoelectric points were 5.85 and 5.55, respectively, and they possessed antigenic similarity when examined by the immunoblotting. From the results, it seems likely that these proteases, produced by *S. aureus* from diseased chickens, might belong to the thiol protease group.

Kawano J et al. (1996) reported that Methicillin-resistant coagulase-negative staphylococci were isolated from the nares and skin of 1- to 8-week-old healthy chickens in three flocks from a farm. Isolation of methicillin-resistant coagulase-negative staphylococci was positive for 72 (25.7%) of the 280 chickens tested, with the frequency varying from 2.2 to 100% according to flock. A total of 45 appropriate isolates were selected and subjected to identification. Of the 45 methicillin-resistant coagulase-negative staphylococcal isolates selected, 37 were identified as *Staphylococcus sciuri*, 5 were identified as *Staphylococcus epidermidis*, and 3 were identified as *Staphylococcus saprophyticus*. The distribution of the species was different among the flocks. Comparative analysis of

the SmaI-digested chromosomal DNA by pulsed-field gel electrophoresis revealed that the isolates could have originated from a single clone of each of *S. sciuri* and *S. saprophyticus* and three clones of *S. epidermidis*. By two methods based on the PCR technique, the *mecA* gene was detected in all five representative isolates of each methicillin-resistant coagulase-negative staphylococcal clone. This is the first evidence of the existence of methicillin-resistant coagulase-negative staphylococci from animals possessing the *mecA* gene.

Andreasen JR Jr et al. (1993) studied that heterophil chemotaxis using heterophils isolated from the peripheral blood of five commercial broiler chickens naturally infected with staphylococcal bacteria was compared by the modified Boyden-chamber technique with chemotaxis of heterophils from two chickens from the same flock not infected with *Staphylococcus* (field controls) and from four healthy laboratory control broiler chickens. The infected chickens had gross and histologic lesions of staphylococcal tenosynovitis and osteomyelitis. Staphylococci were isolated from the lesions. Hematologic parameters and histologic lesions of infected chickens also were examined. Compared with field and laboratory controls, *Staphylococcus*-infected chickens had heterophilic leukocytosis. The heterophils of *Staphylococcus*-infected chickens had significantly lower chemotactic activity than both control groups in terms of random movement and directed chemotactic movement in response to stimulus. Toxic changes were observed in heterophils of some of the *Staphylococcus*-infected broilers.

Reece RL et al. (1985) reported that the resistance to anti-microbial agents of bacteria isolated from pathological conditions of birds in Victoria, 1978 to 1983, was determined for isolates of *Escherichia coli*, *Salmonella* species, *Staphylococcus aureus*, *Pasteurella multocida*, *P. anatispestifer*, *Yersinia pseudotuberculosis* and *Haemophilus*

paragallinarum. The isolates of *E. coli* had a high prevalence of resistance to tetracycline and sulphonamides, and a lower prevalence of resistance to furazolidone and sulphamethoxazole-trimethoprim. The isolates of *Salmonella spp* commonly had resistance to tetracycline, sulphonamides, furazolidone and sulphamethoxazole-trimethoprim. Almost half the isolates of *S. aureus* showed resistance to lincomycin and many showed resistance to penicillin. Resistance to tetracycline was found in isolates of *P. multocida*, *P. anatepestifer* and *Y. pseudotuberculosis*. Some isolates of *H. paragallinarum* showed resistance to sulphonamides, streptomycin and sulphamethoxazole-trimethoprim.

Hooshyar P et al. (1982) reported that effects of storage at 5 and -18 C for up to 28 days and high and low inoculation levels on survival of *Salmonella infantis* and *Staphylococcus aureus* on smoked broiler halves were investigated. *S. infantis* and *Staph. aureus* counts were significantly reduced during storage. The reduction in counts of *S. infantis* could not be attributed solely to either temperature or inoculation levels. Storage at 5 C significantly (P less than .05) reduced *Staph. aureus* counts on smoked broiler halves as compared to storage at -18 C. For *Staphylococcus aureus*, low inoculation levels (10^3 to 10^4 organisms/ml) resulted in significantly higher mortality rates than did high inoculation levels (10^7 to 10^8 organisms/ml). At both storage conditions and inoculation levels, *Staph. aureus* was able to maintain higher populations than *S. infantis*.

Hummel R et al. (1979) reported that the ecological and epizootiological aspects relating to *Staphylococcus aureus* in domestic fowl are expounded, on the basis of literature, with particular reference being made to five topics: occurrence of these conditionally pathogenic bacteria in domestic fowl, point of attack and routes of infection, conditions for

possible occurrence of infections, reciprocal infection between man and animal, as well as aspects of therapy and prophylaxis.

Hummel R *et al.* (1979) reported that the ecological and epizootiological aspects relating to *Staphylococcus aureus* in domestic fowl are expounded, on the basis of literature, with particular reference being made to five topics: occurrence of these conditionally pathogenic bacteria in domestic fowl, point of attack and routes of infection, conditions for possible occurrence of infections, reciprocal infection between man and animal, as well as aspects of therapy and prophylaxis. were present in the intestinal tract. Birds with staphylococcal synovitis had higher numbers in all sampling regions. The counts were very high in subjects suffering from *staphylococcal dermatitis*. Higher rates of positive birds and higher numbers of staphylococci were detected with a whole body sampling method than with a nasal swab technique. The isolates obtained in this study belonged to the *S. aureus* biotype B which is associated with poultry and pigs.

CHAPTER 3

MATERIALS AND METHODS

CHAPTER -3

MATERIALS AND METHODS

The present study was carried out at the Department of Microbiology, Hajee Mohammad Danesh Science and Technology University during the period from March 2011 to August 2011. The detailed outline of the Materials and Methods are given bellow.

3.1. Materials

3.1.1 Selection of Study area

This study was conducted during the period from March to August 2011 at Sajahanpur of Bogra District. The sample were collected from the selected breeder birds of the breeder farm and brought to the Department of Microbiology HSTU and quality breeders' laboratory for analysis.

3.1.2 Farm Selection and observations

“Quality Breeder Farm and hatchery” at Sajahanpur in Bogra district was selected for study areas because this farm is located near to QBL laboratory and it was easy to transport the sample to the laboratory. It was also advantagious for close supervision and monitoring of the flock. Proper management practice was maintained in the farm. The breed was Hubbard classic. No antibodies were used in the selected flocks for the prevention of any diseases.

3.1.3 Experimental birds

The birds were divided into the three groups categorized as group A, B and C on the basis of their age by following way –

Group-A: 0-14weeks

Group-B: 15-24 weeks

Group-C: Above 25 weeks

3.1.4 Collection of Sample

Sample collected from the Quality breeder farm. Collecting sample was pus&synovial fluid from different group of diseased broiler breeder bird. Thus the total number of sample is 122.

3.1.5. Bacteriological media

3.1.5.1 Nutrient agar

28 grams nutrient agar (Hi-media, India) powder was added to 1000 ml of distilled water in a flask and the mixture was heated by boiling to dissolve the medium completely. The medium was then sterilized by autoclaving at 121°C allowed them to cool to temperature of 45°C in a water bath. After cooling the medium was then distributed to sterile Petri dishes and allowed to solidify. After solidification of the medium, the plates were allowed to incubation at 37°C for over night to check their sterility and then stored in a refrigerator for future use.

3.1.5.2. Nutrient broth

Thirteen grams of dehydrated nutrient broth (Hi-media, India) was dissolved into 1000 ml of distilled water and heated to boiling to dissolve it completely. The solution was then distributed in tubes, stopper with cotton plugs and sterilized in autoclave at 121°C and 1.2 kg/cm² pressure for 15 minutes. The sterility of the medium was checked by incubating at 37°C for overnight and stored at 4°C in a refrigerator for future use

3.1.5.3. Blood Agar (BA) medium

Fourty grams BA base (Hi-media, India) powder was added to 1000 ml of distilled - water in a flask and heated until boiling to dissolve the medium completely. The medium was then sterilized by autoclaving at 121°C maintaining a pressure of 15pound/sq inch for 15 minutes. The autoclaved materials were recommended allowed to cool to a temperature of 45°C in a water bath. Defribinated 5% blood was then added to the medium aseptically and distributed to sterile Petridishes and allowed to solidify. After solidification of the medium, the plates were allowed to incubation at 37°C for over night to check their sterility and then stored in a refrigerator for future use.

3.1.5.4. Mannitol Salt Agar

Contains mannitol as a fermentable carbohydrate, the indicator phenol red for detection, and a high concentration of salt to inhibit undesired organisms. It has been observed that coagulase positive staphylococci grew luxuriantly, producing yellow-gold colonies with yellow zones. Nonpathogenic staphylococci produce small colonies with no color change of the surrounding medium. Other bacteria are generally inhibited, making possible the use of a heavy inoculum without danger of overgrowth. Incubation for 24-36 hours at 37C is recommended.

3.1.5.5. Staphylococcus Agar No. 110

Selective medium for the isolation of pathogenic staphylococci.

Preparation

Suspend 149.5 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Sterilize in autoclave at 121°C for 15 minutes. Cool to 45-50°C, Mix well and dispense into plates. The prepared

medium should be stored at 8-15°C. The color is amber, slightly opalescent. The dehydrated medium should be homogeneous, free-flowing and clear in color

3.1.6. Bio-chemical media

3.1.6.1. Triple Sugar Iron (TSI) agar slant

A quantity of 6.5 grams of Bacto TSI agar (Oxoid, England) was suspended in 1000 ml of distilled water and heated to boiling to dissolve the medium completely. The solution was then distributed in tubes plugged with cotton plugs and sterilized in an autoclave at 121°C at a pressure of 15-pounds/sq. inch for 15 minutes. After sterilization, the tubes were kept in slanting position in such a manner so as to allow a generous butt after solidification. The tubes were then incubated at 37°C for overnight to check their sterility and then stored in a refrigerator for future use.

3.1.6.2. Methyl-Red Voges-Proskauer (MR-VP) broth

A quantity of 3.4 grams of Bacto MR-VP medium was dissolved in 250 ml of distilled water dispensed in 2ml amount in each test tube and then the tubes were autoclaved at 121°C maintaining a pressure of 15 pounds/sq. inch for 15 minutes. After autoclaving, the tubes containing medium were incubated at 37°C for overnight to check their sterility and then stored in a refrigerator for future use.

3.1.6.3. MIU (Motility, Indole, urea) medium

A quantity of 18 gram MIU medium was suspended in 950 ml of distilled water and heated for boiling to dissolve the medium completely. Dispense in 95 ml amounts into flask and sterilize by autoclaving. Cool to about 50-55°C and aseptically add 5 ml sterile 40% urea solution per 95 ml basal medium. Mix well and dispense into sterile test tubes. Allow cooling in an upright position. The tube was then incubated at 37°C for 24 hours to check the sterility.

3.1.6.4. Indole test

Two ml of peptone water was inoculated with 5 ml of bacterial culture and incubated for 48 hours. 0.5 ml of Kovacs reagent was added, shaken well and examined after 1 minute. A red color in the reagent layer indicated indole positive. In negative case there is no development of red color.

3.1.6.5. Methyl red solution

The indicator phenyl red solution was prepared by dissolving 0.1 gm of bacto methyl red in 300 ml of 95% alcohol and diluting to 500 ml with the addition of 200 ml distilled water. The test was performed by inoculating a colony of the test organism in 0.5 ml sterile glucose phosphate broth. After overnight incubation at 37°C, a drop of methyl red solution was added. A positive methyl red solution was shown by the appearance of the bright red color, indication of acidity. Yellow or orange color indicates negative test.

3.1.7.1 Appliances

The different types of glass ware and appliances used for the experiment were as follows: Test-tube, Petri dishes, conical Flask,

pipette, slides, microscope, cotton, immersion oil, water bath, thermometer, incubator, ice boxes, balance, syringe and needle, centrifuge tube, centrifuge machine, vials, micropipette, stirrer sticks, stop watch, scissors, scalpel, blade and handle, hand gloves, spirit lamps, bacteriological loop.

3.1.7. 2. Reagents and Chemicals

The reagent and chemicals used in this study were Methyl red, Phenol red, Gram's Stain, alcohol, spirit, liquid paraffin wax and other selective media.

3.1.7.3. Antibiotic discs

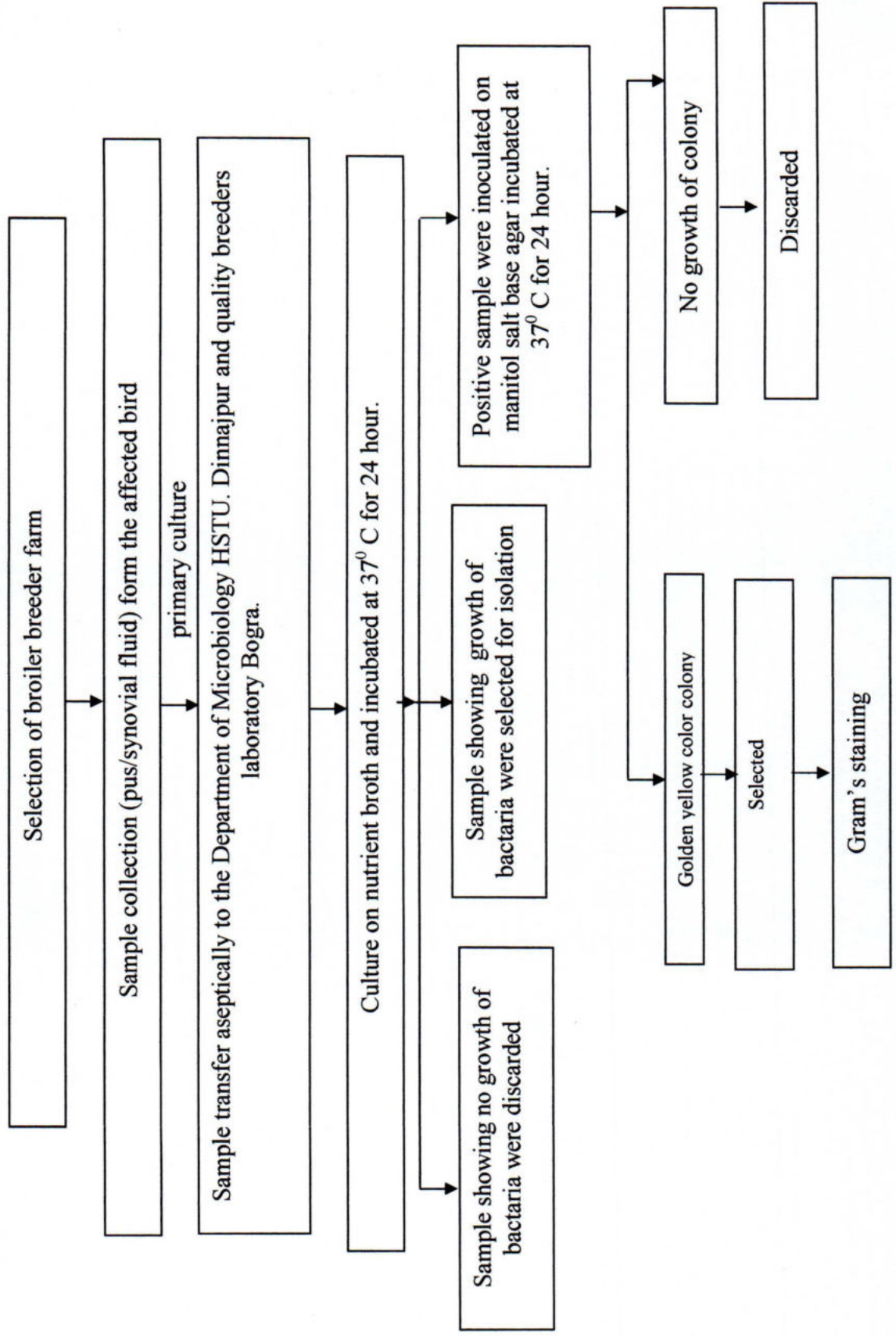
To determine the drug sensitivity pattern of different bacterial isolates with different types of antibiotics. Commercially available antibiotic discs (Oxioid Ltd., UK) were used. The method allowed for the determination of the drug by measuring the diameter of the zone of inhibition that results from different diffusion of the agent into the medium surrounding the disc. The followings are the antibiotics that were tested against the selected organism with their disc concentration.

Table 1: Antibiotics with their disc concentration

Sl. No.	Name of the antibiotics	Disc concentration (µg/disc)
1	Amoxicillin (AML)	10
2	Ciprofloxacin (CIP)	5
3	Chloramphenicol (C)	30
4	Nalidixic Acid (NA)	30
5	Gentamicin (GN)	30
6	Streptomycin (S)	10
7	Cephadrin (CN)	30
8	Cephalexin (CL)	30
9	Tetracycline (TE)	10
10	Kanamycin (K)	30

3.2. METHODS OF ISOLATION

3.2.1. The experimental design of the present study is given below:



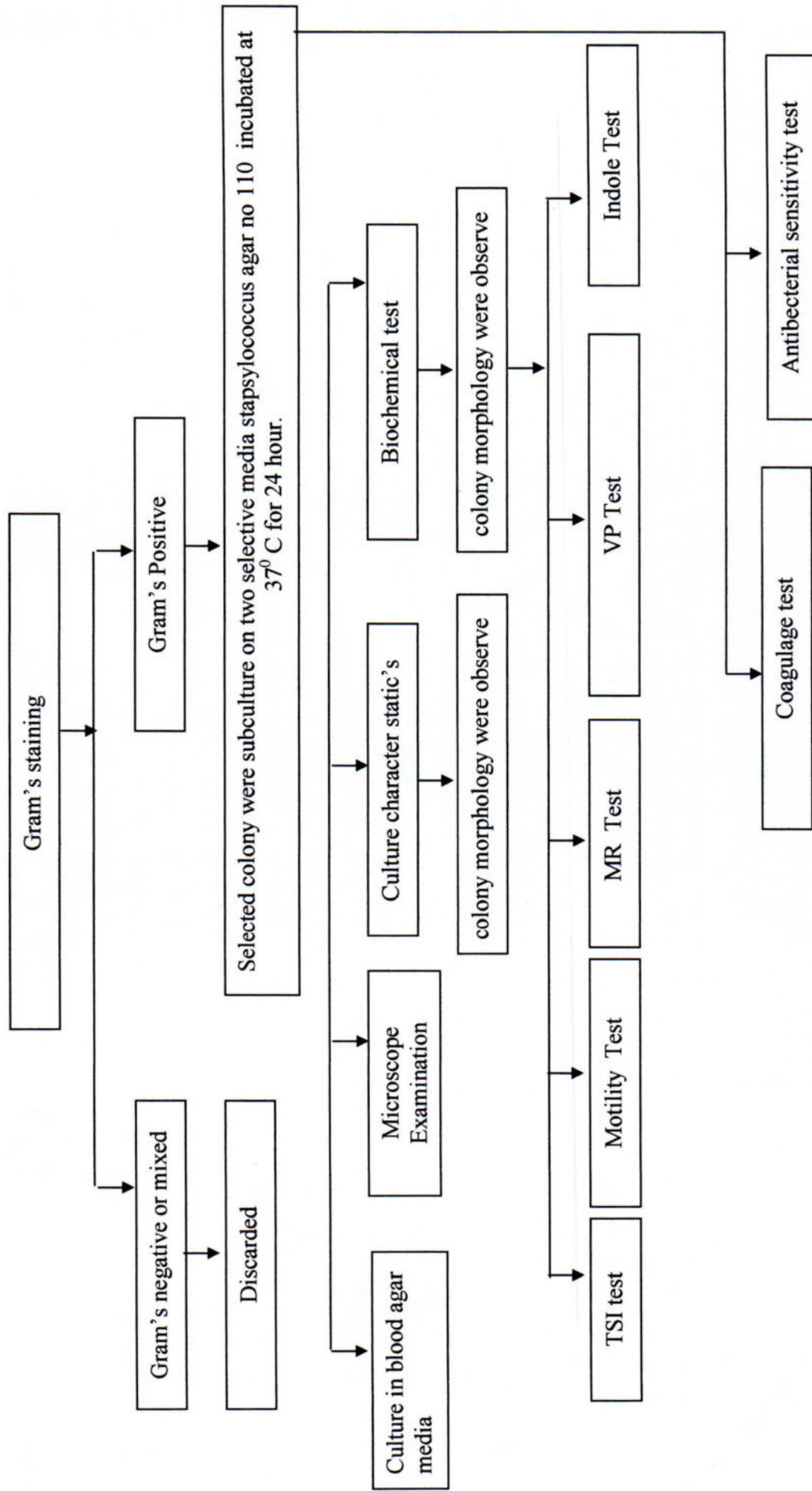


Figure 1. Schematic illustration of the experimental design

3.2.2.Methods of cultural characterization of *Staphylococcus* spp.

3.2.2.1 Nutrient agar (NA)

The sample was inoculated into the nutrient agar with sterile bacteriological loop and was incubated for 24 hours at 37⁰C.

3.2.2.2 Mannitol salt base agar

This is specific media for isolation of pathogenic staphylococci .The sample was inoculated into the Manitol salt base agar with sterile bacteriological loop and was incubated for 24 hours at 37⁰C.

3.2.2.3 Staphylococcus agar no.110.

This is specific media for isolation of pathogenic staphylococci. The sample was inoculated into the Staphylococcus agar no.110. With sterile bacteriological loop and was incubated for 24 hours at 37⁰C.

3.2.2.4 Blood agar (BA)

Suspected colony from nutrient agar was inoculated in to blood agar and was incubated for 24hours at 37⁰C, if positive for *Staphylococcus* spp. showed white to golden yellow, smooth, shiny, round and convex colony. Some strains produce (β) hemolysis around the colony. (Plate-13,14. Page-57.)

3.2.3 Morphological characterization of using by Gram's staining method

- A loopful of sterile distilled water was placed in the center of a clean sterile slide.
- A small colony was picked up with a bacteriological loop and was mixed with distilled water of a slide.
- The colony was made to thin smear on a slide
- The smears were fixed by air drying
- 0.5% crystal violet solution was then applied on the smear for one minute
- Lugols iodine was then added to act as mordant for one minute
- Acetone alcohol was then added to decolorize for 1-2 second
- Then the slide was then washed with water.
- 2% Safranin solution was added as counter stain and allowed for one minute
- Then the slide was washed with water
- Then the slide was blotted with blot paper and was allowed to dry
- The slide was examined under microscope with high power objective (100X) using immersion oil.

3.2.4 Method of Biochemical characterization

3.2.4.1 Methyl-Red (MR) test

- Suspected colony was inoculated in to tube containing 5 ml sterile glucose phosphate broth.
- Then the tube incubated at 37°C for 24 hours
- After 24 hours a drop of methyl red solution was added.

3.2.4.2 Voges-Proskauer (VP) test

- Suspected colony was inoculated into tube containing 2 ml of sterile glucose phosphate peptone water.
- Then the tube incubated at 37°C for 24 hours
- A small amount of creatine was added and mixed.
- Then 3 ml of sodium hydroxide were added and shaken well.
- Then the bottle cap was removed and left for an hour at room temperature.

3.2.4.2. Indole test

- Suspected colony was inoculated into tube containing of 2 ml of peptone water.
- Then the tube incubated at 37°C for 48 hours.
- After 48 hours 0.5 ml of kovac's reagent was added.
- Shaked well and examined after one minute.

3.2.4.3 Triple sugar iron (TSI) agar

- Suspected colony was inoculated into the surface of the slants and stabbed into the butt of the tubes containing TSI agar.
- Then the tube was incubated at 37°C for 24 hours.

3.2.4.4 MIU (Motility, Idole, Urea) medium.

- Suspected colony was inoculated into the tube containing MIU medium.
- Then the medium was incubated at 37°C for overnight.

3.2.4.5 Manitol salt agar base.

- Suspected colony was inoculated into the Manitol salt agar medium.
- Then the medium was incubated at 37°C for overnight.

3.2.5 Coagulage test

Small amount of bacterial colony diluted with distilled water on slide and add one dropped of sheep plasma was added.

3.2.6 Maintenance of stock culture

During the experiment it was necessary to preserve the isolated organism for longer period. For this purpose, pure culture of the isolated organism stored in sterilized 80% glycerin and bacterial culture were mixed and sealed with paraffin wax and store at 37°C. The isolated organisms were given code name for convenience.

3.2.7 Hemolytic activity

To determine the hemolytic property of isolated bacteria, the colonies of bacteria were inoculated on to BA media and inoculated at 37°C for 24 hours. Various types of hemolysis were observed after development of bacteria colony on the BA. The hemolytic pattern of the bacteria was categorized according to the types of hemolysis produced on BA and this was made as per recommendation of Carter (1986) and was listed as mentioned below.

3.2.8 Isolation of bacteria in pure culture

For isolation of bacteria in pure culture, the mixed culture was inoculated into Nutrient agar media by streak plate technique to obtain isolated colonies as per suggested by Poindexter (1971).

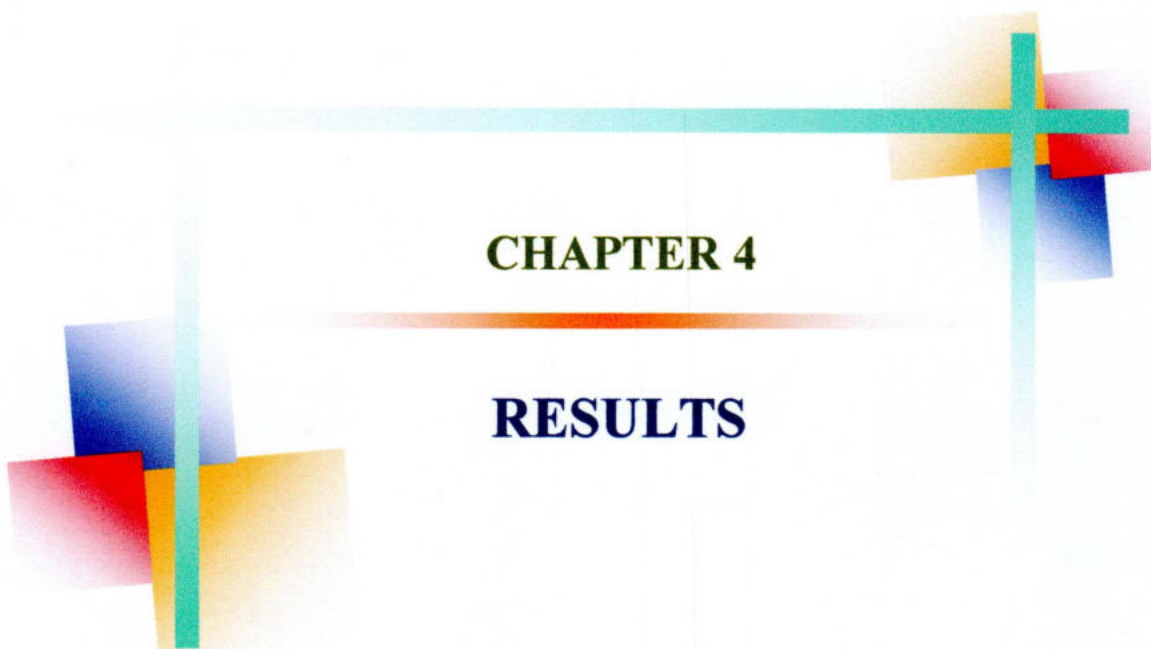
drawing the cooled parallel line.

This method was repeated as many times as necessary to obtain a culture containing only one type of colony and usually at least two more times to ensure purity.

3.2.9 Comparative antimicrobial sensitivity pattern of *Staphylococcus sp.*

Susceptibility and resistance of *staphylococcus sp* to different antibiotics was measured in vitro by employing the modified Kirby-Bauer (Bauser *et al.*, 1966) method. This method allows for the rapid determination of the a drug by measuring the diameter of the zone of inhibition that results from diffusion of the agents into the medium surrounding the disc.

A suspension of test organism was prepared in nutrient broth by over night culture. By sterile pipette 0.1ml of of test culture was poured on nutrient agar plate. Sterile glass spreader was used to spread the culture homogenously on the medium. Antibiotic discs were applied aseptically to the surface of the inoculating plates at an appropriate special arrangement with the help of sterile forceps. The plates were then inverted and incubated at 37°C for 24 hours.

A decorative graphic consisting of two sets of overlapping squares in blue, red, and yellow, connected by a horizontal line with a green-to-yellow gradient. A vertical green line passes through the center of the squares.

CHAPTER 4

RESULTS

CHAPTER- 4

RESULTS

The present research was carried out to study of the characterization of *Staphylococcus sp* from arthritic broiler breeder with antibiogram study of identified field isolates. Samples collected from the 122 affected birds of different age. The positive samples (pus/fluid) were subjected to bacteriological examination for isolation and characterization of bacteria. During collection of samples, health condition and type of infection was investigated according to different group and age.

4.1.1. Isolation and identification of *Staphylococcus sp* from breeder birds

A total of 122 samples (pus / fluid) from affected breeder birds belonging to A,B and C and comprising 89, 21 and 12 birds respectively were inoculated into different bacteriological media. The result is presented in Table 1. It was observed that high percentage of occurrence (52.8 %) of *Staphylococcus sp* was obtained from group A followed by group B (42.8%) and group C (16.67%).

Table 1. Isolation and identification of *Staphylococcus sp* from live affected birds on the basis of inoculation of sample in to bacteriological media and normal biochemical media.

Group of birds	Name of the sample	No sample tested	Grand total of sample	Positive case	% of Positive case	Total no. of positive isolates
A=(0-14wks)	fluid/ pus	89	122	47	52.8	58
B=(15-24wks)		21		09	42.8	
C=(≥25wks)		12		02	16.6	

4.1. Results of isolation and identification of pathogenic *Staphylococcus* sp.

4.1.1. Results of cultural examination

4.1.1.1. Culture on nutrient broth

Nutrient broth inoculated with the sample revealed the growth of bacteria after 24 hour of incubation at 37°C aerobically and was indicated by the presence of uniform turbidity. (Plate- 9, page 56).

4.1.1.2. Culture on nutrient agar

Nutrient agar plates streaked separately with the sample revealed the growth of bacteria after 24 hour of incubation at 37°C aerobically on nutrient agar the organism produced circular, small smooth, convex, and yellowish colonies. (plate-1,4, page 54).

4.1.1.3. Culture on Manitol salt base agar

Manitol salt base agar plates were streaked separately with the sample revealed the growth of bacteria after 24 hour of incubation at 37°C aerobically the organism *Staphylococcus aureus* produced golden yellow manitol fermenting colony with yellowish media and *Staphylococcus epidermidis* produced manitol nonfermenting white colony with pink color media. (Plate-6,8, page 55).

4.1.1.4. Culture on Staphylococcus Agar No.110

Staphylococcus Agar No.110 plates were streaked separately with the sample revealed the growth of bacteria after 24 hour of incubation at 37°C aerobically the organism produced golden yellow color colony. (Plate-10,11, page 56).

4.1.1.5. Culture on blood agar media

Blood agar plates were streaked separately with the sample revealed the growth of bacteria after 24 hour of incubation at 37°C aerobically the organism produced smooth, shiny, round and convex colony with haemolysis. (Plate-13,14. page 57).

4.1.1.6. Results of Grams staining technique

After Grams staining the organism revealed under microscope Gram positive, cocci shape, grape-like cluster. (Plate-12, page 57).

4.1.2. Results of biochemical test

Presumptively selective colony taking by sterile inoculating loop and repeatedly streaked on the selective media Staphylococcus Agar No.110 to check and confirm their purity. For identification, a series of biochemical test especially selective for *staphylococcus sp* were performed.

4.1.2.1. Results in TSI agar media

After inoculation and incubation in TSI agar media produced acid without gas. Acid production was indicated by the color change from reddish to yellow, indicate TSI positive. (Plate-15, page 58).

4.1.2.2. Results in MIU medium

After inoculation and incubation in MIU medium produced no turbidity. Which indicate the organism is non-motile and MIU negative. (Plate-16, page 58).

4.1.2.3. Results in Manitol salt base agar

After inoculation and incubation in Manitol salt base agar the media becomes yellow due to manitol fermentation. (Plate-17, page 58).

4.1.2.4. Results in MR-VP medium

After inoculation and incubation in MR-VP medium red color indicate MR test positive no color change indicate VP test negative. (Plate-19,20, page 60).

4.1.2.5. Coagulase test

Small amount of bacterial colony dilute with distilled water on slide and add one dropped of sheep plasma. (Plate-21, Page-61).

Table-2. General cultural media and colony character

Name of the media	Colony character
Nutrient broth	Uniform turbidity
Nutrient agar	Circular, small smooth, convex, and yellowish colonies
Manitol salt base agar	Golden yellow manitol fermenting colony
Staphylococcus Agar N°110	golden yellow colony
Blood agar media	Smooth, shiny, round and convex colony with haemolysis.

Table- 3. Biochemical characteristics of *Staphylococcus sp*

Biochemical test	Result
Fermentation reaction with sugar	
Glucose	+
Sucrose	+
Fructose	+
Mannitol	+
Fermentation reaction with dulcitol	
Indole	-
MR	+
VP	-

(+) = positive, (-) =Negative, MR= Methyl red, VP= Voges proskaure

A total of 122 samples (pus /fluid) collected from the bird affected with the susceptible infection of different age for isolation and characterization of *Staphylococcus* organism. Out of 122 samples 58 were *Staphylococcus* positive.

Table -4.Frequency response of staphylococcus isolates to microscopic examination and specific biochemical test

No. of tested isolates	Test performed	observation	Response				Indication
			Positive isolates	% of Positive isolates	Negative isolates	% of Positive isolates	
122	Microscopic examination by Grams staining	Showing gram positive, cocci shape, grape-like cluster	58	100	0	0	<i>Staphylococcus sp</i>
	TSI agar slant reaction	Acid production becomes uniformly yellow color	58	100	0	0	<i>Staphylococcus sp</i>
	Motility test by MIU medium	Absence of turbidity	58	100	0	0	<i>Staphylococcus sp</i>
	Indole test	no pink color ring at the adjacent	58	100	0	0	<i>Staphylococcus sp</i>
	MR test	Red color indicate MR test positive	58	100	0	0	<i>Staphylococcus sp</i>
	Mannitol salt fermentation	The media color becomes yellow	30	51.72	28	48.27	<i>Staphylococcus aureus</i>
	Coagulase test	Coagulase positive	30	51.72	28	48.27	<i>Staphylococcus aureus</i>

It was observed that all isolates were Gram positive, Spherical shape, grape-like structure visualized under microscope using Grams staining technique and the table shows that all the organism non-motile, MR positive, indole negative and coagulase positive. The observation shows that *staphylococcus aureus* mannitol fermentation positive. (Plate no 8, page 57).

4.1.3. Results of antibiotic sensitivity pattern of isolated bacteria

Of all bacterial isolates, two isolates of *Staphylococcus aureus* and *Staphylococcus epidermidis* were selected randomly for the antibiotic sensitivity and resistance pattern against commonly used antibiotic. The results of sensitivity against antibiotic discs (zone of inhibition) were categorized as resistance (R), less sensitive (+), moderate sensitive (++) and highly sensitive (+++). The results of antibiotic sensitivity are given in Table 4.

4.1.3.1. Antibiotic sensitivity pattern of *Staphylococcus aureus*

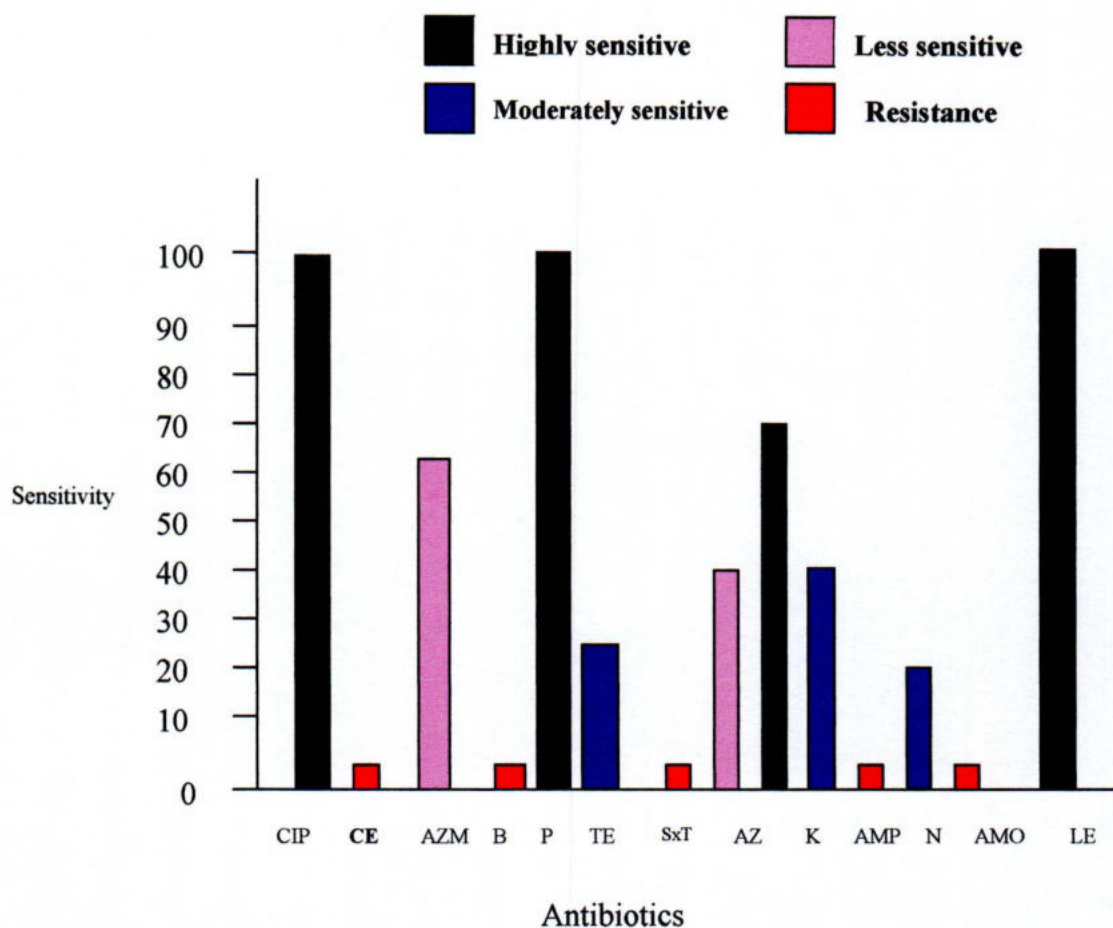
The antibiograms of various isolates of *Staphylococcus aureus* were found to be highly sensitive (+++) to Ciprofloxacin (100%), Nitrofurantion (70%), Levofloxacin. (100%), Gentamycin. (100%) and penicillin (70%). Doxycycline (100%) and Azithromycin (60%). Less sensitive (+) to, Tetracycline, Neomycin, Kenamycin and Erythromycin, (40%). Resistance to Cephradine, Ampicillin, Bacitracin, Amoxycillin and Sulphamethoxazole x Trimethoprim.

Table 4: Results of antibiotic sensitivity test of the isolated bacteria obtained from arthritis and bumble foot infection of broiler breeder.

Organisms	<i>Staphylococcus sp</i>
Antibiotics (µg)	
Amoxycillin-10	-
Cephadrine-30	-
Ciprofloxacin-5	+++
Gentamicin-10	+++
Kenamycin-30	+
Nitrofurantion	+++
Penicillin	+++
Levofloxacin	+++
Tetracycline-10	+
Doxycycline	++
Azithromycin	++
Sulphamethoxazole x Trimethoprim.	-
Ampicillin, Bacitracin	-

Here: µg: Micro gram, +++: Highly Sensitive, ++: Moderately sensitive, +: Less sensitive, -: Resistance.

Fig 1: Graphical presentation of antibiotic sensitivity pattern of isolated *Staphylococcus sp.*



Legend:

CIP=Ciprofloxacin, CE=Cephadrine, B=Bacitracin, AMP=Ampicillin.

AZM=Azithromycin, NF= Nitrofurantion DO=Doxycycline,

E=Erythromycin, TE=Tetracycline ,LE=Levofloxacin, P=Penicillin, G=

Gentamycin. Multidrug resistance was detected in case of *Staphylococcus* infection in Broiler parent stock. The organisms are highly (70%) sensitive to CIP, P, G, NF and LE. The organisms were moderate about (40%) sensitive to Doxycycline and Azithromycin. The organism shows resistance to Ampicillin, Bacitracin, Sulphamethoxazole x Trimethoprim and Amoxicillin.

Table.5. Antibiotic sensitivity test of the isolated *Staphylococcus sp*

Antibiotic disc	No. of isolates		Percentage (%)	
	Sensitive	Resistance	Sensitive	Resistance
Ciprofloxacin	58	0	100	0
Cephadrine	0	58	0	100
Bacitracin	0	58	0	100
Ampicillin	0	58	0	100
Azithromycin,	35	23	6.03	39.65
Nitrofurantion	41	17	70.68	29.31
Doxycycline	58	0	100	0
Erythromycin,	24	34	41.37	58.62
Tetracycline	18	40	31.03	68.96
Levofloxacin	58	0	100	0
Penicillin	58	0	100	0
Sulphamethoxazole x Trimethoprim	0	58	0	100

2 mm and above clear zone around the antibiotic disc indicated positive case.



Figure-1. Selection of Breeder flock. Name of the breed 'Hubbard classic'.



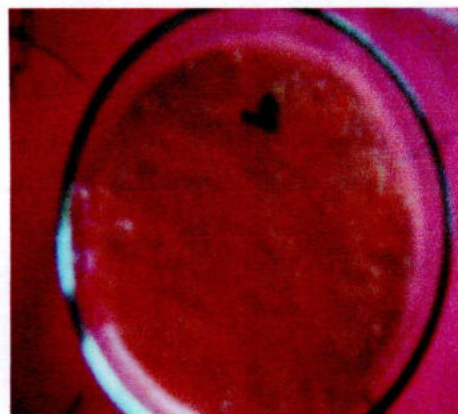
Figure-2. Affected leg, foot pad affected with Staphylococcus infection.



Figure-3. Joint affected with Staphylococcus infection



Plates -1. Fresh nutrient agar before incubation.



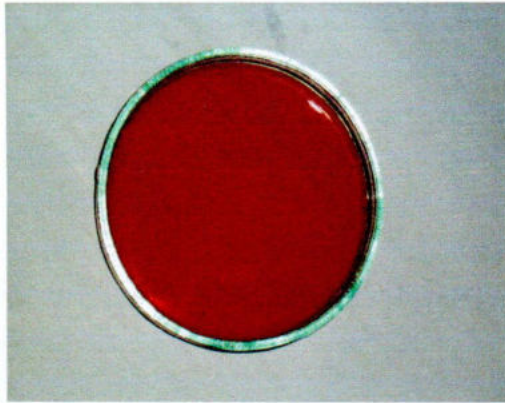
Plates -2. The organism produced circular, small smooth, convex, and yellowish colonies on nutrient agar.



Plates -3. Fresh nutrient agar before incubation.



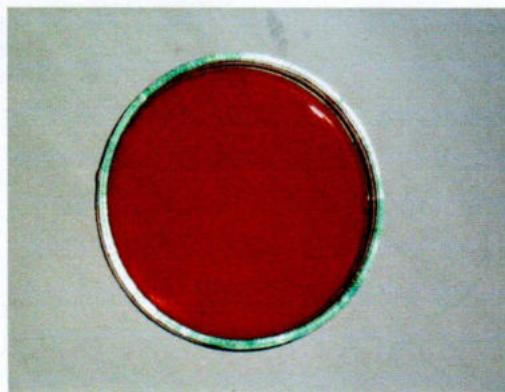
Plates -4. The organism from the primary culture produced circular, small smooth, convex, and yellowish colonies on nutrient agar.



Plates -5. Fresh manitol salt base agar before incubation.



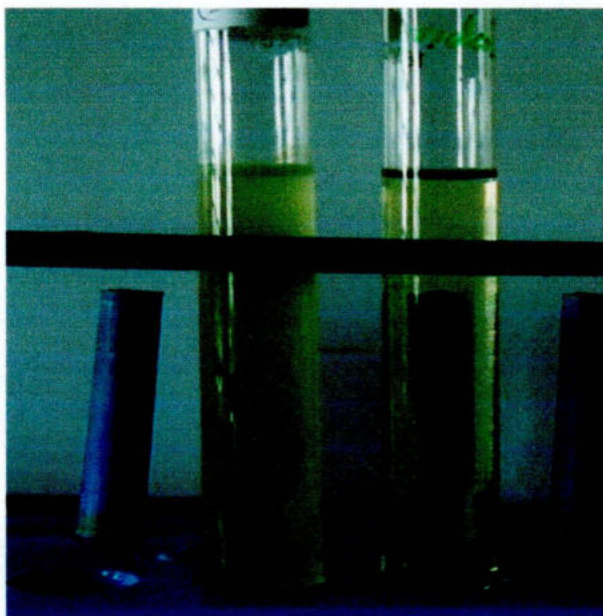
Plates -6. Manitol salt base agar after incubation produced golden yellow mannitol fermenting colony with yellowish media (yellow) is *S. aureus*



Plates -7. Fresh manitol salt base agar before incubation.



Plates -8. Manitol salt base agar after incubation produced mannitol non fermenting white colony with pink color media is *S. epidermidis*.



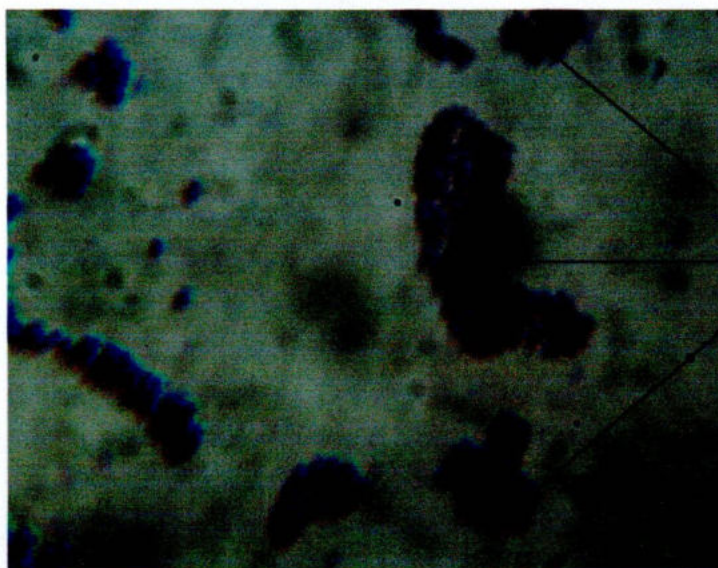
Plates -9. Presence of uniform turbidity throughout the medium (left) and un inoculated control (Right) in nutrient broth.



Plates -10. Fresh Staphylococcus Agar N°110 before incubation.

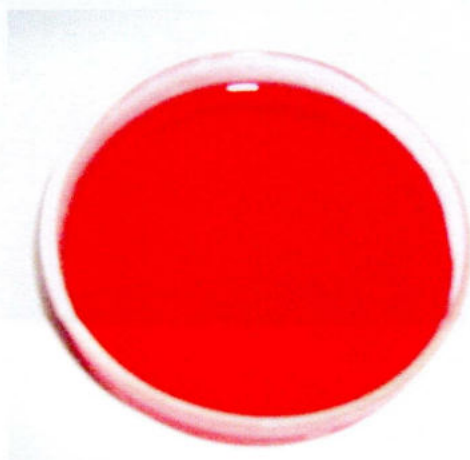


Plates -11. Staphylococcus Agar N°110 media after incubation produced golden yellow color colony.



gram positive,
grape-like
cluster .

Plates -12. Microscopic feature of Grams staining of the *Staphylococci sp* isolates showing gram positive, cocci shape, grape-like cluster .



Plates -13. Fresh blood agar media before inoculation and incubation.



Plates -14. Blood agar media after incubation produced smooth, shiny, round and convex colony with haemolysis.



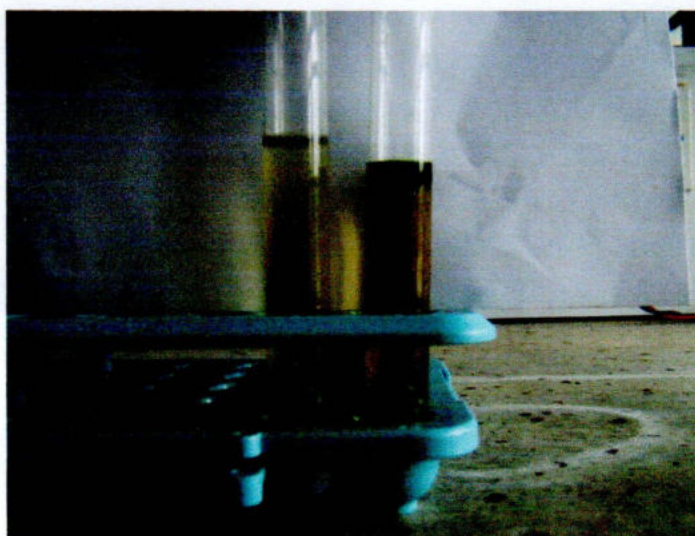
Plates -15. Bio-chemical test showing Triple sugar iron test positive (TSI test, +ve). Normal color (left) becomes yellow (Right) due to acid production.



Plates -16. Bio-chemical test showing MIU test negative (MIU test, -ve), after incubation no turbidity at (left) control at (Right).



Plates -17. Bio-chemical test in Manitol salt base agar showing media becomes yellow due to manitol fermentation.



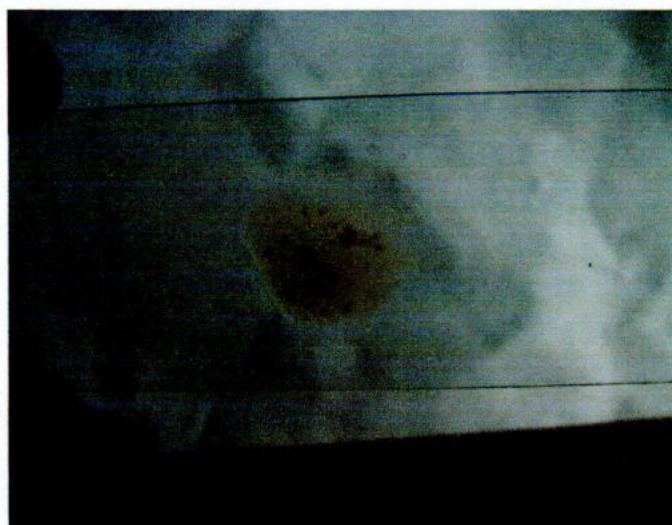
Plates -18. Bio-chemical test showing indole negative, absence of pink color ring (Right) at the adjacent.



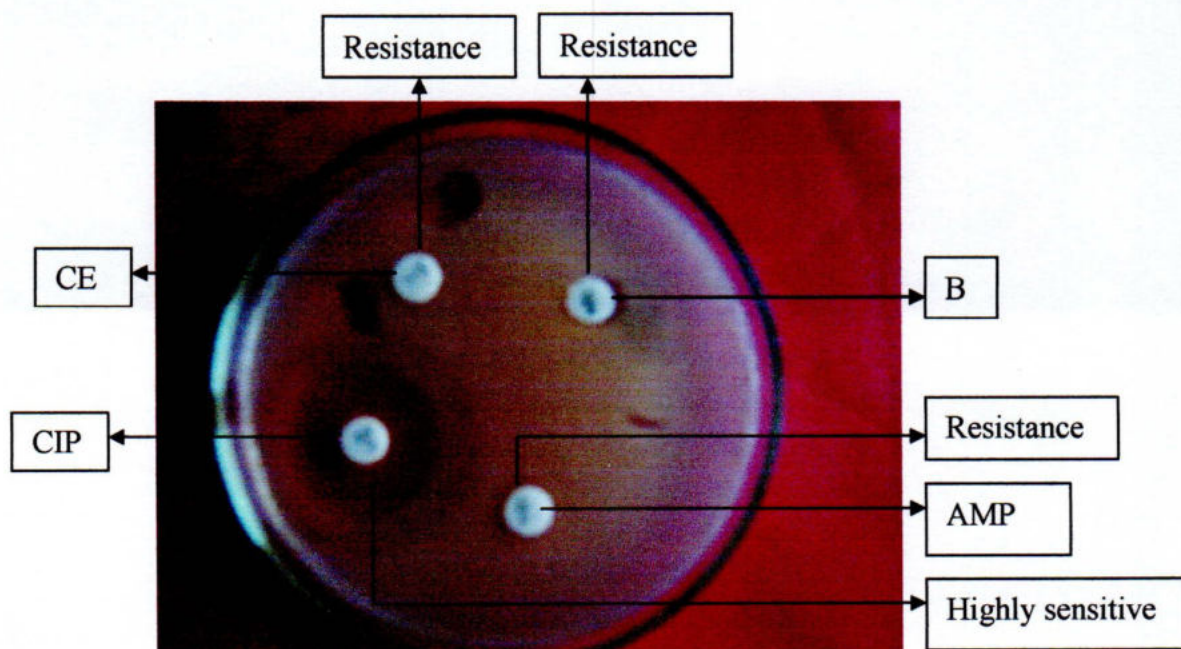
Plates -19. Bio-chemical test showing MR test positive red color (left) and control (Right).



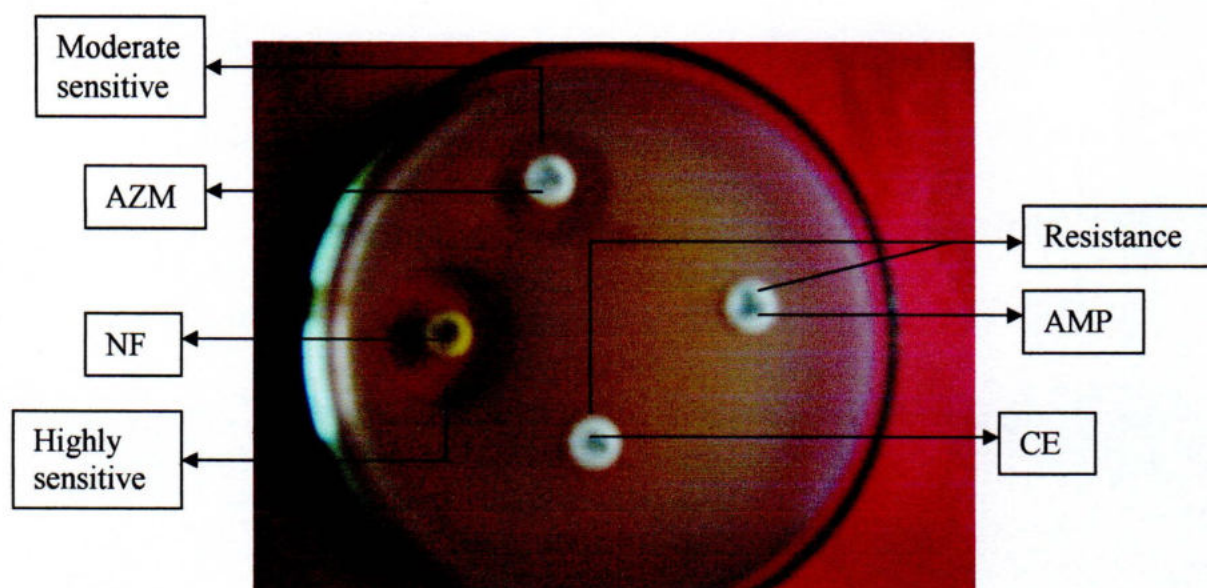
Plates -20. Bio-chemical test showing VP test negative (no color change after adding reagent)



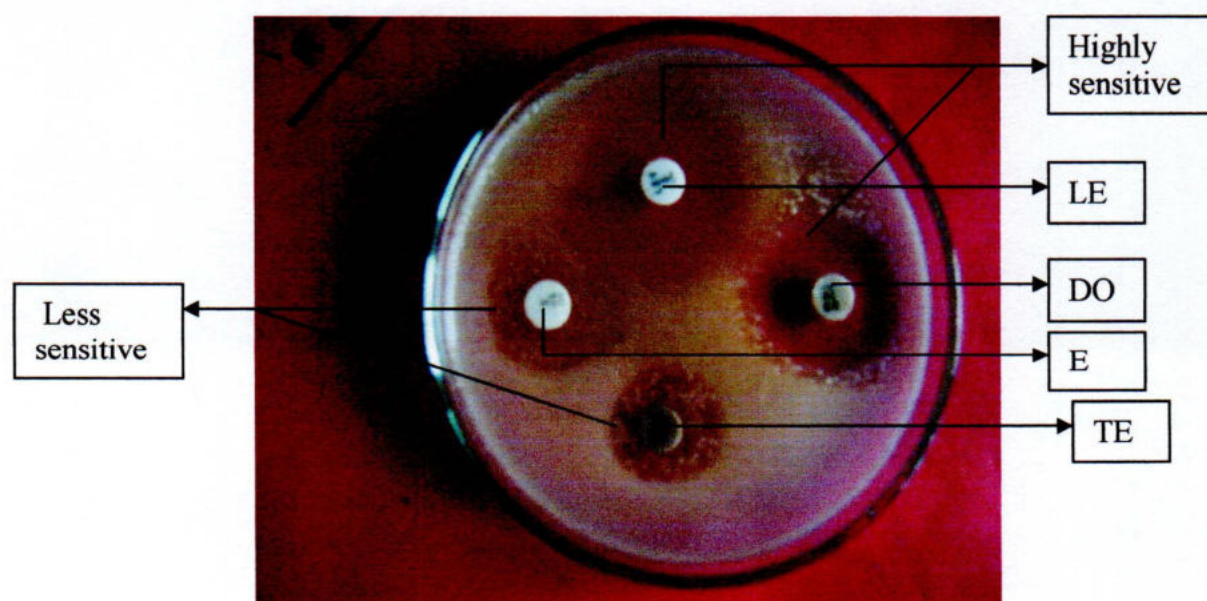
Plates -21. The test showing coagulage test positive.



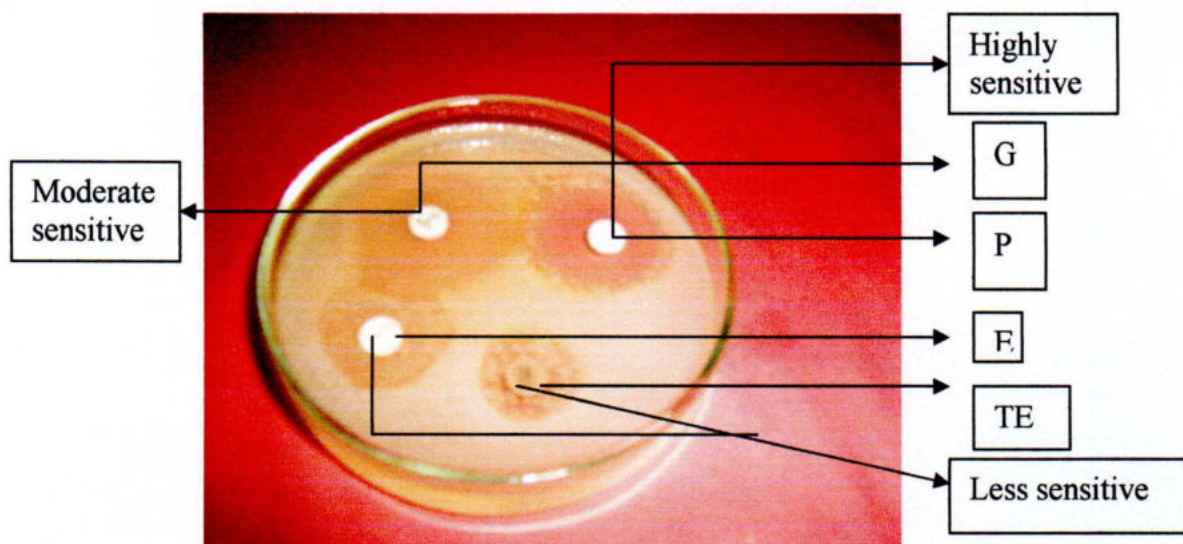
Antibiotic sensitivity
Plates-22 **Legends:** **CIP=Ciprofloxacin,**
CE=Cephadrine , B=Bacitracin, AMP=Ampicillin.



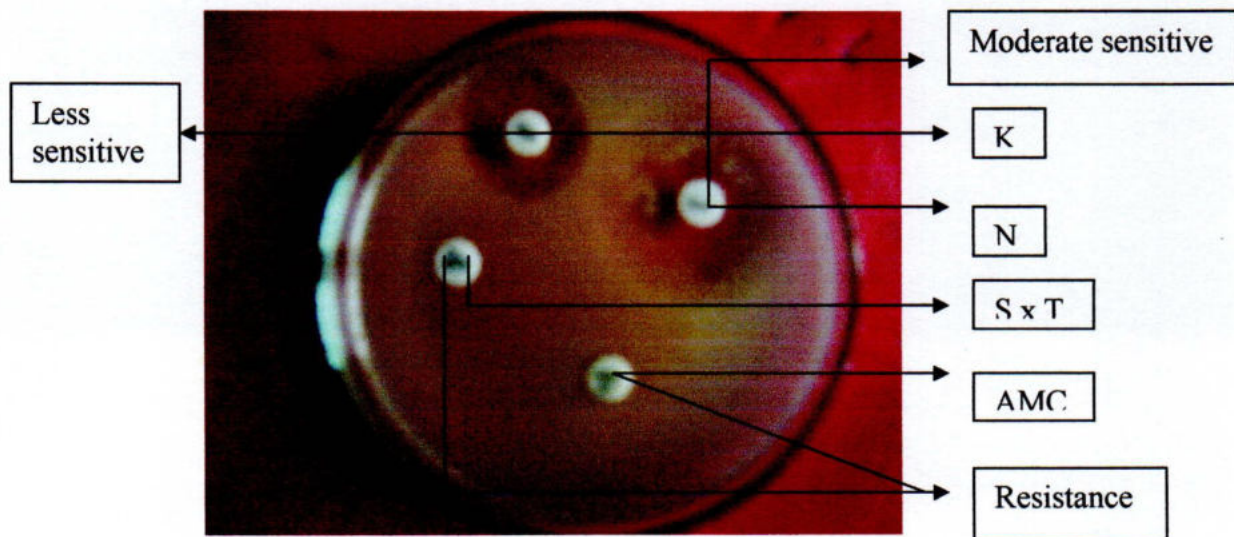
Antibiotic sensitivity
Plates -23 .Legends: AZM=Azithromycin,
CE=Cephadrine , NF=Nitrofurantion , AMP=Ampicillin.



Antibiotic sensitivity
Plates -24 Legends: DO=Doxycycline,
E=Erythromycin , TE=Tetracycline , LE=Levofloxacin.



Antibiotic sensitivity
Plates -25.Legends: P=Penicillin, G=Gentamycin,
 TE=Tetracycline , E= Erythromycin.



Antibiotic sensitivity
Plates -26.Legends: K=Kenamycin, N=Neomycin,
 SxT=Sulphamethoxazole and Trimethoprim , AMC=
 Amoxycillin.

CHAPTER 5

DISCUSSION

CHAPTER-5

DISCUSSION

The present study was aimed at characterization of the *staphylococcus sp* from arthritic broiler breeder of the Quality breeder farm, Bogra. The study was also extended to isolate and characterize the *Staphylococcus sp* employing cultural studies, microscopic examination and different types of biochemical tests with antibiogram study by using different anti bacterial agents available in the market.

In the present study, out of 122 arthritic samples (fluid /pus), fifty eight samples were identified as positive for *staphylococcus sp*. among all of sample 89 were collected from group-A (0-14 week), 47 revealed staphylococcus positive. 21 sample from group-B(15-24weeks), 9 were detected to be positive for *staphylococcus sp*. the prevalence of Staphylococcosis in broiler breeders 52.8%, in group A 42.86 %, in group-B 16.66% and in group C (table 1,page-45).

The most frequent sites of *Staphylococcus aureus* infection in poultry were bones, tendon sheathes and joints especially tibiotarsal and stifle joints. In the present study *staphylococcus aureus* was isolated at the percentage of 52.8% in 0-14 weeks old broiler breeders similar to the findings of other researchers (B.Y. Rashed, 2011; Shareef *et al.*2009).

The bacterium is considered to be normal resident of the chicken, located on the skin, feathers and on the respiratory tract. The organism must enter the circulatory system to cause disease. The probability of the infection is increased by any injury

that provides the bacteria with route of entry. Arthritis is most commonly caused by *Staphylococcus aureus* in association with other organism (B.Y. Rashed 2011).

The results of this study were more or less in agreement with the findings of previous workers who also conducted research investigation on staphylococcus. But in this present study higher percentage of *Staphylococcus aureus* were recorded from 0-14 week's old birds. The difference of prevalence percentage of bacteria was probably due to the differences of age, breed and different environmental factors.

In the present study, specific culture media and biochemical tests which were used for the detection of *staphylococcus sp*, were also used by a number of scientists (Shareef *et al.*, 2009; Rashed.B.Y.2001; Bergmann V *et al.*,1980 and Terzolo.H.R *et al.*,1979).

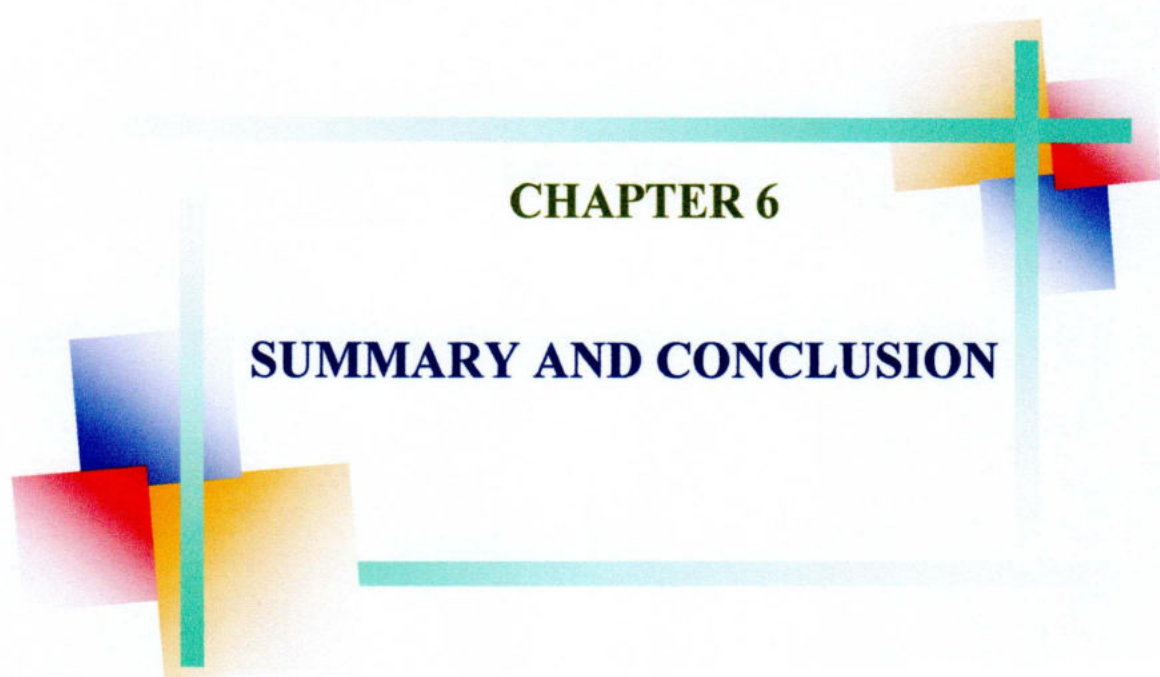
In this study the colony characteristics of the *Staphylococcus sp* observed on different media were similar to the findings of other authors (Buxton and Fraser, 1977). In Grams staining the morphology of the isolated bacteria exhibited Gram positive, spherical shape, grape like cluster which was supported by several authors (Freeman, 1985and I.A Marchant and Packer 1967). The isolated staphylococcus organism were recorded as non-motile. The fundamental basis for the detection of motility of bacteria was the motility test (Buxton and Fraser, 1977and freeman,1985). The non-motile organisms which were isolated in this investigation were also recorded as *Staphylococcus aureus* and *Staphylococcus epidermidis* In this study the organism were isolated and characterized by both cultural and biochemical characteristics. This procedure is similar to Capita R *et al.*, (2002) studied that ninety-six *Staphylococcus aureus* isolates from retail chicken carcasses

in Spain were characterized using cultural and biochemical tests. The organism showed the coagulase test positive (plate.21 page 61).

Antibiogram test means, the test that is done in laboratory or in- vitro condition to detect the sensitiveness of antibiotics against certain bacteria which is responsible for specific disease. On the other hand this test is done to measure the ability of the antibiotics to prevent the growth of bacteria in vitro condition or in suitable environment outside the body. Due to following importance this test was done. Antimicrobial resistance testing was performed by disc diffusion method using 16 different antibiotics. The recent antibiogram study revealed that the isolate were highly sensitive to ciprofloxacin (70%), Nitrofurantion (70%), Levofloxacin. (70%), Gentamycin. (70%) and penicillin (70%) (Table No-4. Page 50, plate no.11,12,13,14,15 Page No.58,59,60). All the isolates were Moderately sensitive to Doxycycline (40%) and Azithromycin (40%) (Table No-4. Page No.50), less sensitive to Tetracycline, Neomycin, Kenamycin and Erythromycin, (20%)(Table No-4.Plate No.22-26,Page No.61-63). All the isolates were resistant to Cephradine, Ampicillin, Bacitracin, Amoxacillin and Sulphamethoxazole x Trimethoprim. The study was more or less similar to Miranda JM *et al.*, (2008); Nemati M , *et al.*, (2008) ; Zhou Q *et al.*, (2007) ; White DG *et al.* (2003)

From the present study it could be concluded that staphylococcal infection is the most real important causes of arthritis leading to economic losses in the broiler breeders as high morbidity swollen joint thus we must take care to control the staphylococcus infection in poultry. The selected farm should be periodically checked for the prevention of staphylococcus infection and positive reactor should be culled and biosecurity plan of the farms should be followed strictly, from research interest point of view the following task may be scheduled for future study.

- Molecular characterization and pathogenicity studies of the identified isolates
- Detailed genomic analysis to obtain the idea about molecular basis of pathogenicity and drug resistance
- Development of effective measures against identified field isolates
- Development of toxoid from the isolated organism.



CHAPTER 6

SUMMARY AND CONCLUSION

CHAPTER-6

SUMMARY AND CONCLUSION

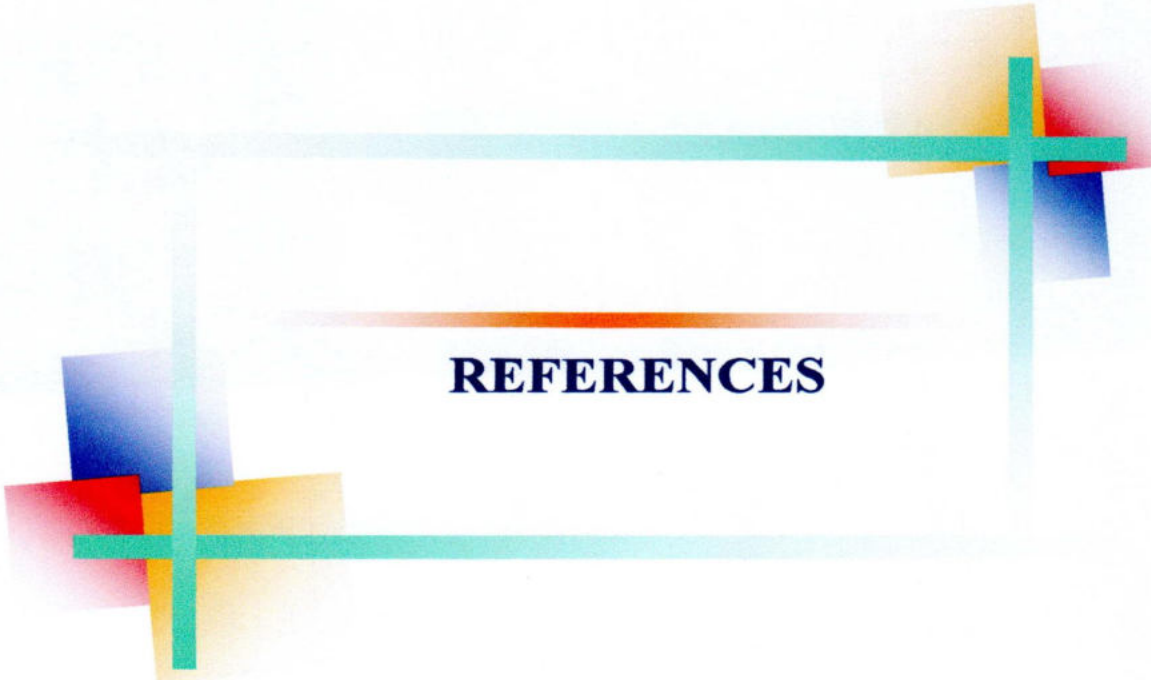
The present research work was conducted for the isolation and characterization of *Staphylococcus sp* in broiler breeder flock with antibiogram study of identified field isolates in Quality breeder Farm Sajahanpur, Bogra and Isolates of the *Staphylococcus Sp*, from the sample (pus/fluid) collected from arthritic and bumble foot infection. Sample from breeder chickens were collected and the laboratory works were conducted in the Department of Microbiology, HSTU and QBL laboratories Sajahanpur, Bogra.

Among economically important diseases of poultry *Staphylococcosis sp* is one of the most well recognized bacterial disease in poultry industries causing losses through mortality and reduced production. The result of study demonstrated that *Staphylococcus sp* organism could be detected in deferent ages of broiler breeder, particularly during 0-14 weeks of age 52.8% in compared to growing stage 42.86% and laying stage 16.66% the infection rate is high growing stage.

Staphylococcus sp is a normal inhabitant of the skin and upper respiratory tract of diseased and healthy chickens. *Staphylococcus sp* is an important opportunist that can cause superficial to life-threatening illnesses in poultry, this organism has been implicated in osteomyelitis, synovitis, and cellulites, arthritis, osteomyelitis, dermatitis, endocarditis, septicemia, wound infection and bumble foot disease. Which causing continuous economic loss in breeder farm. But there is no effective vaccine available in our country. The infection can be controled by using different antimicrobial agents.

Staphylococcosis being controlled primarily following maintenance of strict hygienic and sanitary measures. Antibiotics are widely used on diseased birds for the treatment of staphylococcosis. In case of treatment antibiotic sensitivity test is more effective.

The disease can be controlled by decreasing the stress by reducing overcrowding, insufficient feed and water, feed restriction, vaccination and other handling, faulty debeaking, poor litter management, onset of sexual maturity and egg production, inadequate clean out and biosecurity procedures, high temperature and poor ventilation.



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APPENDICES-I

Composition of different media

1. Nutrient broth	
Peptic digest of animal tissue	5.00gm
Sodium chloride	5.00gm
Beef extract	1.50
Yeast xtract	1.50
Distilled water	1000ml
Final PH(at 25°C)	7.4±0.2
2.Nutrient agar	
Bacto-nutrient broth	2gm
Distilled water	250ml
3.Blood agar	
Bacto tryptose	10gm
Beef extract	1.5gm
Sodium chloride	2.5gm
Glucose	0.15gm
Dextrin	0.25gm
Para-aninobenzoic acid	10gm
Agar	7.50gm
Distilled water	500ml
Defibrinated blood	50ml
3.Triple sugar iron (TSI) agar slant	
Lab-lemco powder	3.0gm
Yeast extract	3.0gm
Peptone	20.0gm
Sodium chloride	5.0gm
Lactose	10.0gm
Sucrose	10.0gm
Glucose	1.0gm
Ferric citrate	3.0gm
Sodium thiosulphate	3.0gm
Phenol red agar	q.s
agar	12.0gm
Distilled water	1000ml

distilled water to make 300ml. stored in amber bottle .

d. Ethyl alcohol

250ml

e. Acetone

250ml

f .Counterstain

Safranine

2.5ml

Ethyl alcohol (95%)

100ml

Safranine working solution : the stock safranine is diluted 1:4 with distilled water .

4. MR-VP medium	
Buffered peptone	7.0gm
Dextrose	5.0gm
Dipotassium phosphate	5.0gm
Final PH(at 25°C)	6.9±0.2

APPENDICES-I(contd)

5. Staphylococcus agar media 110	
Agar	
Di –potassium phosphate	15gm/lit
Sodium chloride	5.00gm
d- mannitol	75.00gm
lactose	10.00gm
gelatin	2.00gm
yeast extract	30.00
Casin enzyme hydrosate	2.80
PH	10.00gm
	7.0±0.2at 25°C
Distilled water	1lit
6. MIU medium base	
Casin enzymatic hydrolysate	10.0gm/lit
Dextrose	1.00gm
Sodium chloride	5.00gm
Phenol red	0.01gm
Agar	2.00gm
7. Buffered peptone water	
Proteose peptone	100gm/lit
Sodium chloride	5.00gm
Di –sodium phosphate	3.50gm
Monopotassium phosphate	1.50gm

APPENDICES-II

Preparation of reagent

1.Peptone water Peptone Distilled water	1gm 1000ml
2.Kovacs reagent preparation for indole p-dimethyl aminobenzal dehyde Amylalcohol Conc.HCL	5gm 75gm 25ml
3.V-P reagent 1 5% alpha –naphtholin absoulate ethyl alcohol	
4. 40%potassium hydroxide containing 0.3 creatine .the ingridents were dissolved by heating gently over astem bath. When in solution add 0.05gm of cotton blue dye	
5.phosphate buffer saline Sodium chloride Disodium hydrogen Phosphate Potassium chloride Potassium hydrogen phosphate Distilled water to make	8gm 2.8gm 0.2gm 0.2gm 1000ml



APPENDICES-II (Contd)

6. Methyl red Methyl red Ethanol (absoulate) Distilled water	0.05 28ml 22ml
7. Phenol red solution 0.2% aqueous solution of phenol red .	
8. Gram stain solution a. Stock crystal violet crystal violet ethyl alcohol(95%) b .Stock oxalate solution Ammonium oxalate Distilled water Crystal violet working solution :20ml of solution no.1 mixed with 80 ml of solution no.2 additional dilution was made when desired . c. Lugols iodine solution iodine crystal potassium iodide dissolved completely in 10 ml of distilled water, then added to distilled water to make 300ml. stored in amber bottle . d. Ethyl alcohol e. Acetone f .Counterstain Safranine Ethyl alcohol (95%) Safranine working solution : the stock safranine is diluted 1:4 with distilled water .	10gm 1000ml 1gm 1000ml 1gm 2gm 250ml 250ml 2.5ml 100ml