Studies on Aerobic Bacterial Flora of Gastrointestinal Tract of Poultry in Health & Disease with Special Reference to *E. coli*

Thesis
Submitted to Magadh University in Partial Fulfilment
of the Requirements for the Degree
of
M. SC. (VET.) IN BACTERIOLOGY

BY
ATAM SINGH NARULA
Post Graduate Department of Bacteriology
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Prof. P.B. Kuppuswamy,
B.A., G.M.V.C., B.V.Sc.,
P.G.(Newzealand), M.S.(Missouri),
Principal and Head of the Department.

Post Graduate Deptt.of Bacteriology,
Bihar Veterinary College,
PATNA.

This is to certify that the entire work presented in the Thesis entitled " Study of aerobic bacterial flora of the gastrointestinal tract of Poultry in health and disease condition with special reference to E.coli" is the bonafide work of Shri A.S. Narula, a candidate for the degree of M.Sc. (Vet.) with Bacteriology as his major subject, which was carried out under my supervision and guidance.

P.B. Kuppuswamy

(P.B. Kuppuswamy)

11/12/66.

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(A.S. Narula)

Bihar Veterinary College,
Patna
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C O N T E N T S .

INTRODUCTION.

-:: I N T R O D U C T I O N ::-

t-::- t -::-t

In a country like ours with varied dietary systems and sentimental disparities, and also suffering from severe malnutrition especially due to non-availability of the vital tissue building Protein in enough quantities, Poultry development is a major field which can bring about a revolutionary change in the improvement of Nation's health within a short span of time. Poultry is considered as one of the major converters of grains and other materials including waste products which are not usually used for human consumption, into highly valuable protective proteinous food.

Unfortunately the progress of the Poultry development is hit badly by heavy mortality occuring in the poultry, annually resulting not only in colossal economic loss but also leads to retarded growth , poorly finished sale birds and decreased egg production. More than 50% of the birds die annually and out of the 40% die of bacterial and viral diseases (Naidu- 1959). Since the fowls are more vulnerable to diseases , our greatest success in poultry farming lies mainly on the proper diagnosis and control of the same.

The birds which recover from the attacks of disease remain as 'Carriers' and these will be a permanent source of infection in the flocks and would contaminate poultry houses , drinking water, eggs and food stuff

directly or indirectly. The diagnosis of carrier stage in such animals though rather cumbersomi is also more important from public health point of view as most of the Salmonella strain possess the same degree of pathogenicity for birds as well as to human beings. Poultry constitutes the greatest single host reservoir for Salmonella (Buxton- 1957). More than 100 serotypes have been isolated from fowls Williams (1959). Salmonella Enteriditis was first isolated by Gaerter (1888) from an infection in men and since then this organism has been found to be associated with fatal disease in man, animals and birds specially in the form of outbreaks of food poisoning in man following consumption of meat of affected birds. Similarly infected eggs from a diseased bird or a "carrier" hen are the cause of the dissemination of causual organism leading to epidemics.

Among other diseases known to be transmitted from bird to man mention may be made about T.B.,Psittacosis, H.S.Asporigillosis etc. (Thomas G.Hull - 1955) because from point of zoonosis since in rural areas fowls are sometimes kept in the same living rooms as men, the chances of infections spreading to man and vice-versa will be great.

In case of poultry the respiratory tract and the gastrointestinal tract form the main systems which require exploration for the purpose of diagnosis. Before a diagnosis could be achieved, a thorough knowledge of the normal microflora of that particular system is essential which would help to arrive at a definite conclusion.

Smith (1962) , Smith and Jones (1963) and Smith(1965)

showed that although the bacterial population of the faeces closely resembled that of the large intestines there could be a great change in the microflora of the stomach and small intestines without any appreciable deviation being noticed in the faeces.

Smith (1965) while studying the normal flora of the gastrointestinal tract of healthy fowls at different ages and in different regions of the digestive tract has shown that the normal microflora of the system consists of E.Coli welchii , Streptococci , Lactobacilli Yeast and Bacteriodes. Though they mostly form the normal flora , most of them may gain pathogenicity when the circumstances are congenial for them leading to fetal results. E.Coli frequently has been isolated from the diseased fowls. It is usually considered to be a secondary invader although it has been incremmiated as the cause of death in some of the flocks, Number of investigators have reported the association of this organism with Septicaemic conditions in fowls Ligneris (1894) Davis (1938), Qureshi (1957), Gunmmurthi and Panduranga Rao (1962), Verma (1964) , and Sarkar (1966). The well known condition 'Coligranuloma' , at first reportedly by Hjarre and Wramby (1945) has now been reported by many workers Weakman (1948), Lisset (1949), Koblar (1957) and Ramchandran et al (1965).

Keymer (19) enumerating the causes of mortality among birds in British Islands has shown that Welchii , E.Coli , Listeria , Salmonella and Streptococci

have been associated with the severe outbreaks with heavy loss of birds. Even the Pseudomonas which for sometimes was only considered to be of not much pathogenic importance has been shown to be the cause or mortality of chicks (Heram E.Essex at al 1930), Cprice (1958) and Valdao (1961).

Under modern system of poultry farming lot of antiboitic feeding is being practiced for the prevention of diseases and also as a feed supplement in chicks to promote growth. This has arisen an interest among the Scientific workers as to whether the continuous use of these antiboities in any way would lead to the development of some resistant stains of bacterial population (Barnes - 1958) found that the inclusion of chlortetracyclin in the feed did not alter the total number of faecal Streptococci present. However, it lead to the development of a different very resistant strain of Strept. faecalis which replaced the normally present strain. This development of a new pathogenic strain may be the cause of the disease itself. He further observed that the implication of these results to the preservation of meat and poultry with Tetracyclin compounds is that the use of an antiboitic in the feed may altogether nullify its effects as a preservative.

In the present study an attempt has therefore been made to find out the normal bacterial flora of the gastrointestinal tract of poultry in health and diseased condition, study of their pathogenicity and antiboitic

sensitivity to corelate them with the factors responsible for mortality in the poultry to facilitate control measures, against the same.

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REVIEW OF LITERATURE.

-: ESCHERICHIA COLI :-

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Escherichia Coli was for the 1st time reported by Escherich from the faeces of a breast fed infant and since then it has gained world wide importance.

The organism is found as a member of the normal microflora of the gastrointestinal tract of man and almost all the animals. It is found even on the first day gaining its entry shortly after birth and continuing its existence throughout the life.

Seers and Brounbe (1954) and Seers et al (1956) described two groups of the organism durin g his study of E.Coli in the intestinal tract of man and dog. One was a transient group of organisms found only for a few days after birth whereas there was a resistant group which had its role after sometimes and continued it throughout the life or atleast for over a long period. Attempts to re-establish new resident strains in the dog failed. Sears et al (1956). The whole environmental change was required to bring about the change of the resident strains.

Smith and Crabb (1961) Smith (1966) studying the normal bacterial flora of faeces and the gastrointestinal tract of the animal and man reported the presence of E.Coli , Clostridium Welchii, Streptococci , Lactobacilli , bacteriodes and staphylococci. The E.Coli , streptococci and lactobacilli formed the major part of the flora. Smith-(1965) described the presence of more number of E. Coli

in the anterior part of the stomach than in the posterior part. Their number increased from subsequent portions of small intestines towards the posterior side , the number being the highest in the large intestine. The fact was due to the pH variation in the gastrointestinal tract which was more acidic in the gizzard portion and increased in alkanity on the posterior side.

Gaut at al (1943) while describing the useful role , the colon organisms play associate it with the probable suppressing of the growth of some of the proteolytic organism , and also in the synthesis of appreciable amount of 'B' Vitamins.

The organism though a normal inhabitant of the gastrointestinal tract is associated with many of the pathological conditions in man , animal and birds. In human beings it is described as a cause of the Infantile gastroenteritis and other conditions affecting the Urinogenital system, peritonium , gall bladder , respiratory system , and meninges (Dudgeon at al 1923 Mayer at al 1924 , Bray - 1945, Chamber- 1955 and Rees - 1957). It has been reported from the condition 'white Scours' of calves Jenson (1893) , Lovell and Huggs (1935) and Smith (1960). In older animals Jenson (1896) , Frey (1953) , Merchan at al (1956) have associated it with cervicitis, cystitis , mastitis , and metritis . In lambs it has been reported to be responsible for the diarrhoea (Charles - 1959) ; Oedema disease in pigs

has been reported to be due to Coli infection Sojka et al (1957) , Rees (1959) and Szabo (1962) .

In case of poultry it has been associated with many pathological conditions besides the well known condition ' Coli granuloma ' or Hjarre's .

Davis (1938) reported the disorders due to the organism mainly pertaining to the digestive system with enteritis and necrotic liver as the common symptoms. Davis et al (Loc. Cit.) demonstrated its pathogenesis in relation to healthy and weak chicks. They found low pathogenecity for healthy ones than the weak ones. Bueno (1940) attributed the organism to be the primary causual agent of the various pathological conditions of the poultry.

Hajarre and Wramby (1945) described for the 1st time a condition characterised by tuberculosis like granulomatous lesions in the liver and caeca of fowls. A pure culture of mucoid Bact coli was isolated from the lesions and when , the tissue pulp of the granulomatous lesion and the culture isolated, was inoculated I/V the disease was set up experimentally. The disease could be produced in mice as well as the rabbits. Digestive tract was the via media of the infection but the disease failed to be produced by feeding the cultures. Weakwan (1948) from Canada and Lisset (1949) from France reported the condition identical to Hjarre's disease.

Koblar (1957) found the condition of Coli granuloma in 95 cases out of 2,583 post mortem cases.

They were found most commonly in the liver and intestine. Kidney , skin, lungs and spleen were less frequently affected. Pure cultures of B.coli was isolated and the diesease could be produced when the culture was inoculated but the oral administration had no effect. Kohlar attrivutes primary cause to be prasitic enteritis. Similerly the coligranuloma was absorved in 4 cases over a period of 5 years by Gourdon and Devos (1954). Venulsen (1953) reported 8 cases of the so called granulomas of viscera in fowls which corrosponded to the lesions of Hajarre's disease. Bandernakaya (1954) reported the condition from Ceylone. Joubert at al (1964) reported the disease in 11 cases.

Biely and March (1963) described the role of the Genetical factors in the causation of the disea-se 'Coligranuloma! The diseasewas diagnosed by him in three generation of a strain of white leg horn fowls. The disease could not be observed in the other strains of fowls which were kept in the same conditions in the same premesis.

In India Ramchandran at al (1965) for the first time recorded the disease and studied its histopathology. The disease could not be reproduced in the same pattern as observed in the original case by oral route. Prashad, Shrivastava and Prashad (1966) in Bihar have reported a case of Coli granuloma from a white leg horn hen

Studying 30 E.Coli cultures from the internal organs of fowls, died in widely separated areas in USA, Edward and Ewings (1954) reported that all belonged to single biochemical variety O2:K1:H5. This type of culture was not found among 200 culture from the intestines of fowls and other

domestic animals nor it could be found in the stools of the food handlers or persons affected with diarrhoea.
Ulbrich (1954) isolated serologically related strains from fowls showing granuloma. These strains differed from those strains isolated from healthy ones. 53% of the 176 stains isolated from sick fowls, piglets, calves and lambs could be serologically typed with Group 'O' Sera whereas only 9% of 136 stains isolated from healthy animals could be typed in this way. Gomes (1955) reported the death of day old chicks following transportation by plane. The cause of the death was attributed to be the septicaemia due to B.Coli. Laboratory tests in mice and G.pigs confirmed the virulence of the organism.

Gross (1957) isolated E.Coli from fowls and turkeys showing lesions of aerosacculitis, fibrinous pericarditis, perihepatitis salpingitis and pano phthalmitis from experimental chicks. Natural infection had always been there in combination with chronic respiratory diseases. Gross (1957) experimentally produced panopthalmitis in association with severe bacteriamia. The chief symptom was blindness associated with hypopyon.

E.Coli. had been reported as the cause of an infectious enteritis (Colibacillosis) in two flocks of birds in West Pakistan by Qureshi (1957). These birds had been inoculated with new castle disease vaccine. The disease was reproduced by inoculation of the suspension of the organism from the affected birds E.Coli was isolated from 207 out of

the 378 chicks with enteritis. Cross and Siegal (1959) produced peritonitis in chicken by inoculating sterile youlk I/P and at the same time a pathogenic stain of E.Coli I/P or into the vagina. They suggested that peritonitis actually occurs when peritonial cavity is contaminated with youlk and E.Coli enters through vagina.

Reid at al (1961) reported a strain of E.Coli which was pathogenic to chicks. This strain infected hatching eggs by shell penetrations on the 1st day. The eggs which were infected on the 1st day had 79% hatchability as compared to the 83% of the controls and a total mortality upto 9th day of life was 8.2 as compared to 1.8%. There was no difference of mortality after 10 days but infected chicks weighed significantly less after 9 and 80 days.

Mushin and Ashburner (1962) investigating the cause of limy disease of mutton birds in Australia observed that examination of 1274 specimen from affected birds failed to give any indication of pathogenic bacteria. Proteus species and other intermediate coliform types occurred frequently though the isolation of E.Coli was rare.

Gurumurthi and Panduranga Rao (1962) from India observed Coli bacillosis causing heavy mortality in brooder chicks under one to four weeks of age. Death was seen in a few hours after the 1st symptom. White pastey, diarrhoea, blocking of the vent with driet faeces and welting of the surrounding area was noticed in about 30% of the affected chicks. Non haemolytic strain of E.Coli was isolated from

the affected animals. The strain was pathogenic for swiss albino mice and had a variable pathogenicity for chicks but was non-pathogenic for rabbits. Verma (1964) also reported Coli bacillosis from Bihar taking a tall of about 958 chicks between the age of 2-9 weeks. Sarkar (1966) is isolated E.Coli from chicks in one of the farms in Bihar which had high mortality. Yadava (1966) attributed Coli bacillosis to be the cause of mortality in chicks in one of the Poultry Farms in U.P. (India).

Savoo (1963) isolated 21 strains, belonging to group 'O'1, 'O'2 and 8 to 'O'78, out of the 48 strain of E.Coli causing coli septicaemia in chicks. The disease could be reproduced in 3-6 and 21 days old chicks when they were given 6 hour old broth culture containing 10^{-9} organisms/ml. at a dose of 0.2 ml. S/C 0.2 ml. I/P or 0.8 ml. by mouth on 3 successive days. The mortality was higher when coccidial infection was also associated with it. The death of the embryos was observed when the hatching eggs were immersed in 24 hours broth culture for 1-2 minutes. The chicks hatching out of such eggs were carriers of the disease. Hardy (1964) has reported the outbreaks of Coli septicaemia in 7-9 weeks old chicks. He showed the affinity of the organism to the pericardial tissue forming fibrinous lesions in the pericardium. The strains of E.Coli isolated from systemic lesions in chicks were found serologically related with the human strains and all showed the same pathological characters. Eight strains of E.Coli involved in the enteric

infections in man sheep and cattle were serologically unrelated to the avian septicaemic strains.

Gross (1964) reported a disease of chicken characterised by reduced weight gain and caseous youlk sac which was set up by dipping the incubating eggs in a culture suspension of E.Coli serotype '0'103 obtained from a field flock affected with a similar condition.

Woloszyn et al (1964) described Vitamin 'A' deficiency and feeding errors as the contributing factors to the winter out break of Coli infection. In acute infectious diarrhoea and the chronic infections emaciation and arthritis were the symptoms. The predominent serotype were '0' 1:K1, '0'71:K?. Experimentally acute and chronic infection could be produced by these serotypes.

Ciosek (1965) studied 110 E.Coli strains isolated from diseased fowls and 396 strains from healthy fowls with 10 '0' and '0K' stains. Of the 44 typed strains from the diseased fowls 19 were 02 :K1 :H4. The other belonged to group '0'8, '0'1, '0'3, '0'22 , '0'78 and OF 42. The birds with 02: k1 H4 had a syndrome characterized by Pneumonia, pericarditis , air sacculitis, hepatitis , enteritis and oedema of the spleen and kidney. The 23 typed strains from the healthy fowls belonged to group '0'2, '0'8, '0'78 , '0'3 and '0' 22.

(b) Physiological and other related Characters :-

It was since early times that use of fermentation tests was considered as a key-note for the different members of E.Coli group. It was Theobald Smith who gave

the fact that E.Coli was lactose fermenter whereas the Salmonella was a non-lactose fermenter.Escherich (1885) described the occurrance of two types of biochemical groups in his organisms one B.Coli or (E.Coli) formed fairly long rods, was motile and clotted milk slowly, the other type formed plumpy rods was non-motile and clotted milk more actively, the group was named as Bact. lactous aerogenes. Kruse (1894) emphased the heterogenicity of the group covered by the term B.Coli as usually employed since it included a related species widely distributed as intestinal organism and the organism present in water and soil. Smith (1895) observed that B.aerogenes produced more gas than B.Coli and Co2Hgratio was more in the former than in later. On the basis of series of formation test viz.:-

dextrose, lactose, sucrose, starch,inulin, action on litmus milk and indole formation resulted in the recognition of certain primary divisions within the group itself (Refik- 1896, Grimbert and Legros-1900,Dieshem-1901 and Gordon - 1903). One group B.lacti aerogenes fermented starch and inulin, was indole negative. The word lactus usually omitted from the name. The second and the third group differed from Bact.aerogenes in failing to ferment starch and inulin and in forming indole. They differed from each other in their action on the fermentation of sucrose. The sucrose negative strains corrosponded to the early strains of Escherisch (E.Coli Commine). The sucrose positive strain was latter named as Bact.Coli Communis (E.Coli Communis) by Durham (1901).

Voges and Proskauer (1898) described the differentiation of B.Coli organisms from the organisms belonging to other groups by the colour reaction which is positive in case of many other bacteria but negative in case of E.Coli. Durham (1901) showed that the Bact. aerogenes was V.P.+ ve whereas B.Coli was -ve. MacConkey (1909) observed the great predominence of V.P.negative reaction from the strains isolated from faeces. Positive reactions were given by 11 out of 178 human case.

Clark and Lubs (1913) devised the Methyle Red test for the differentiation of the members of Coli typhoid group of organisms. The B.Coli gave a positive reaction (red colour) whereas the other group Bact. aerogenes gave a negative reaction (yellow colour). Lavaine (1916) found a high negative correlation between M.R. and V.P. test. On the basis of Co_2H_2 ratio, M.R. & V.P.reactions, the organisms belonging to this group could be divided into two primary divisions. The first group giving $Co_2:H_2$ ratio of about 2:1 V.P. + MR-ve comprised strains which were isolated from plants, gains and unpolluted water or soil. This group could be further sub-divided into A.aerogenes and Bact.cloacae on the basis of gelatine liquifactin. The second division comprising of the ratio strains isolated from the intestines of man, animal and birds was having $Co_2: H_2$ ratio of 1:1, V.P.negative and MR+. This was typical of E.Coli Communa. Intermediate reaction between the two groups were seen.

Brown (1921) elucidated the usefulness of the medium containing citrate for distinguishing Bact. Coli from Bact. aerogenes. Koser (1923,1924,1926 a and b) gave the well known Kosers Citrate medium providing citrate as the main source of carbon. Coliform bacilli could thus be divided as Coli type (Mr = VP-Citrate-) Aerogenes type (MR-VP+Citrate+) Intermediate type(MR+ VP-Citrate+) Levine et al (1934) correlated the intermediate type with the production of H_2S in the ferric citrate agar.

Gale (1946) describes a number of decarboxylase enzymes in bacteria. Schafnitz (1951) used KcN. to check the resistance of the respiratory system of the organism to KcN in differentiating the A.aerogenes and Friendlander's bacilli from E.Coli, which was later modified by Moller (1954). The medium was useful due to the fact that E.freundii, Ballerup-Bathesda , Klebsiella and Proteus grew well in this medium, whereas Salmonella, Arizona, Shigella and E.Coli did not grow in the media.

Charter and Taylor (1952) carried out series of Biochemical tests of E.Coli strains from two nurseries. They showed that the strains which were antigenically related gave different fermentation reaction. Groups of strains of E.Coli gave three different patterns of fermentation though on agglutination

absorption test they were found to have identical 'O' and 'B' antigen. The variation led to the indication that the classification of the organism of this group on the basis of their biochemical reaction might lead to erratic results and was unreliable but the result of this pattern could lead to the indication of the different origin of the serologically identical strains. Edward et al (1955) also described different biochemical pattern of 58 Coli cultures from monkeys within 'O' group III. Similar observations were made by Wilson and Miles (1955) and Reed (1960).

Lovell et al (1960) studied haemolysin production in four E.Coli strains. Haemolysin infiltrates from cultures 4-24 hours old with highest titre in culture 6-10 hours old was demonstrated. The haemolysin was destroyed by heating and could be removed from the filtrate by 30% Ammonium Sulphate Solution.

Smith (1963) identified two different haemolysins alpha and beta from the haemolytic E.Coli strains from cattle, pig, sheep and men. The alpha haemolysin was filterable and identical to that described by Lovell et al (1960) whereas the beta haemolysin was not filterable. The haemolysin could not be distinguished on their affect on blood agar. The alpha haemolysin was toxic for mice, rabbit, guineapigs on intravenous inoculation but had no toxic effect when injected directly in the rectum of duodenum.

Martin and Obrien (1965) applied flourascent antibody technique to the culture of faeces on blood agar plates for the detection of enteropathogenic E.Coli. The study carried out on 364 faecal specimen by the conventional cultural method and the F.A. technique gave many advantages of the latter over the former. The results could be interpretted easier and quicker new serotypes were isolated than shown by the other cultural method. The inability to confirm serotypes which had been found by F.A. method was attributed to the inefficiencies in the cultural method.

(c) Serological characters of E.Coli :-

Jensen (1897) reported the presence of several different types of coli bacteria with fermentative main group A and B. Cross protection could not be achieved when sera against different strains of E.Coli was used by Joest (1903) who pointed the limitations of the biochemical tests in the typing of different E.Coli organism. Christzensen (1917) continuing the work of Jensen (1897) on typing of E.Coli found the differentiation of pathogenic from non-pathogenic strains impossible on the basis of morphology cultural biochemical and serological tests. Since the pattern of biochemical and serological tests. Since the pattern of biochemical and serological characters of the both was more or less similar.

Lovell (1927) successfully prepared antisera

and was able to classify E.Coli strains on the basis of their serological behaviour. The sera was prepared from rabbits by inoculating rabbits. Smith (1928) prepared sera out of the two different types of the colonies. Capsular and non capsular. The antisera prepared against one showed a high titre for the culture from the homologous daughter stain but had a low titre for the other.

Lovell (1937) described the presence of two different antigens in E.coli. One a soluble specific polysaccharide substance associated with the capsule 'K' and the o ther somatic 'O' antigen. He further demonstrated that the mucoid bacteria produced two corresponding antibodies when injected into rabbits. Kaufmann (1943) further working on the serological typing of E.coli described the presence of an 'L' antigen which prevented the agglutination of 'O' antigen in the hitherto inagglutinable strains. The antigen was thermolabile. He prepared sera against 'O' and 'L' antigen Kaufmann examined Ninetytwo E.coli cultures and found three flagellar 'H' antigen from 56 motile strains - 19 more flagellar antigens were added by Kaufmann and Vehlne (1944) and Vehlne (1945). The flagellar antigens of E.Coli showed little or no overlapping and were thus monophasic. Kaufmann (1945) selected 20 'O' groups and repeated the 1st antigenic scheme for E.coli typing. Same year he reported the presence of 'A' antigen in some of the strains which prevented the agglutination of 'O' antigen also. The colonies having 'A' antigen were

found to be whiter, denser and more opaque than those without such antigen and produced transluscent outgrowths of left for sometimes.

Knipschildt (1945) demonstrated the presence of heat resistant 'O' agglutination inhibiting antigen which was not destroyed by heating at 100°C. to which he named 'A' antigen in the capsulated bacteria Vahlne (1945) demonstrated that the inagglutinability could be removed if the same was autocloved for 2 hours at 120°C. thus rendering the typing of coliform according to their resistant 'O' antigen possible.

Kaufmann and Vahlne (1945) suggested the designation of thermolabile as well as the thermostable antigen of the capsule by a common term capsular antigen 'K'.

Knipschildt (1946) found 'B' antigen. This antigen differed from 'L' by retaining its antibody binding properties inspite of heating at 100°C, while it differed from 'A' antigen by losing its 'O' agglutination inhibition properties on heating to 100°C. Kaufmann (1947) formulated the elaborate antigenic schema for the E.coli group on the basis of his work and the work of Knipschildt (1945-46) and Vahlne (1945).

Since 1947 additions in the different serotypes have been done by the workers. More than 145 'O', 100 'K' and 50 'H' antigens have been recognised. Wramby (1948) showed a greater antigenic uniformity on the strains of E.coli isolated from diseased animals than those from the normal animals.

Guerdon et al (1957) studied III E.coli strains and found that type 08 was isolated from a calf, 2 sows , 2 fowls type '0' 25 from a sow with perpural sepsis type 086 and 026: B6 from 2 hens with granuloma and type 055 : B5 from pigs and 3 dogs.

(D) Bacteriophage Typing :-

The history of bacteriophage starts from the time when Twart (1915) while, working with staphylococci isolated from calf lymph vaccine, observed a visible degenerative change in some of the colonies. He named it as a Disease of bacteria. The filterate of these changed cultures initiated the disease of staphylococci. deHerelle (1917) demonstrated the occurence of rapid and generalised lysis of the shiga bacillus or broth to which some of the original mixed culture had been added. Transmission of the lytic agent to the susceptible cultures in series was also reported. He gave the name 'bacteriophage' to the lytic agent. Hylurgo (1931) described a method for the isolation of bacteriophage for various types of bacilli.

Luria et al (1943) described 3 phages of E.coli strain 'B'. Demerac and Fano (1945) studied a collection of seven coli phages and designated them as T_1,T_2,T_3, T_4,T_5, T_7.Anderson showed the presence of tail in all the phages except $T_3$and T_7. The latent period of T_2, T_4 and T_6phages vary from 20-25 minutes and T_1, T_3 and T_7, 18 minutes and for T_5 the latent period was 40 minutes (Delbruck -1946).

Nicolle et al (1952) concluded that E.coli strains of Infantile gastroenteritis could be classified by means of phage typing as some of the isolated phages were found to be showing lytic action over the strains of E.coli belonging to serotype) 111:B 4 and O 55 : B 5. Fraser and Williams (1953) described short tail on T_3 and T_7 phages. Morphology of all the seven T phages was studied by Electron microscope by Williams and Frazer (1953). The size of the various parts of the seven phages was also studied.

Smith and Crabb (1956) classified the E.coli strains of animal origin by the 16 phages viz.,A,B,C,D,E,G, H,L,M,O,T,X,Z_1,Z_2,Z_3 and Z_4 from the animals. Nicolle (1957) from the animals. Nicolle (1957) emphasized the importance of phage typing in the epidemiology of infantile gastro-enteritis. Smith (1960) studying the complecities of phage typing in animals stated that the bacteriophagic method of classification would only yield information on the predominent types of E.coli present.

Kasatiya and Singh (1961) carried out phage typing of 200 stains of animals and human origon with 43 phages isolated locally and 21 obtained from other places. The strain of E.coli from animal origon belonged to different phage types.

Ansari (1965) studied the susceptibility of 174 E.coli strains to T series of phages. He could not find any relationship between biochemical type, serotype and phage type between the different strains. Serologically identical strains belonged to various biochemical type

and phage type whereas serologically unrelated stains were similar in biochemical types and phage types.

(E)Antibiotic Sensitivity: of E.coli :-

Pulvertaft (1952) made tests with penicillin, streptomycin, auromycin, chloromphenicol and tetramycin on growing culture of E.coli. With Penicillin enlargement of the organism occured at all concentrations followed by lysis with the other four antibiotics death occured at high concentrations and at low concentrations enlargement of the organism and monster formation without division occured.

Smith and Crabb (1957) studied the effect of administering diets containing low levels of tetracyclin on the incidence of drug resistant Bact.coli in the faeces of pigs and chickenns. He observed a much higher proportion of tetracyclin resistant E.coli in the faeces of pigs and fowls given diet, containing low levels of tetracyclin than in the faeces of pigs and fowls living on farms where these agents had never been fed.Corey and Byrnes (1963) found oxytetracyclin resistant strains in the commercially processed chickens. They showed that these resistant forms arose in the intestines as a result of feed containing antibiotics.

Warden and Schaible (1960) showed that the pure cultures of E.coli introduced into the digestive tract of poultry via crop had no effect on the antibiotic growth stimulating mechanism in turkeys, poultry or heavier chickens. Lambelin (1961) studied the bactericidal effect of Kanamycin in concentration of 12.5-25 Mg./Ml. for

33 of the 40 pathogenic stain of E.coli.

Gross (1961) studied the effect of chlortetracyclin, erythiomycin and nitroforans for the purpose of treatment for experimental air sac disease. He observed that the infections associated with air sac disease could be controlled by Furaltadone in the feed or water. Furaltadone allowed better weight than Furazolidine. Chlortetracyclin and erythromycin reduced the severity of the lesions caused by P.P.L.O. alone but did not control the disease caused by E.coli alone. High levels of chloretetracyclin were required to render air sac infected with PPLO and infectious bronchitis virus resistant to E.coli invasion. Glautz (1962) tested 287 stains of E.coli, isolated from animal and poultry, for sensitivity to different antiboitics. The most effective compount were colistin chloramphenicol, furszolidone, N-1-amino-2 pyrrolidone, thiofuradene, and polymysin. Intermediate in activity were dihydrostreptomycin, chloretetracycline, tetracyclin and oxytetracyclin. The least effective were penicillin, oleandomycine, furaltadone, and nidroxyzone. Malik (1963) showed that the most effective out of the 8 antiboitics used by him against E.coli cultures , were Neomycin, tetracyclin, oxytetracyclin and chloramphenicol. Streptomycin and chloretetracyclin were only moderately effective, whereas penicillin and erythromycin had no action. Baxocsai and Szabadfy (1964) successfully controlled a dysentry due to E.coli with Neomycin.

Matvienko and Rudenko (1964) studied the sensitivity of 148 strains of E.coli isolated from dead, sick and healthy animal, with different antiboitics by

the paper disc method. Effective treatment for the Coli infection was with 75000 Units of Colisten orally, 200,000 Units of Mycerin orally or 50,000 Units of Monomycin I/M. 3 times a day for 3-5 days. Satisfactory results were also found on treatment with 0.2-0.3 gm. of furazolidone orally twice a day for 3 days repeated after 3 days interval.

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-: S A L M O N E L L A :-

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The genus Salmonella belonging to the family Enterobacteriaceae has a world wide distribution. There was a conception that the organ ism is host specific but the theory has altogether changed since any serotype pathogenic to an animal or a bird may be equally pathogenic to man Dolman (1954). The general habit of the genus is that it causes mortality in the young while the adults are much more resistant and often become carriers which remain a permanent source of danger in conveying the infection. The pathogens invariably affect the gastrointestinal tract causing varying degree of inflammation.

Domestic poultry forms the single greatest host reservoir for Salmonella (Buxton - 1957) and more than 100 serotypes have already been reported from poultry Williams (1959). It is most common in chickens, ducks and turkeys, it is quite often found in rhodents , less frequently seen in swines, commonly found in cattle , sporadic incidences are recorded in sheep and goats and occasionally in various wild animals and birds.

The historical background of the genus remained dormant till the end of 19th century though the clinical description of the enteric fever in man could be traced

as early as from the time of Hippocrats. As early as 1856, 1873. William Bud described the infective nature of the causal agents of the enteric fever and its properties. The desctructive action of the disinfectants on the contagion was also described by him.

Eberth (1880-81) was the first to observe typhoid bacilli in infected tissue and Gaffky (1884) who, succeeded for the 1st time in isolating the organism by cultural methods, named the organism as Eberthella typhosum which got the credit of being the 1st isolate of the genus. It was during that period that Salmon and Smith (1885-86) reported the isolation of Salmonella cholarae suis (Bacillus suipestifer from pigs.)

In human beings the 1st isolation of Salmonella was reported by Gaertner (1888) from one person who had died after the consumption of beef in Germany. The organism was named as Bacillus enteritidis (Salmonella enteritidis).

Every year several new organisms are reported at present more than 600 serotypes are known to be responsible for the diseases in human beings and animals or in both , Taylor (1960). Salmonellosis in animals occurs in almost all parts of world. The incidence being more high in poultry and is associated with wide variety of serotypes. It is significant that outbreaks are mostly caused by S.pullorum, S.gallinarum and S.typhimurum.

Edward and Bruner (1940) have described multiple type of paratyphoid bacilli in infections of fowls. They have

reported two new serological types of Salmonella, S. Saintpaul and S. lichfield Jangher (1940) observed that S.typhimurium was the most frequent organism found in birds and next in order of frequency was S. anatum. A varient of S. typhimurium was the cause of infection in pigeons (which had lost heat stable antigenic factor V) flies and others insects were reported to have acted as mechanical carriers of infection but possibility of the rhodents acting as intermediate between different hosts should be carefully observed.

Broomhead and Mann (1969) reported that, in an outbreak, source of infections was a dish prepared out of raw eggs. S. thompson was the organism isolated from the batch of eggs and serological examination of hens gave evidence of infection in the flock.

Walker (1960) has described the methods of production and marketing of broilers and their possible effect on public health. The use of antiboitics in the broilers ration results in the emergence of resistant stain which might cause an increase in the carrier rate. Spink (1960) has traced the origin of Salmonella thompson infections in 35 people to a brioler shop the organism could be isolated from cooked chickons and from the faeces of the manager and his assistant. The organism was isolated from living birds at a packing station supplying the shop.

Vala-dao (1961) reported the isolation of Salmonella stains from birds and animals in Mozambique

since 1937 as follows S.gallinarum (27), S.dublin (16) S.typhimurium (8) , S. enteritidis and pullorum (7 each), S.braenderup (4) , S. typhinewington , S.heidelberg , S. binz , S. anatum , S. montevideo , S. infantis , S.kottbus and S. muenchen (1 each). Thal et al (1961) isolated Salmonella lille, manhatten , montevideo and typhimurum from the liver and intestines of 25 of about 1600 fowls examined at Stockholm Veterinary Institute, Sweden. S.senftenberg , S.montevideo and S. manhatten were found present in the faecal samples from 4 of 146 apparently healthy flocks Fowls infected experimentally with S.montevideo excreted Salmonella intermittently with their faeces and yelded negative tube agglutination reaction and no postmortem lesions could be observed.

Cosgrove and Lindenmaier (1961) isolated organisms belonging to genes Salmonella from the lesions including the joint swellings from birds aged 3-4 weeks with a high incidence of swelling of hock joints and occasionally swollen foot pads, Colusi and Sequeira (1964) also reported an atypical stain of S.gallinarum from the outbreaks of hock joint of fowls which responded well to the treatment with furazolidone and chloramphenicol.

Kulasegaram (1963) has recorded S.hereilly and S. typhimurium infections in poultry in Ceylone. The source of infection has been attributed to the import of bird. Carrini et al (1963) recorded the isolation of S.braenderup , S. Schwarzengrid and laifa for the first time in Italy from broilers. The percentage of

isolation from liver and gall bladder of partially eviscarated birds was higher than from peritonial fluids. Simmon at al (1963) commenting over the isolation of Salmonella from fowls and different animals in Queensland noted that S.typhimurium was the most frequent species representing 28.7% of avian , 52.4% bovine , 48.3% of ovine and 14.2% porcine stains.

Gordon and Tucker (1965) have compared the cycle of infection of Salmonella menston and S. thomnson. They found similerity in them in the way that S. menston is present in adult fowls , their eggs, chicks hatched, and the eggs laid by their progeny at maturity. The importance of the occurrence of infection through water and feed, keeping in mind the fact that S.menston occurs in poultry, poultry products as well as man.

Salmonella serotypes isolated from birds in India :-

S.Jallinarum was isolated for the 1st time in India from a chicken at Bhowali - a hill resort in Kumaon Hills by Cooper and Naik (1931). Two isolation of the S.jallinarum were further recorded at Panjab and Mukteswar by IVRI workers. In the same year organism resembling S. anatum was isolated from dead chicks (1-17 days old) in the neighbouring areas of Mukteswar - Kumaon.

In 1947-48 S.bovis morbificans was isolated by IVRI workers at Delhi. S.typhimurum was reported by

Iyer and Rao (1950) from the birds at Izatnagar. Rao at al (1952) isolated S.gallinarum from an outbreak at I.V.R.I., Izatnagar.

Dixit (1952) isolated S.typhimurium and S. anatum which took a heavy lot of chicks at Poona. Pande and Nilkanthan (1953) isolated S. bovismorbificans from septicaemic cases killing a heavy number of chicks at Delhi. S. alachua from one outbreak in Poultry Farms at Bombay Das and Jayaraman (1955).

Rao (1956) and Ganguli (1958) reported the isolation of S. litchfield from chicks. Salmonella pullorum was reported to have been isolated at I.V.R.I. from the material recovered from Allahabad same year Ganguli (1958) reported S. enteritidis from fowls. Occurance of pullorum disease in the State of West Bengal was also reported by Das at al (1959). S. gallinarum was also isolated by them.

Gupta and Rao (1960) recorded an outbreak of paratyphoid in chickens and isolated S. concord and S. newport. An outbreak among chicks due to S.typhimurium was also reported by the same worker. Rao and Khera(1960) reported the isolation of S. dublin from carrier birds. Rao and Gupta (1961) reported the occurrence of S. dublin and S.waltevreden from outbreaks among fowls Izatnagar. Mullick and Rao (1962) isolated S.enteritidis from chicks at Izatnagar same year an outbreak

due to S. pullorum was reported by the same workers from M.P.

Sharma and Singh (1961) isolated S.chester, S. sandiego, S. richmond, S. anatum, S. weltevreden, S. hvittingfoss, S. champaign, S. pomona, S.matopeni during their survey of Salmonella S.Richmond and S.sandiego were also reported to have been isolated by them from dead embryos (in shell) and ducks respectively. Sharma and Singh (1963) reported isolation of S. bareilly and S. Chester from chickens of 1-15 days with typical lesions of necrotic foci on the surface of liver and enteritis.

Khera, Rao and Aggarwal (1965) isolated S. stanley from the cases of egg peritonitis in laying birds. The lesions observed were characterised by necrotic and hyperplastic lesions in oviduct and suppurative and necrotic lesions in ovaries. Nitrofurazone and Furazolidone were of no avail though immunization with autogenous vaccine was of some use.

Dutta and Singh (1965) for the first time recorded the isolation of S. Senftenburg from fowls in India while examining the faecal contents from pigs, fowls, ducks and pigeons.

Methods of Isolation:-

There is a large number of E. coli and other usual flora in the digestive tract of all the animals which usually out-numbers the pathogenic organism thus rendering their isolation difficult Von Drigalski(1904).

The need for the development of an enrichment medium was felt. The enrichment media inhibits the growth of most of the organism and provide better chances of growth to Salmonella organism.

Lentz and Tietz (1903), Loeffler (1906), Browning et al (1913) and Krum-weide and Pratt (1914) studied the growth inhibitng properties of various dyes so that they could be used in the isolation of enteric pathogens.

The widely used enrichment broth was formulated by Muller (1925) which was later modified by Kaufmann (1930-31). He suggested the use of bile and brilliant green to the Tetra-thionate broth. Studying the efficacy of the medium Kaufmann (1936) reported that the medium in conjunction with phenol red brilliant green agar of Kristensen et al (1925) increased to 100% the isolation of Salmonella paratyphi B and isolation of Salmonella from gastroenteritis case became 500% more than it should have been when no enrichment medium was used. Galton and Quan (1944) gave the credit of 64% increase in the isolation of Salmonella to the use of this media.

Though tetrathionate broth was widely employed still some workers prefer the use of salenite broth formulated by Leifson (1936) as enrichment medium when the material is suspected for Shigella and Salmonella typhi which are usually inhibited when tetrathionate broth is used in combination with brilliant green agar. Hobbs and Allison (1945) comparing the two enrichment

broth commented that Leifson salenite broth was more superior to tetrathionate broth in the isolation of Salmonella typhi and as good as the tetrathionate broth in the isolation of S. paratyphi B. Cook et al (1945) Armstong (1954) and Thomas (1954) found the salenite broth useful for the isolation of S.sonnei.

Smith (1952-59) described the essential use of brilliant green MacConkey broth for the isolation of S. cholerae suis since the organism did not give satisfactory growth when salenite broth was used.

Galton et al (1952) advocated the use of 0.125 gm. of Sod. Sulphathiazole/100 ml. of the tetrathionate broth. This greatly helped in checking the multiplication of Proteus organisms. Improved results were found when Pottasium tetrathionate was used in the place of Iodine and Sodium thiosulphate in the usual tetrathionate broth by Preuss (1949).

Nagel (1950) and Zschucke (1951) recommended the use of Streptomycin in Salenite broth. Since the Salmonella and shigella vary to a great extent in their antiboitic sensitivity use of streptomycin in enrichment medium is not very much recommended.

Bregman (1953) recommended the use of addition

of Magnaecium chloride to tetrathionate broth Rappaport at al (1956) formulated a Magnaecium chloride melachite green enrichment broth which when inoculated with faeces (diluted 1 in 1,000) gave more satisfactory results in the isolation of Salmonella organism other than S. typhi than the normal tetrathionate broth and salenite broth.

Hajna (1955-a) used a broth containing mannitol, glucose, sodium desoxycholate, sodium citrate and phosphate buffers in the enrichment medium given by him. This broth was GN (Gram Negative broth). Increased number of Salmonella and Shigella organisms were isolatedby this method.

Among the plating media Endo (1904) developed a selective agar which was later improved by Mayfield (1933) making it more selective. The addition of bile salt into the media made it more selective in action. Among them are MacConkey Agar and desoxycholate agar of Leifsons (1935). More differential plating media like that of S.S.Agar (Difco), KLJ (Agar Kristensen Lester and Jurgans Agar, 1925). Bismuth sulphide agar of Wilson and Blair (1926, 1927, 1931). The majority of the organism of the coli group and those of the proteus

group are inhibited by the use of this media.

Different authors have expressed varied views regarding the use of these media.

Hobbs and Allison (1945) have attained good results by the use of Salenite broth, Tetrathionate broth and MacConkey agar, Wilson and Blairs agar and Leifsons desoxycholate agar. Thompson (1953) have reported the satisfactory use of Brilliant green, MacConkey agar for isolation of paratyphoid organisms.

Hormaecher and Peluffo (1959) giving a scheme for the isolation of Salmonella and Shigella reported to have got excellent results with Wilson and Blairs Bismuth Sulphate agar but advocate the Wilsons formula recommended by Mackie Macartney (1953). He gave the advantage of the fact that it was easier in preparation.

Dexon (1962) while discussing the efficacy of Brilliant green MacConkey agar reported that satisfactory results were obtained with it when selenite broth was used for primary inoculation along with it.

Taylor (1962) has given DLFM medium (Dulcitol, Lactose, Iron, Agar medium)for the rapid identification of Salmonella and Shigella. According to him the medium off-ers primary recognition characteristic of Salmonella, Shigella and arizona early in analysis and greatly reduces

the number of identifications media necessary subsequently. It permits the determination of lactose and dulcitol fermentation, production of H_2S and motility simultaneously.

Miller and Banward (1965) studied the effect of various concentrations of brilliant green and bile salt on Salmonellae and other micro-organisms and according to them the inhibitory effects of brilliant green decreased as the concentration of bile salt increased. Staphylococcus aureus and Proteus were inhibited by all test media. E. coli was inhibited on all but two combinations of brilliant green and bile salts. Acrobactor aerogenes generally followed a pattern of growth similar to that of three species of Salmonella. Three of the 24 combinations of brilliant green and bile salt had little or no inhibitory effect on Salmonella but inhibited the other organisms.

Advocating a good combination of the media Edward and Ewings (1962) said that enrichment of plating media to be used are dictated by the particular circumstances under which one is working. If one is attempting to isolate only Salmonellae other than S.typhi then Tetrathionate broth which contains 1 in 10,000 dilutions of Brilliant green should be plated on Brilliant green Agar. The addition of Sulphonamides to one or both media may be found useful. On the contrary if one is interested in the isolation of S.typhi direct plating on bismuth sulphide agar is

indispensible and the use of varying amounts of inoculation is highly desirable salenite broth also has been found useful in isolation of S.typhi.

The use of different media was given by different workers to study the biochemical reactions in case of Salmonella before the use of serological methods. The media given were dextrose lactose agar (Russal- 1911), Sucrose and Mannitol agar (Kindall and Rayan -1919), Triple sugar agar (Krumwide and Kohn- 1917),Iron agar (Kligler - 1917). Medium to find out the citrate utilization as a sole source of carbohydrate was given by Koser (1923) and Simmons (1926).

Sturat et al (1945) gave urea medium for the differentiation of proteus and Salmonella.Christensen (1946) gave a medium for the determination of urease production modified by Kristensen (1948) and Hormache and Munnilla (1957).

Jacques and Singer (1950) gave two media for differentiating between Salmonella and Shigella from other members of Enterobacteriaeae.

Moeller (1954) gave the KCN inhibition test to differentiate the different organisms belonging to the family Enterobacteriaceae.

Trabulsi and Edwards (1962) discussed the

differentiation of Salmonella pullorum and gallinarum by biochemical methods. In addition to other usual tests the cystein gelatine medium and ornithine decarbozylase tests were found valuable. They concluded that Salmonella pullorum and gallinarum constitute two distinct biochemical types.

Since the first isolation of the organism belonging to the genus Salmonella S. typhi (Erberth-1880, Gaff.Ky.-1884). Many organisms have been isolated which had been a source of confusion for many workers as to their correct classification as most of them belonged to almost one biochemical pattern. Schuetze (1920) explored the possibility of the use of absorbed serum. It was the work of white (1925-1926), who recognised the importance of the necessity of considering recent discoveries concerning bacterial variation in the differentiation of bacilli, that the classification of Salmonella was placed upon a sound footing. Kaufmann (1941) modified, systemetised and extended it to form the present classification of the genus. This greatly helped in the rapid and accurate recognition of serological types.

Kaufmann (1942, 1950) described Polyvalent 'O' and 'H' serum which facilitated the recognition of Salmonella four 'O' serum (OA, OB, OC and OD) and four 'H'

serum (HA,HB,HC abd HD) were described.

Kaufmann (1954) modified the method of preparation of polyvalent sera.

Edward and Ewings (1962) approached the subject of polyvalent sera in a different manner. Only one serum was prepared and in this serum it was attempted to incorporate agglutinins for all the 'O' and 'H' antigen of the genus. Satisfactory results were observed.

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-:: P S E U D O M O N A S ::-

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The blue pus organism whose importance as a human pathogen was established about 84 years back(Gessard, 1882) are principally the bacteria found in water and in soil. They are characteristically known by the yellowish green pigment diffusing throughout the medium. They are known world wide. The literature contains numerous references as to its association with local and generalised infection in wide variety of animals and man.

Pseudomonas pyocyanea (Synonym Ps.aeriginosa)was recorded in association with the Tuberculous bacillus from a human case of pericarditis(Ernst,1893). Lartigan(1898)isolated the organism in an out break of epidemic of dysentry limited to 15 persons. Only in 4 out of those fifteen cases the disease proved fatal. The source of infection was attributed to be the water supply. The Pseudomonas infection as a secondary infection to Staphylococci leading to fetal results was reported by Brill and Libman (1899)from a case in which they could isolate the organism from the blood during life.

Pous (1927) reported that Ps.aeriginosa was more pathogenic in tropics where it may be responsible for typhoid like symptoms and abscess of liver. Severe generalised infection of infants particularly weak and premature ones resulted in multiple abscess formation and Bronchio-pneumonia In some cases the portal of entry was umbilicus whereas in other

cases the gastrointestinal tract or the respiratory tract was to be blamed for it. It has, however, been found in pure cultures in abscesses in different parts of the body and in man, cases of endocarditis and pneumonia have been reported in which Pseudomonas aeriginosa seems to have been the sole responsible micro - organism (Jordon and Burrows—1949).

The presence of Pseudomonas aeriginosa in a disease of equine had not been reported uptill 1949 when Doll, Brainner and Kinskaid (1949) isolated the organism from an aborted equine foetus. According to their report the organism had been previously isolated from an aborted equine foetus at Kentucky Station in 1944. The organism was frequently encountered in the genital tract of both equines and bovine species.

Knox (1953) reported enzootic Pseudomonas infection on three minks Farms in Denmark.

Barick and Benner (1920) isolated the organism from the cases showing Pneumonia in pigs. These pigs had been vaccinated against Hog cholera. Attempts to reproduce the disease failed on oral or parenteral administration(S/C or I/V route) or by direct contact. However, paralysis of the hind limbs could be observed following I/V injection, of a large dose of the broth culture though in those cases also death did not follow.

Pickens at al (1926) reported the presence of this organism as the cause of recurring mastitis in cattle. The source of infection could not be known nor the disease could

be reproduced. Cases were recurrent type and the calves from the affected breed showed symptons of fetal diarrhoea.

Essex et al (1930) reported Pseudomonas pyocyanea in an epizootic form of disease in a flock of about 400 white leg horn and plymouth rock chicks 5 - 9 weeks old. Only 25% of the chicks could survive.The possibility of the death due to coccidiosis and other helminthic worms was ruled out by the faecal examination.The organism was recovered from cultures of heart,liver,spleen and brain from about 95% of the fifty chicks under study.No gross lesions were observed except the congestion throughout the viscera.The experimental reproduction of the condition was not very successful,however,the adult birds were found to be more resistant than the young chicks.

Farrag and Mahmond (1953) reported the association of the Ps. aeriginosa organisms with a high percentage of cases of ottorrhea in dogs in Cairo,Egypt. Purulent discharge was seen in the ears of those dogs.

From India Azizuddin and Chandrashekharan Nair (1954)reported the isolation of the organism from calves in Madras State.It was the cause of enteritis in these animals. ElNasri (1959)reported the outbreak of enteritis from a dairy herd in Sudan.

De Narasquez (1956) reported the experimental production of Pyelonephritis in rabbits by inoculating Ps.pyocyanea in association with E.coli. The lesions could,hoever,be established in animals with pre-existing renal scars due to causal previous staphylococcal infection.The relationship between renal scarring and subsequent infection was regarded as established both in rabbits as well as man.

Calaprice (1958) reported the outbreak of a disease in chickens. The disease affected chickens aged 40-45 days. Mortality was first low which reacted high after sometime. About 1,000 chicks died in a few days. The postmortem changes included congestion of the visceral organs enlargement of the spleen, catarrhal, and haemorrhagic enteritis and in some cases minute abscesses in the kidney were also observed. On microscopic examination of liver and spleen, kidney and lung Ps. pyocyanea was observed. Intraperitoneal or intravenous injection of the culture was fetal for rabbits and guinea pigs in three days. Chickens died 15-24 hours after I/V or I/P injections. Streptomycin was the antibeitic which gave successful treatment.

Williams, Williams and Hyams (1960) described the Ps. aeriginosa to be an important cause of infections acquired in hospital in infants as well as adults who had undergone operations or had been trea-ted with broad spectrum antimetabolites.

Valdao (1961) while describing the septicaemia due to Pseudomonas infections in poultry reported that birds 4-6 weeks old were susceptible whereas the birds over 6 months old were resistant similarly the transmission could be successfully achieved in young birds but not over three months. Pigeons and rabbits were resistant whereas G.pigs and mice were highly susceptible and succumbed to the experimental infections. Oral dosage of sulphanilamides gave satisfactory results.

Gorrill and DeNavQuez (1964) successfully produced infections of the normal urinary tract of mice by I/V

inoculation of E.coli, Ps.aeriginosa and proteus mirabilis. The ability to establish urinary infection was described to be greater in Ps.aeriginosa and least in E.coli.

Haogsma and Pereboom (1965) described an acute haemorrhagic purulent pneumonitis due to Ps.aeriginosa in minks in Netherlands taking a toll of about 900 minks which was nearly half the number of the total strength of the Farm. The formalized vaccine, prepared out of the strain of Ps.aeriginosa isolated from the farm, prevented the further lossess.Administration of various antiboitics was ineffective and the source of the infection was believed to be the contaminated water.

Mulcock at al (1965) reported a blue colouration of the fleece wool caused by the pigment of Ps.indigofora.

Gy.Gaidaes (1966) observed in a large dairy herd the excretion of Ps.aeriginosa from 19 clinically affected cases of parenchymatous mastitis in Hungry. 34 strains of the organism were isolated from the milk samples of clinically symptomless animals. The source of infection was found over to be water used for the washing of udder during milking. Since the Pseudomonas with identical boilogical properties were found to be isolated from the milk as well as the water used for washing of udder.

STREPTOCOCCI:-

Rivolta (1873) described chain forming cocci in the pus from cases of strangles in the horse. Rosenbach (1884) isolated from an abscess in man a coccus to what he gave the name Strept - pyogenes thus establishing the genus name 'STREPTOCOCCUS'. Klein (1886) demonstrated the relationship of the Streptococcus to Scarlet fever.

It is present as a normal inhabitant of the faeces and the gastrointestinal tract of almost all the animals. Lev and Briggs (1956) and Smith (1965). According to Smith (1965) they were present in much smaller number in species other than carnivora and herbivora. The number of the Streptococci declined with the age except in the case of caeca.

Streptococcal infections occur sporadically in fowls. Norguard and Mohler (1902) isolated for the 1st time Streptococci from the Septicaemia cases in chickens. Dammann and Mangold (1905) described an encapsulated Streptococci associated with an epizootic attack of sleeping sickness in chickens. They gave the name "Strept. capsulatus gallinarum".

Grave (1908) and Magnusson (1910) and Hudson (1933) reported the highly acute speticaemia of fowls caused by a streptococcus. Kernkamp (1927) associated the organism with the chicken died of an idiopathic peritonitis. It has been associated with many chronic infections also (Dammann and Manegold - 1905, Edward and Hull - 1937).

Gibbs (1931) isolated haemolytic streptococci from the inflammatory exudate from the cases of Pharyngeotracheitis in fowls. Appolosora (1938) observed streptococcal pneumonia

in fowls as a complication of Nuttaliasis.He assumed that the outbreak was due to mixed infection and Str.pyogenes,not normally pathogenic,induced disease due to the low resistance of the animals which had been weakned by the Nuttalia invasion.

Polgov at al (1940) observed enzootic purulent bronchopneumonia in fowls.Diplococci similar to pneumoncoccus were isolated in all cases.Harms(1941)obtained the pneumoncoccus in almost pure cultures from organs of fowls suffering from Pneumonia.

Moore B(1948)reported an outbreak of food poisoning in a School affecting several children apparently caused by a haemolytic streptococci.The source of infection was pudding prepared a day earlier,served to the children.The extract of the streptococci produced symptoms in human volunteers,whereas washed viable organism did not produce the symptoms, cause is attributed to be the toxin produced.

Agrimi(1956)gave a short description of six outbreaks of streptococcal infection in fowls in Tuscany.The mortality in adults was 5-10%,in chicks 50-60% and in embryos 16-19 days old about 70%.The strains isolated were Str.zooepidemicus.One case was due to Beta haemolytic Stren.faecalis.

Describing the effect of antiboitic supplements on the faecal streptococci Lancefield groups of poultry,Barnes(1958) reported the presence of two faecal streptococci in the gut of 10-12 weeks old bird.There were Str.faecalis var liquifaciens and Str.faecium. The inclusion of chlortetracyclin in the feed throughout the life or for a short period did not alter the total

number of faecal streptococci present. It led to the development of a large number of resistant, non-proteolytic strains of streptococci faecalis which replaced the other strain normally present. Three months after discontinuing the chlortetracycline supplements faecal samples indicated that the atypical strains had disappeared again from the birds previously given the chlortetracycline Str. faecalis var liquificiens once more being dominating. Caecal contents examined after 5 months confirmed this. Elliott and Barner (1959) studied the serological types and antibiotic resistance of lancfield group D Streptococci in chicks receiving dietary chlortetracycline. They showed that the controls in which only chlortetracycline sensitive streptococci were present initially strantococcus faecium predominated and Str. faecalis was present in small numbers representing three serological types(Type 1169D5, D15 and D76). Administration of chlortetracycline whether at low concentration throughout life or intermittently at a high concentration led to the emergence of a highly resistant non-proteolytic strain of Stren. faecalis becoming predominent in the treated birds.

Studying the causes of mortality in chickens upto 10 days old in South Australia Walts and Rae (1958) reported that the greatest single factor was Omphalitis 48.7% followed by unabsorbed yolk sac 30.7%. These conditions were due to streptococci. In certain cases it was in conjunction with E. coli and Pseudomonas.

Sato at al (1960) isolated Str. zooepidemicus from lungs and peritonial exudate of a number of fowls from a flock of 56, Six of which died of septicaemia or accompanied by

respiratory symptoms. Six of 13 adult fowls injected I/V or I/P with freshly isolated strains or cultures 18 months old or suspension in saline of naturally infected yolk died with similar clinical symptoms one day to 5 weeks later. Mice and rabbits were susceptible, G.pig appeared to be resistant.

Andrews at al (1961) reported that chicks less than three hours old were uniformly killed by infection with relatively large number of virulent pneumococci. When infected at 4 or 5 days of age nearly all survived and resistence apparently persisted for life. Serum of freshly hatched chickens did not protect mice against Pneumococcal infection but when mice were treated with serum of chicks 4 or 5 days old nearly half of them survived. They suggested that the fowls resisted pneumoncocca-l infection either by inhibiting multiplication or by lysin of the organism.

Gross and Domermuth (1962) reported the presence of Str.faecalis from bacterial endocarditis in poultry in conjunction with Staph. aureus and Past.multocida. The above cultures were also isolated from the liver of chicken and turkeys. The each of the culture was capable of reproducing the disease.

STAPHYLOCOCCI:-

Ogston (1881) pointed out the constant presence of Staphylococci in the acute and chronic abscesses. Rosenbach (1884) obtained pure cultures of Staphylococci and gave the generic name"STAPHYLOCOCCUS" as proposed by Ogston.It was divided into species Staph.pyogens aureus and Staph.pyogenes albus. Passet (1885) added the third species Staph.pyogenes citreus.Later workers found it difficult to place the isolates in different groups,Wilson and Miles (1964) proposed to recognise a genus Staphylococci including yellow and white Staphylococci commonly found in animals forming grape like clusters in solid medium and a genus micrococcus containing yellow white and red cocci occuring in tetrads and leading Saphophytic type Staph. aureus contained almost all the pathogenic cocci of animals.

Miller (1948) described the case of food poisoning in the form of vomitting and diarrhoea, due to the consumption of meal containing guineafowl gravy and sausages.Coagulase +ve type of Staphylococcus aureus was found in the faeces of two patients out of seven examined Staph.aureus was held isolated from the unconsumed food also.Staphylococcal toxin have been associated with the cases of food poisoning in man (Hange- 1953), Roseti (1953). Food of the animal origin held responsible for the complication in Italy.

Staphylococci are the organisms found almost in all the situations. In the animal body they are normally found in the nose, on the skin, in saliva, and in the intestinal contents. Hallman (1937), McFarlan (1938), Gillespie et al

(1939), Routree and Barbour (1951) have shown that they are present in the anterior nares of a high proportion of normal persons and that 30 to 60% of the persons are nasal carriers of potentially pathogenic Staphylococci.

It has been found as a normal inhabitant of the gastrointestinal tract during the study of the normal flora of gastrointestinal tract by Lev and Briggs (1956) and Smith (1965).

Staphylococci has been associated with many chronic or acute conditions of fowls although the cases may be sporadic. Outbreaks also occured in which upto one half of the total flock was affected. The conditions have been mostly associated with poor husbandry conditions in which wounding specially of the feet was liable to occur and it could be reproduced by I/V injection of Staphylococci cultures (Hola and Purchace-1931) Jungher and Plastridge-1941 and Smith-1953). Gibbs (1931) isolated 73.8% of Staphylococci from the respiratory tract of domestic animals.

Smith (1954) produced acute diseased conditions in fowls by inoculating I/V stains of Staphylococci isolated from poultry , vaccination by the S/C injections of live cultures did not prevent chicken developing the disease when challanged but it reduced its mortality rate. The disease was staisfactorily treated with Penicillin, Streptomycin, Aureomycin and teramycin but not chloramphenicol.

Fahey (1954) described an arthritis due to Staph. Pyogenes Var aureus amongst 400 turkey poults of 10 days age in which 81% of the birds were affected. Mondini and Quaglio- (1959) have reported the cases of Osteo arthritis

in 100 outbreaks in Italy. The causal organism being Staph. aureus. The disease could be produced by the I/V or S/C inoculation or by scarification.

BlancoLoiselier & Vindell Caurin (1955) studied the Staphylococceal infections in fowls. They isolated several strains of Staphylococci and on study divided them into two types. The acute form which was characterised by inappetance, pallor of the mucosae, diarrhoea, ruffling of feathers and death in 12-48 hours. In some cases haemorrhagic oedema and loss of feathers were also seen. The subacute type was characterised by lameness due to inflammation of the tibiometatarsal and planter joints Ankylosis followed and there was death due to cachexia. Moudini and Quaglio (1956) gave an account of widespread generalised infection of fowls with Staphylococcus aureus mainly Penicillin resistant strains and occasionally with Pseudomonas pyocyanea also resistant to antibiotics. Such cutaneous lesions were found in the neck round the eyes, back, thighs claws and interdigital space and characteristically on the wings. Miliary nodules were found in liver and spleen. Ducci and Rosatelli (1959) treated the wing gangrene, in two flocks, caused by Staph. pyogenes with Trisulfan (consisting of Sulphadimidin, Sulphadiazin and Sulphamerazin). Mortality which was 3% completely ceased 3 days after the treatment.

Dalsanto (1959) investigated the cause of death of 250 chicks aged 2 days which died within a few hours after a train journey lasting 20 hours. He attributed the, cause of death to be due to Staphylococcus pyogenes isolated from the caeca, yolk sac and heart blood.

Smith and Crabb (1960) studied the antiboitic sensitivity of Staph. aureus isolated from the nose and other organs of pigs and chickens kept under commercial conditions and from their attendant. The stains isolated from the animal or birds which were kept on diets containing antibiotic were more resistant to the action of antiboitic than those isolated from animals, birds not kept on antiboitic diet . This also applied to their attendant. Phage typing and other tests showed that the antiboitic resistant stains from the attendants were usually identical with those from their pigs.

Gross (1962) isolated strains of Staphyloccoccus aureus and one of Pasterella, along with strains of Str. faecalis from the liver of chicks and turkeys with endocarditis. Each of the strain was capable of reproducing the disease in chickens and turkeys when inoculated I/V.

LACTOBACILLUS:-

Kern in 1881 for the 1st time isolated the organism from the fermented milk of the Concasus. Similar bacillus was observed by Do'derlein (1892) from the acid vaginal secretion of pregnant women. Moro (1900) cultivated a similar bacilli from the faeces of breast fed infants the organism was named as B. acidopilus also confirmed by Finkelstein (1900). Tissier(1900) isolated two new organisms of the same group from the faeces of two infants to which he gave the name B.bifidus and B.exilis. VonFrendenreich and Thoni (1903) isolated a number of bacilli forming lactic acid from the cheese. This was named as B.casie by Orla - Jensen (1904). Sherman and Stark (1927) showed the presence of these organisms in the milk.

Mereshkowsky (1905, 1906) and Petiow (1907) isolated the organism from the faeces of a large species of invertebrate, fishes,and mammals.Heinemann and Hefferan (1909) isolated the organism from the human saliva and gastric juice.

The number of lactobacilli increases on the intestines when lactose or dextrin is given in considerable quantities in the diet Rettger and Cheplin (1921) Cannon and McNease (1923) Kligler (1915) Snyder (1939) and Harrison and Opal (1944) noted the increased numbers of L.acidophilus in the mouth of people

Smith and Crubb (1961) has shown the presence of lactobacilli in this faeces of almost all the animal and man. Smith (1965) while working on the normal microflora of the gastrointestinal tract of different animals has shown that lactobacilli constituted the major component of the microflora of the stomach. Small intestines, and in the some cases large intestines in most of the animals and birds particularly those whose diet was mainly cereal.

MATERIALS AND METHODS.

-:: MATERIALS AND METHODS ::-

Source of Collection:-

The material in the present study was collected from apparently healthy, carcasses of the diseased birds and from the slaughtered birds. The entire gastrointestinal tract starting from the proventriculous at its junction with the crop, to the last portion of colon at its junction with the cloaca was taken in the study. Faecal samples were also collected for examination from healthy birds and birds showing symptoms of diarrhoea (3o and 20 birds respectively).

Details of the samples collected from different sources and different regions of the gastro-intestinal tract are tabulated as under in Table No: 1.

TABLE NO: 1.

Sl. No.	Source of Collection.	Carcasses of the diseased birds.		Healthy		Cloacal Swabs.
		Ant. Reg.	Post. Reg.	Ant. Reg.	Post. Reg.	
1.	Central Poultry Farm, Patna	50	50	25	25	25
2.	Private Poultry Farm	50	50	-	-	25
3.	Slaughter House	-	-	25	50	-
	TOTAL:-	100	100	50	50	50

Anterior region:- Proventriculous and gizzard.

Posterior region:- Small intestine at its junction with the gizzard to the large intestines at the junction of the colon with the cloaca.

In the case of Central Poultry Farm and the Private Poultry Farm the white leg horn and the Rhod Island Red breeds of the fowls are maintained. In case of the Central Poultry Farm they are given the usual feed mixture consisting of maize, bran, ground nut cakes, fish meal, burseem meal, limestone, bone meal, common salt and mineral mixture. In addition to this sufficient quantities of Vitamin A_1, B_2 and D are added. In case of chicks TM_5 and Bifuran are also added to the above mixture, though the TM_5 and Bifuran was occasionally fed. Piperazine adepate was used for the occasional deworming of the fowls The feeding at the Private Poultry Farm was also mainly on the same lines little change of constituents. The antiboitic feeding was not practiced at the Private Poultry Farm. In case of poultry birds from the Private slaughter house no idea about their feed or breed could be had as the birds were purchased from different sources with different management. The age of the birds in all the cases ranged from few days old chicks to adult birds. The material was collected during the period from the month of April to September, 1966.

In all, materials from 100 dead birds and 75 healthy birds besides the 50 cloeal swabs were collected

as detailed in Table No: I. Twenty samples from the diseased birds were discarded due to the fact that the material was highly putrified and was not worth processing. That way eighty samples each from the anterior and posterior portion in case of dead and 50 and 75 samples from the anterior and posterior portion respectively were collected from the healthy birds totally 285 samples +50 swabs.

Methods for Collection:-

Both the anterior and the posterior portion of the gastrointestinal tract was tied with a piece of thread to avoid further contamination and leakage of the material. The material was kept in sterile petridiobes and taken to the laboratory and processed as early as possible. In case of cloacal swabs the cloaca was first cleaned thoroughly with a clean sterile cotton. The previously sterilised cotton swab was then inserted into the cloacal region taking care that the intestine was not injured and theswab wasl also well smeared with the faecal contents. The swabs were then again plugged back into their original tubes, which contained plain broth.

At the time when the material was collected from the private poultry farm there was a mortality of about 10 - 15 birds daily in the farm where there was a flock of about 900 adult birds. Even with the usual antiboitic medication there was no check of martality.Fowls of all ages were affected viz., adults, layers and chicks.

The fowls did not show much of conspicous symptoms except keeping away from the rest of the flock. Dull, diarrhoea and listlessness were accompanying symptoms. On postmortem examination the enteritis was observed along with congestion of the visceral organs. On incising through the intestine a yellow viscid fluid was seen in the whole intestinal region giving a very foul odour. The blood smears were also collected from such cases and were stained with Gram's and Leishman's method.

Inoculation into Different Media:-

Before inoculation the contents of both the anterior and the posterior group were collected in separate sterilized tube after properly cleaning the surface with 70% alcohol. After the intestinal contents were removed the whole organ was opened with a sterile scizzor to check gross changes, if any. Pieces of mucus from different regions was scrapped with a sterile scalpel and was added to the respective tubes containing removed from the intestinal tract. This was done to get as much of the organisms as possible. The faecal swabs were immersed in plain broth tubes which were incubated for sometime and then put to process.

The culture from this collected material was attempted in the following media——

i) Blood Agar;

ii) Plain Broth;

iii) Tetrathionate Broth; and

iv) MacConkey Agar.

The isolates were further processed as and how it was required by a particular group of organisms as described.

E. COLI

The red lactose fermenting colonies from the MacConkey's Agar were picked up and subcultured on plain agar slants which were incubated for 24 hours. The morphological characters of the organism were studied after staining with Gram's method of staining. The motility was checked with the solid motility medium. The usual Biochemical tests viz., Indol, M. R. V. P. Nitrate reduction test growth in citrate media (Koser's Citrate Media) were studied after 4 days of the incubation. The gelatine liquifocation and the H_2S production were checked in the combined media.

Peptone water culture was used for the inoculation of different sugar tubes. Sugar used were Glucose, Lactose, Mannitol, Sucrose, Adonitol, Dulcitol, Salicin and Inostol. A drop of liquid paraffin was added to each of the sugar tubes to check the presence and absence of gas in addition to the acid formation. Andred's

indicator was used for checking results. The sugar tubes were incubated for 24 hours, the tube showing fermentation reactions were noted and the remaining tubes were further incubated upto 10 days and the results checked every 24 hours.

The Eosine methylene blue agar plates were also streaked with the culture to check the characteristic metallic shear.

Cultures which were Indole + MR + Nitrate + VP -ve, non - gelatin liquefiers - ve were selected for other tests of E. coli.

Bacteriophage Typing:-

T_1 to T_7 series of Bacteriophage were received with the kind courtesy of Dr. B. N. Singh and Shri J. N. S. Yadava of the Department of Microbiology, Central Drug Research Institute, Lucknow. The phage was propagated with the method advocated by Smith and Crubb (1956). To the 18 hours old broth culture of the Bacteriophage propagating strain (E. coli - B) two loops full of the phage were added. The tubes were incubated at 37° C. for 24 hours and then kept in room temperature for another 3 days. Lysis was observed in the culture. Again two loops full of the phage were added to the lysed culture and the process repeated 3 - 4 times till appreciable lysis was observed in the tubes. The phage preparations were then

centrifuged at high speed and the supernatent was collected. 0.1 ml. of chloroform was added to 10 ml. of the phage preparation to kill the bacterial growth. The phage was diluted then in 10 fold dilutions with phosphate buffer and the critical dilution at which maximum confluent lysis was seen was calculated and the phage was accordingly diluted with phosphate buffer. The diluted phage which was 10^{-7} in concentration was checked for its efficiency. One of the phage was mixed with 10 ml. of the broth which had been cultured with phage propagating strain B. The mixture was spread over a dried plain agar plate and was incubated for 24 hours after the fluid portion was drained out. More than 200 plaques could be counted. The propagated phage was stored in the referigerator though the period to avoid the chance of the growth of the varients in it.

For actual typing of the culture the 6 hours old culture was uniformly spread over a dried plain agar plate. The excess of the culture was removed with the help of a sterile pipette. The plates were incubated at 37°C. for an hour or so for the drying of the fluid portion. Each of the seven phages were put with the help of Platinum loop on the inoculated plates taking care that the drops do not collase with each other. The circular areas were marked on the outside of these plates before the phage was put. These plates were kept in the same position for about half an hour so that the phage sticks to the plate and then the plates were incubated at 37°C. for 6 hours and then kept at

room temperature. Those showing confluent lysis were marked as ++++ whereas those showing semi - confluent lysis were marked as +++, ++, + according to the extent of lysis produced.

Serological Typing:-

Method adapted for the determination of serotype was the same with little modification as given by Kaufmann (1947) and Barua et al (1956).

Antiboitic Sensitivity:-

Neomycin, Tetracyclin Hydrochloride (Achromycin), Demethyl Chlortetracyclin Hydrochloride (Ledermycin), Chlortetracyclin Hydrochloride (Aureomycin), Dihydrostreptomycin Sulphate and Furadentin (Nitrofurazone) were used for the antiboitic sensitivity. All the antiboitics except Neomycin, Furadentin were received from the College Pathology Laboratory who in turn had received from M/S Cynamide India Ltd., and Sarabhai Chemicals. The other two antiboitics viz., Neomycin and Furadentin were purchased from the local market.

Disk method for the antiboitic sensitivity as described in Cruickshank's Medical Microbiology (1965) was adapted with slight modification. Filter paper discs of the of the size 6.5 m.m. were cut with the help of a paper hole punch using Whatmann's No: 1 filter paper. The discs numbering 100 were put in empty cleaned Penicillin Phials,

and was sterilised in dry heat at 150°C. for one hour. The antiboitics were diluted with sterile distilled water. One ml. of each of the antiboitic solution was added to each of the phial of 100 discs so that each of the disc contained approximately 0.01 ml. The dilutions were made in a way that each disc contained 10 megms., 25 megm., 50 megm. and 100 megm. The antiboitics were kept in the referigerator throughout the period of study.

18 hours old broth culture of the organism was spread over the dried and incubated nutrient agar plates. The plates were dried for sometimes in the incubator. Each of the disc from different dilutions of each antiboitic was kept on circular areas previously marked in the plate with the help of sterilized forceps. The forceps after use every time was put in 70% alcohol and heated in the gas flame. The plates were incubated for 24 hours and the reading was taken next day. The area of the zone of inhibition was measured. This area included the diameter of the disc as well as the surrounding zone of inhibition. The particular dilution of any antiboitic giving an inhibition zone of less than 10 mm. was declared as resistant whereas the sensitivity of the antiboitic was accordingly put as ++++, +++, ++ and +.

Pathogenicity Test:-

The pathogenicity of E. coli was done in the white

albino mice. In case of two cultures the pathogenicity was tested in the rabbit and fowls after the pathogenicity was established in mice. These two cultures were isolated from cases of coligranuloma. The mice were inoculated intraperitonially with one ml. of 10^{-9} 18 hours old culture. The rabbit and the fowls were inoculated intravenously, dose being 1 ml. In case of two fowls the culture was given directly into the digestive tract with the help of a pipette for 3 days at the rate of 1 ml., care was taken so that the culture did not enter the trachea. Two fowls were kept as controls. The fowls used were of 'white leghorn' and were 8 - 12 weeks of age. After a particular experimental animal (fowl, rabbit or mice) had died the postmortem was performed and attempts to isolate the cultures were made. The culture isolated was again inoculated into one mice to complete the requirements of "Koch's Postulates". After all these tests were completed the cultures were maintained after sealing with paraffin in the refrigerators and sub - cultures were made at regular intervals.

SALMONELLA.

The enrichment broth used was Tetrachionate broth prepared by the method given by Muller (1925) modified by Kaufmann (1930 - 31). Since the ready made media loses its efficacy on keeping all the ingredients required viz., Sodium Thiosulphate in 60% solution fresh bile after filteration, calcium carbonate, equal to the

amount required for about 250 ml. of the broth was separately autoclaved and kept as the stock requisites. At the time when the material was to be inoculated the ingredients were mixed, the required quantity of iodine solution and brilliant green was added so as to make the final concentration of 1 : 10,000 of the brilliant green and the prepared media tubed in sterile test tube about 8 ml. in each tube. The tubes were boiled for sometime cooled and then inoculated. The tubes were incubated for 24 hours but in certain cases the incubation was extended for another three days. In some cases the contamination was much in that case a second sub - culture was used to give more enrichment to the Salmonella organism and reduce the contamination.

For primary culture brilliant green (Kristensen, Lester and Jurgens - 1935 modified by Kaufmann, 1954) was used. To this brilliant green agar medium, sodium sulphadiazine at the rate of 2 - 16 mg/ 100 ml. of the medium was added as suggested by Galton et al (1954) to avoid the growth of Pseudomonas. Drigolski's Agar was also used in certain cases.

The positive colonies were picked up from the plates and sub - cultured on plain agar. While picking up the colonies care was taken not to touch the platinum loop with the medium in the plate as some of the organism, which remain viable in the selective medium but do not multiply

due to the inhibitory effect of some of the chemicals, start multiplying in the plain agar which is without any inhibitory compound and give erratic results in their biochemical and sugar fermentation behavior (Hormache *et al* - 1959). The slants were incubated for 24 hours at 37°C.

The purity of the cultures was checked on the morphological characters using "Gram's method" of staining. The growth was again checked by plating the growth on MacConkey agar media in which lactose was substituted with mannitol. The non - lactose fermenting colonies in MacConkey agar (used for *E.coli* isolation) were also processed and checked for the presence of Salmonella organism.

From the pure growth on the plain agar slants tests for biochemical and sugar fermentation were put as detailed in *E. coli*. The sugars used were glucose, lactose, mannitol, lactose, adonitol, dulcitol, salicin, sucrose and inositol.

The Hydrogen Sulphide production was checked by inoculating the growth on Triple Iron Sugar Agar. The citrate utilization activity was checked in Koser's Citrate medium (1923). Christensen urease media as modified by Hormache and Munnilla (1967) was used for the checking of urease activity. The cultures were also tested for gelatin liquefaction and motility in the same semi -solid

media for motility as in case of E. coli.

Glucose, mannitol, dulcitol, positive cultures with + ve citrate, H_2S and M.R. reaction and negative V.P. gelatine and urease reaction were selected for further studies.

'O'-1 bacteriophage was tried to check the lysogenic activity in the culture in the same way as done for E. coli. In this case instead of spreading the 6 hours old culture it was put as a drop on the plate of nutrient agar. After the drop was absorbed on the plate drop of 'O'-1 phage was put on the drop of the culture. It had the advantage that on one plate many cultures could be tested. The reaction was observed on the next day after incubating at 37°C.

Antiboitic Sensitivity:-

The antiboitic sensitivity test was done in the same way as that of E. coli on using the same antiboitics and same dilutions.

Pathogenicity test of the culture was done by giving 1 c.c. of 10^{-9} 18 hours old culture I/P to white albino mice. No pathogenicity tests were tried on fowls.

PSEUDOMONAS.

The pale yellow coloured colonies on Brilliant

green agar and MacConkey agar and Blood agar were taken from the plates (This was the case specially for the plates inoculated with the material collected from the Private Poultry Farm where there was mortality). These colonies were sub cultured on plain agar slants.

The tubes showing green pigment changing to brownish after sometime was processed for the typing of Pseudomonas. The organisms were streaked on the blood agar to check the haemolysis. Morphological characters were studied by using Gram's method of staining. The motility was checked by the semi - solid agar method.

Indol production, M. R. V. P. Nitrate reduction H_2S production and citrate utilization test were done in the same way as that of E. coli and Salmonella. The sugar used were glucose, lactose, sucrose, maltose and mannitol.

The pigment production was checked by treating the nutrient agar slant with chloroform. The greenish blue colour obtained was treated with hydrochloric acid to get the red colouration showing the presence of pigment pyocianine characteristic of the organism.

The proteolytic activity of the organism was checked by streaking the growth on the serum agar plate which was incubated at 37°C.

Antiboitic Sensitivity:-

The antiboitic sensitivity was done with Penicillin 100,200,500 and 1,000 I.U. Dihydrostreptomycin Sulphate and Neomycin in the same dilution as that used in case of E. coli and Salmonella cultures. Disc method was used in the test.

Pathogenicity:-

The pathogenicity of the cultures was tested in which albino mice were inoculated I/P with 1 ml. of 10^{-9} 18 hours old culture. One of the pathogenic stain was tested in 2 guinea pigs, 2 rabbits and 4 fowls. In case of rabbits and guineapigs the inoculation was done by I/P route. In case of two of the fowls the culture was given orally at the rate of 1 c.c. of the culture put directly into the digestive tract with the help of a glass pipette daily for three successive days, whereas in case of two other fowl it was given S/C in a dose of 1 ml.

STREPTOCOCCI AND STAPHYLOCOCCI.

From blood agar plates made by using sheep blood collected under sterile conditions haemolysin and oher colony characters were seen. The arrangement of the cocci

was studied by growing the organisms in plain broth and studying their morphological characters after staining with Gram's method.

Glucose, lactose, sucrose, mannitol, salicin, inulin, were the sugars used along with other biochemical tests for checking the H_2S production and gelatine liquefaction common media was used. Pigment production was observed. The organisms were grown on milk agar as suggested by Christie and Keogh (1940) and to the same medium 8% of sodium chloride was also added to check the salt tolerance. Litmus milk was used to check peptonisation, coagulation and acid alkali production in litmus milk. After culturing the organism in the litmus milk incubation was done for 1 - 4 days at 37°C. Hydrolysis of urea to ammonia was tested by a simple buffered liquid media containing urea as the sole source of nitrogen. Culture was incubated for 2 - 10 days.

In case of streptococci the hydrolysis of Sodium Hippurate was seen.

Coagulase test was performed in case of Staphylococci. The rabbit plasma diluted to 15 times was used in the test. For sugar fermentation glucose, maltose, lactose, salicin, trehalose, sorbitol, mannitol, sucrose were used.

LACTOBACILLI.

The material was inoculated in the plain broth containing acetic acid pH being 5 to 6. After incubation sub - cultures were made on plain agar. The morphology was studied by staining Gram's stain.

The DeMan, Rogosa and Sharpe's (1960) medium was also used for the isolation of the organism.

Usual sugar and biochemical tests were used for the identification of the organism.

PROTEUS.

Characteristically discrete colonies with spreading tendency were taken and sub - cultured on plain agar slants. Morphological cultural and biochemical characters were the yardstick for the identification and typing of the organisms. Sugars used were glucose, lactose, mannitol, maltose along with gelatin, indole citrate and urease reaction for the species differentiation.

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RESULTS.

—:: RESULTS ::—

In the course of the present investigation an attempt was made to isolate the aerobic microflora of the gastrointestinal tract of poultry. Special attention was given to the isolation of the pathogenic strains of E. coli. Fungi were not studied. The antiboitic sensitivity of some of the isolates which could be attributed to the causation of the disease was also studied. The basis of the identification was mainly morphological, cultural and biochemical characters. Due to non--availability of different diagnostic sera, serological studies could not be undertaken. Pathogenicity in cases of some organisms was studied in mice and in few cases other laboratory animals like Rabbits, Guinea pigs and fowl were also used in addition to mice.

In all 370 samples were collected as detailed in the Material Method Chapter. There included 200 samples from the cases which had died and autopsy was performed, 120 samples were collected from the healthy birds and 50 cloacal swabs were taken from diseased birds as well as healthy birds. The whole of the gastrointestinal tract was collected. 20 samples each from the anterior and posterior region were rejected as the samples were highly contaminated and were not worth processing. These samples were from the carcasses of the dead fowls which had died

TABLE NO: 2.

-: TABLE SHOWING THE REGIONWISE ISOLATION OF THE ORGANISM :-

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Name of the organism.	Healthy Fowls			Carcasses of diseased fowl.		
	Anterior region.	Posterior region.	Percentage.	Anterior region.	Posterior region.	Percentage.
1	2	3	4	5	6	7
E.coli ...	30 ...	50 ...	65% ...	52 ...	70 ...	76.0%
E.Fruendii...	4 ...	8 ..	9.7% ...	5 ...	20 ...	15.6%
Streptococcii.	22 ...	40 ..	49.6% ...	38 ...	54 ...	57.5%
Staphylococci.	16 ...	10 ..	20.8% ...	29 ...	39 ...	42.5%
Micrococcus .	4 ...	..	3% ...	8 ...	22 ...	18.7%
Salmonella ...	Nil ...	3 ..	2% ...	Nil ...	Nil ...	0%
Pseudomonas...	6 ...	9 ..	12% ...	19 ...	42 ...	38.1%
Lactobacitti..	26 ...	50 ..	62% ...	55 ...	52 ...	66.8%
Proteges ...	x ...	1 ..	09% ...	8 ...	21 ...	18.1%
Mutyped ...	8 ...	13 ..	16% ...	19 ...	28 ...	29.4%

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TABLE NO:-2.

PERCENTAGE OF THE ORGANISMS ISOLATED FROM THE CLOACAL SWABS OF HEALTHY & DISEASED BIRDS.

Sl. No.	Name of the organism Isolated.	Total swabs collected	Healthy.		Diseased.		
			No:of organisms Isolated.	Percentage	Total swabs collected	No: of organisms Isolated.	Percentage.
1)	E.Coli	30	19	63.3%	20	15	75%
2)	E.fruendii	30	2	6.6%	20	2	10%
3)	Streptococci	30	18	60.0%	20	14	70%
4)	Staphylococci	30	8	26.6%	20	10	50%
5)	Micrococcus	30	6	20.0%	20	8	40%
6)	Salmonella	30	Nil.	Nil.	20	Nil.	Nil.
7)	Pseudomonas	30	1	0.3%	20	10	50%
8)	Lactobacilli	30	14	48.0%	20	14	70%
9)	Proteus	30	Nil.	Nil.	20	2	10%
10)	Untyped	30	4	13.3%	20	6	30%
			72			81	

Total organisms isolated from the cloacal swabs=153.

The percentage of the each organism is of the total number of swabs taken in that particular group.

and postmortem could not be done in time.

The detail of the organisms isolated from the different regions of the gastrointestinal tract and the source of the collection of the specimen are shown in Table No:2. The details of the organisms isolated from the cloacal swabs are detailed in Table No: 3. The results of each organism isolated has been tabulated separately.

E. COLI.

From the material which was streeked on the MacConkey agar plates, single colonies which were red, translucent and convex were collected. Total 202 samples of E. coli from healthy and dead fowls were collected. In addition to this 34 samples from cloacal swabs were also collected totalling the number of the organisms to 236. The position regarding the collection of E.coli from different regions was as under—

Anterior region of healthy fowls -	30
Anterior region of the dead fowls-	50
Anterior region of the dead fowls-	52
Posterior region of the dead fowls-	70
Cloacal swabs of the healthy fowl-	19
Cloacal swabs of the diseased fowl-	15
	236

The percentage of isolation was from 60 to 75 percent of the samples.

TABLE NO:- 4.

-: BIOCHEMICAL PATTERN IN THE E.COLI CULTURES :-

Biochemical Pattern.	Sugar fermentation Glucose	Lactose	Sucrose.	Raffinose.	Dulcitol.	Sorbitol.	Healthy fowls. Ant.	Post.	Carcasses of dead fowls. Ant. Reg.	Post. Reg.	Cloacal Swabs. Healthy.	Diseased.	No. of organism.
1.	2.	3.	4.	5.	6.	7.	8.	9.	10.	11.	12.	13.	14.
1	Ag.	+	+	+	+	+	1	2	2	4	1	1	11
2	Ag.	+	+	+	-	+	3	4	3	2	1	1	14
3	A	+	+	+	+	-	0	2	3	1	2	1	9
4	A	+	-	+	+	+	1	3	2	4	3	1	14
5	A	+	-	-	+	+	1	2	1	2	1	x	7
6	Ag.	+	-	+	-	+	3	3	2	2	1	1	12
7	A	+	+	+	-	-	4	3	2	3	1	1	14
8	A	+	+	-	+	+	4	2	2	4	2	x	14
9	A	+	+	+	+	+	1	3	2	8	1	2	17
10	A	+	-	-	-	+	1	3	4	2	1	1	12
11	Ag.	+	-	-	+	-	1	1	2	4	1	x	9

(Cont....)

1.	2.	3.	4.	5.	6.	7.	8	9	10.	11	12.	13	14.
12	Ag.	+	-	-	+	-	0	1	2	8	1	x	12
13	A	+	-	-	-	+	0	3	3	4	1	1	12
14	A	+	-	+	-	-	4	4	3	1	x	1	13
15	A	+	-	-	+	-	1	1	2	4	1	1	10
16	A	+	+	-	-	+	1	4	3	3	x	x	11
17	A	+	-	-	-	-	NIL	3	3	4	1	1	12
18	A	+	+	+	-	-	1	2	4	2	x	x	9
19	Ag.	+	-	+	+	-	0	1	1	4	x	2	8
20	A	+	+	-	-	-	3	3	6	4	-	-	16
TOTAL Cultures:-							30	50	52	70	19	15	236.

*****
***
*

The anterior region in the case of this experiment consisted of proventriculous and gizzard whereas the posterio region was the whole intestinal region upto the junction of colon with the cloaca.

On checking the motility it was found that 150 strains were motile whereas the remaining 86 strains were non-motile. The Indol, MR, VP and Nitrate reduction tests were done alongwith growth on 'Eosin' methylene blue agar. The colonies giving IMVIC (i.e., Indol + MR + and VP and Citrate negative) character alongwith the production of metallic sheen were put to sugar fermentation reactions.

Sugar Fermentation:- All the cultures attacked lactose, mannitol and glucose. Acid with gas in the case of Glucose was seen in 110 cultures whereas the remaining 126 cultures were producing acids but no gas within 24 hours. The details of the sugar fermentation reaction of the culture is given in the Table No: 4. All the cultures showed great variation in their sugar fermentation reaction. The cultures could be grouped in 20 different biochemical patterns according to the sugar reaction given by them, Table No: 4 (Page No: 77).

In case of healthy fowls in the anterior region in addition to glucose, lactose and mannitol (which was fermented by all the cultures) sucrose was attacked by 18, 25, 29, 8 and 5 cultures of anterior region of

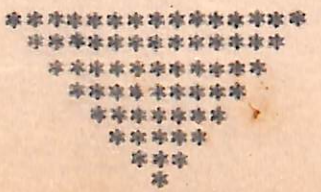
healthy, posterior region of healthy, anterior region of diseased fowls and healthy and diseased cloacal swabs. Similarly Raffinose was attacked by 18, 27, 25, 31, 70 and 11 cultures in the same order as for sucrose. The number of cultures attacking Dulcitol and Sorbitol were 10, 18, 19, 43, 6, 7 and 16, 29, 24, 35, 12 and 8. Most of the sugars were fermented after 24 hours incubation but in case of some of the incubation had to be prolonged upto 10 days. No relation between the fermentative property of pathogenic strains, non-pathogenic strains, their isolation from different regions was observed. A particular biochemical pattern observed in cultures from one region was observed in the cultures from different region. However, more than 55% of the strains from diseased birds were Dulcitol positive.

Bacteriophage Typing:- All the 236 cultures were put to bacteriophage typing against T_1 to T_7 series of Bacteriophages. The plate method was employed. The confluent lysis was given ++++ and semi-confluent lysis were given + to +++ according to the extent of lysis produced by the. On the places were the phage was put. The results of the phage sensitivity of the different cultures have been shown in Table No: 6. Out of the total 236 strains only 180 strains could be typed with different phages while the remaining 56 strains were untypable with these seven phages. Maximum number of strains

TABLE NO: 5.

TABLE SHOWING THE SENSITIVITY OF E. COLI CULTURE TO SERIES OF 'T' PHAGES.

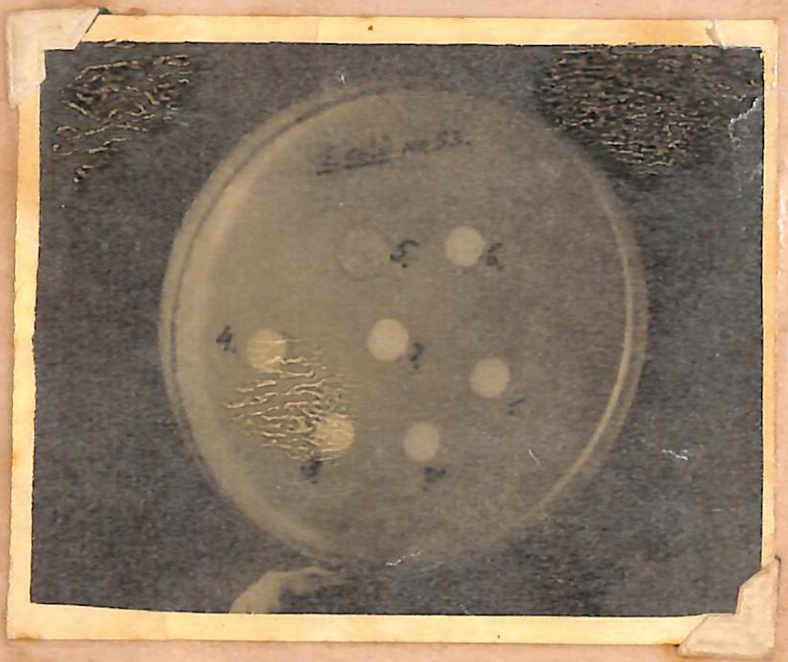
Source of culture.		Sensitivity to phage.								
		T_1	T_2	T_3	T_4	T_5	T_6	T_7	Un-typed	
Healthy Fowls.	Anterior portion.	1	4	3	11	2	2	0	6	
	Posterior portion.	3	8	4	13	4	6	2	9	
Dead Fowls.	Anterior portion.	4	9	3	11	5	6	3	11	
	Posterior portion.	5	15	6	16	5	4	4	17	
Cloacal Swabs.	Healthy.	1	4	0	1	2	1	0	10	
	Diseased.	1	3	1	4	2	1	0	3	
		15	43	17	56	20	20	9	56	Total-236.



PHOTOGRAPH NO: 1.

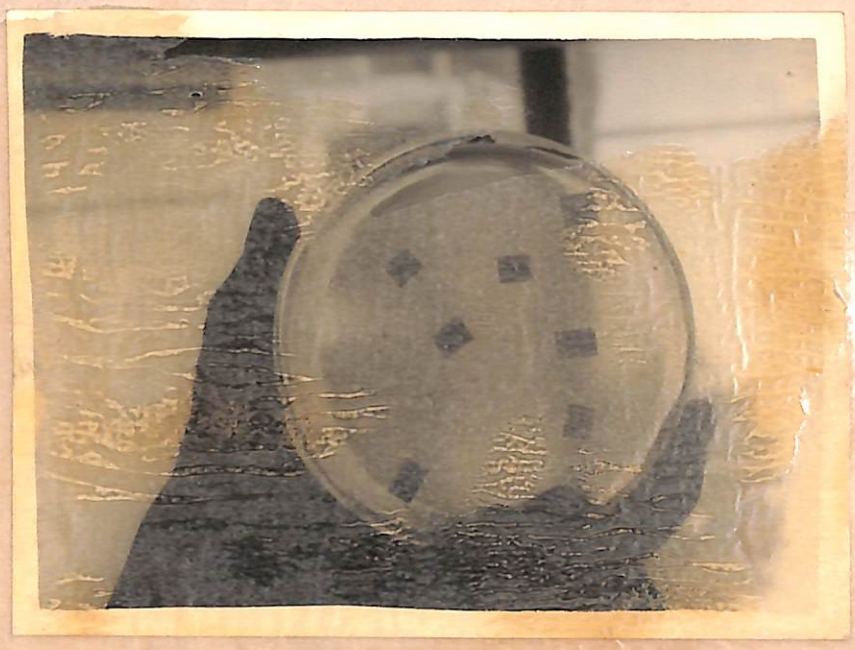
Bacteriophage lysis of culture no: 38.
Sensitive to phage T_2 though T_1 has also shown semiconfluent tyoe of lysis.

PHOTOGRAPH NO: 2.



Bacteriophage lytic action of phage T_5 on culture no: 53.

PHOTOGRAPHS NO: 2.



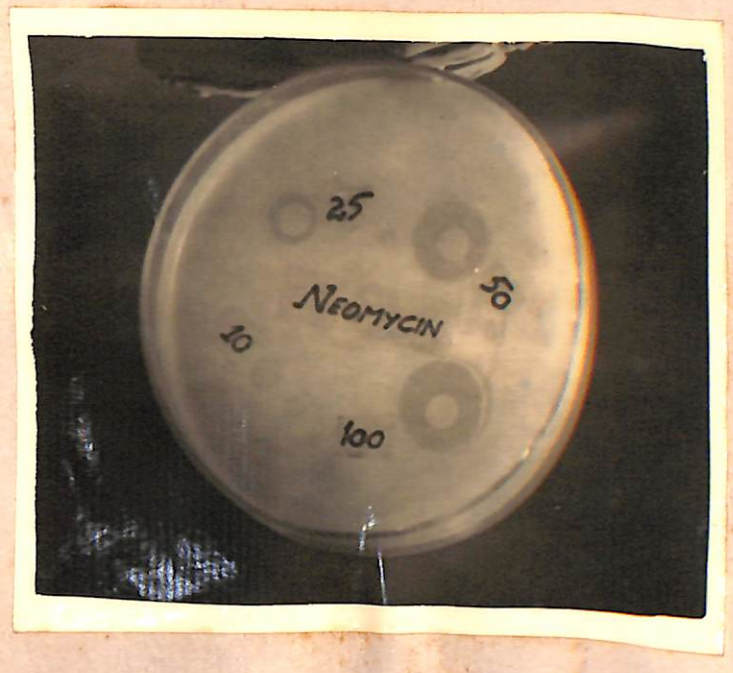
Clear lysis by phage T_2 on culture no: 53.

PHOTOGRAPH NO: 4.

Showing the antiboitic sensitivity of the culture no:38.

The clear zones of inhibition are evident with Tetracyclin Hcl.

PHOTOGRAPH NO: 5.

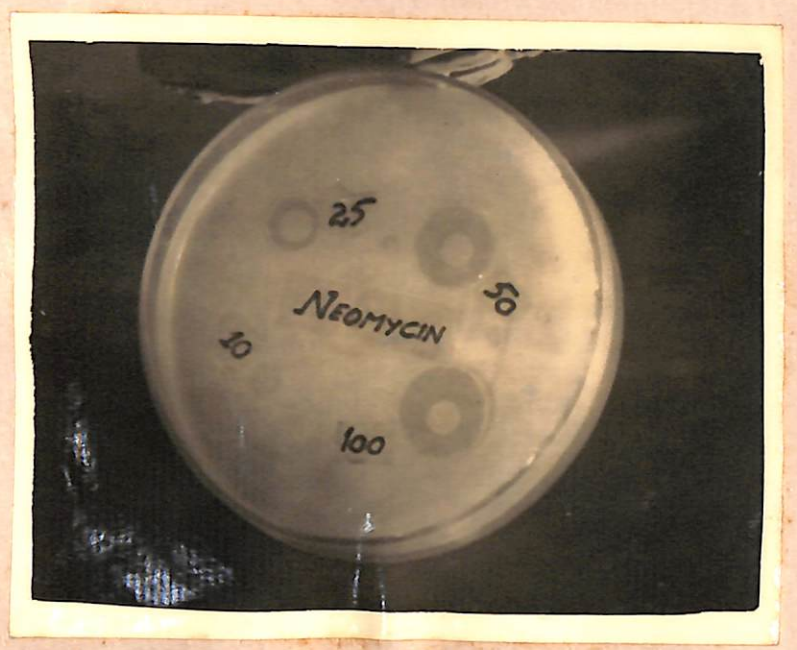


Showing the sensitivity of culture no: 38 to the action of Neomycin.

PHOTOGRAPH NO: 4.

Showing the antiboitic sensitivity of the culture no:38.
The clear zones of inhibition are evident with Tetracyclin Hcl.

PHOTOGRAPH NO: 5.



Showing the sensitivity of culture no: 38 to the action of Neomycin.

antiboitics. The strains which were producing inhibition zone of less than 10 m.m. including the diameter of 6 m.m. of the disc at a concentration of 25 microgram was taken as resistant. The number of resistant strains of different antiboitics (at a concentration of 25 mgm.) has been tabulated in Table No: 6. The percentage of resistant strains to Achromycin, Neomycin, Dihydrostreptomycin Sulph, Furandentin aureomycin and Ledermycin was 16.6, 19.1, 20.1, 22.6, 24.6 and 25.2 respectively. The comparative study of the sensitivity of six antiboitics revealed that the most effective antiboitic for E.coli was Achromycin followed by Neomycin and and Dihydrostreptomycin where the sensitivity was 83.4, 80.9 and 79.9 percent respectively. The percentage of the resistant strains was more in the samples collected from the fowls from places where antiboitic feeding was practiced except in the case of Dihydrostreptomycin Sulphate where the percentage was in the reverse order. The comparison is showed in Table No: 7 (Page Number- 83).

Photograph No: 4 and 5 give the reaction of the antiboitic sensitivity reaction of culture No: 38 with Tetracyclin Hydrochloride and Neomycin respectively. The zone of inhibition in both the photographs clearly indicates the superiority of the former over the later. The zone of inhibition in both the cases was more than 10 m.m. Photograph No: 6 gives resistance of the strain to Lederomycin. 30 strains out of the total 23 strains tested for antiboitic sensitivity were found to be resistant to all of the antiboitic

TABLE NO: 7.

TABLE SHOWING THE PERCENTAGE OF ANTIBOITIC RESISTANCE IN ANTIBOITIC FED AND NON ANTIBOITIC FED FOWLS.

Sl. No:	Name of the antiboitics.	Percentage of resistant strains where antiboitic were not used.	Percentage of the resistant strains where antiboitics in fedd were used.
1	2.	3.	4.
1.	Tetracyclin Hydrochloride (Achromycin)	33%	66%
2.	Chlortetracyclin Aeromycin	40%	60%
3.	Neomycin	45%	55%
4.	Furandentine	43%	57%
5.	Dihydrostreptomycin	60%	40%
6.	Demethyl Tetracyclin (Ledermycin)	42%	58%

Pathogenicity Test:- The pathogenicity test in case of 48 cultures of E.coli was done in white albino mice. Dose given was 1 ml. of 10^{-9} 18 hours old broth culture I/P. The culture tested were from fowls showing the lesions of severe enteritis, or from cases having diarrhoea. 38 cultures out of this 48 were pathogenic for mice. The organism invariably were recovered from the heart blood of the mice when streaked on MacConkey agar. The culture isolated from the injected mice were further given to the

PHOTOGRAPH NO: 6.

The lesions of coli granuloma in the liver.
The tuberculous type of granulomatous lesions were seen all over the surface of the liver.

mice in the same dose to complete the 'Koch' postulates in case of 20 cultures. The mice in all the cases died. The pathogenicity in case of rabbit and fowls were not done in all the cases, due to non-availability of the fowls. However, the pathogenicity of two cultures in fowls of two strains considered to be more pathogenic along with its pathogenicity in rabbit showed that one of the culture No: 38 isolated from a case of coligranuloma was pathogenic for rabbit as well as fowl whereas the other culture No:53 was only pathogenic for rabbit but not for fowl.

A case of coli granuloma was encountered from the postmortem cases from the Central Poultry Farm. The whole intestinal tract was showing small granulomatous nodules. The liver was much affected than the intestinal tract. There were granulomatous nodules of the size of about 8 to 12 m.m.(Photograph No: 6) throughout the surface of liver. These nodules gave tuberculous appearance. The intestines were showing acute congestion. The pure culture of E.coli was isolated from the liver and the intestine. The culture in a dose of 1 ml. of the 10^{-9} of 18 hours old broth culture and a piece of the affected liver after tritration with sterile normal saline solution was inoculated in the dose of 0.5 ml. I/P into two mice in each case. In both the cases the mice died, and culture in pure form was isolated from them. The culture was further

inoculated I/V into rabbit which succumed to the infection in 48 hours. Two fowls were given 1 ml. of the culture S/C whereas two fowls were given the culture 1 c.c. directly into the crop for 3 days consecutively. The fowl inoculated S/C died in 48 hours and the fowl given culture orally died on the 5th day and the pure culture of E.coli was isolated though the typical lesions of the disease could not be produced. The result of the phage typing and antiboitic sensitivity of the culture is as under:-

TABLE NO: 7-A.

TABLE SHOWING THE ANTIBOITIC SENSITIVITY OF CULTURE NO:38 (COLIGRANULOMA)

Concentration of the antiboitic in mcgm.	Achromycin.	Neomycin	Dihydrostreptomycin Sulphate	Furadentin	Aureomycin	Ledermycin
10	9	9	10	8	8	6
25	11	10	11	9	9	8
50	14	12	12	10	11	10
100	17	15	14	13	12	12

TABLE NO: 7-B.

LYSIS PRODUCED BY DIFFERENT BACTERIOPHAGE ON CULTURE NO:38.

T_1	T_2	T_3	T_4	T_5	T_6	T_7
-	++++	++	-	+	-	-

PSEUDOMONAS.

In all 76 strains of Pseudomonas were isolated from the different regions of gastrointestinal tract (Table No: 2 Page No:72 and Table No:3 Page No: 73). The regionwise distribution was as under:—

HEALTHY FOWLS:	a) Anterior region-	6
	b) Posterior region-	9
CARCASSES OF THE DEAD FOWLS:	a) Anterior region-	19
	b) Posterior region-	42
CLOACAL SWABS:	a) Healthy fowls-	2
	b) Diseased fowls-	10

The isolation of Pseudomonas from different sources has been tabulated in Table No: 8.

T A B L E NO: 8.

TABLE SHOWING THE ISOLATION OF PSEUDONONAS FROM DIFFERENT SOURCES.

Condition of the fowls.	Place of collection.		
	Central Poultry Farm, Patna.	Private Poultry Farm.	Slaughter House.
Healthy fowls	11	-	4
Diseased or dead fowls.	18	43	-
Cloacal Swabs.	2	10	-

From one Private owned Poultry Farm the Pseudomonas aeriginosa was isolated as the cause of mortality in the fowls. The farm having a flock of about 900 birds had a mortality of about 10-15 birds daily. The Pseudomonas aeriginosa was isolated invariably from each case. No particular sympton was shown by the fowl except separation from other fowls and showing febrile condition. There was a lose diarrhoea also. The fowls showed watery discharge from the beaks. The infection was seen in fowls of different age groups. The layers as well as non-layers were affected, infection being more prevalent in case of chicks than in adults. On postmortem examination not much of lesions were seen except enteritis and congestion of the different organs . Petechial haemorrhages were also seen on the liver and kidney. On opening of the intestinal canal yellowish viscid fluid was seen. The culture exhibited a great variation in their motility. Pigment production, cultural and biochemical characters as shown in Table No: 9(Page No:88).

PIGMENT PRODUCTION:-

Pigment production was observed by the following method. A few drops of Chloroform were added to the agar slant which turned into blue. On addition of Hcl the colour of the liquid changed from greenish blue to red, showing the presence of Pyocyanin. When the serum liquefaction was done the pigment production changed the colour of the serum plates to greenish blue. The same thing happened when the gelatin was liquefied

by the organism. Except the fermentation of glucose and to some extent maltose the organism did not ferment any other sugar. Indole was not produced by most of the strains.

TABLE NO:2.

SHOWING THE DIFFERENT BIOCHEMICAL VARIATION AND PIGMENT PRODUCTION OF PSEUDOMONAS CULTURES.

Details of the Characters.	Positive reaction.	Percentage of reaction.
Gelatine liquefaction-	88	100%
Pigment production:-		
Brown-	36	40%
Yellow-	25	28%
No pigment-	27	30%
Glucose fermentation-	52	58%
Maltose production-	10	11.5%
Nitrate reduction-	88	100%
Ammonia production-	80	90%
Motility-	82	93%
Serum liquefaction-	60	68%

PATHOGENICITY TEST:-

The cultures from the Private Poultry Farm were tested for their pathogenicity in laboratory animals. Side by side two cultures selected at random from the Central

Poultry Farm and Slaughter House were also tested for their pathogenicity. The culture in case of Central Poultry Farm were non-pathogenic whereas the mice inoculated with cultures from Private Poultry Farm succumbed to the infection within 24 hours and pure culture could be collected which on further inoculation into the mice proved fatal. Two guineapigs, two rabbits inoculated with the culture succumbed to the infection within 48 hours. In one case the guineapig died within 12 hours. Two fowls each were given I/V and orally 1 c.c. of the 18 hours old broth culture, oral administration was continued for three successive days. In case of fowls inoculated I/V the death occurred within 48 hours whereas in case of oral administration the fowl died after four and five days. In both the cases no specific lesions could be observed except enteritis in case of fowls with oral inoculation. Two rabbits kept in the cage where the fowls inoculated originally with the culture were kept also packed up infection and died after showing typical symptoms.

ANTIBOITIC SENSITIVITY:-

Three antiboitics Dihydrostreptomycin Sulphate, Penicillin G. Sodium and Neomycin were tried with the usual disc method, the results were as per Table No: 9-A (Page No: 90).

Dihydrostreptomycin Sulphate was active against

the culture Neomycin also had some activity whereas Penicillin was inactive against the culture.

TABLE NO:-2-A.

Concentration of antiboitic Used.	Dihydrostreptomycin Sulphate.	Neomycin	Penicillin.
10	8	8	6
25	10	9	6
50	13	11	8
100	17	13	8

In case of Penicillin the concentration of antiboitic was 50, 100, 250, 500 I.U.instead of 10, 25, 50 and 100 mcgm.

SALMONELLA.

The Salmonella could be isolated from three cases only. All the three were isolated from the case of the posterior region of the gastrointestinal tract from birds from the Slaughter House. The remaining specimens were negative for the organism. The colonies from Brilliant green agar were sub-cultured on the plain agar slants. The genus Salmonella was identified on biochemical tests.The cultures fermented glucose (with acid and gas production), mannitol, dulcitol and sorbitol.They failed to ferment lactose,sucrose, adonitol and raffinose. Cultures were H_2S positive,citrate +ve MR + nitrate +ve and were negative for gelatine VP and indole production.

The cultures produced lysis with plaque method when '0'-1 phage was used. Though the extent of lysis was not very much well marked yet the lysis could be appreciated and could be classified as ++ (Semi- confluent lysis). The cultures were tentatively typed as Salmonella and serological typing could not be done since the cultures were isolated in the later part of the work and attempts to obtain sera were not successful. The culture has been maintained for further study and serotyping and species typing in some recognised laboratory.

The cultures were pathogenic when inoculated S/C into two mice each a dose of 1 c.c. of 10^{-9} hours old broth culture.

E. FRUENDII (CITROBACTER).

Fifty strains of E. fruendii were isolated from different regions as detailed in Table Nos: 2 and 3 (Page Nos: 72 and 73) and they were identified and typed on the basis of the morphological characters. The strains fermented glucose, mannitol, sorbitol, dulcitol (not fermented in 10 cases), lactose (14 strains not fermented). The strains were indol negative V.P. negative MR +ve Citrate + H_2S positive and Nitrate positive. Three of the strains were indole positive. All the cultures did not liquefy gelatin.

LACTOBACILLI.

Were one of the most common inhabitants in both the regions of the gastrointestinal tract in healthy as well as diseased group. This group of organism was also isolated from the faeces. The plain broth having acetic acid with lowered pH and showing growth were streaked on De'Man, Rogosa and Sharpes medium plates. The pure cultures were isolated. The cultures were identified on the basis of their morphological, cultural and biochemical reactions. The organism was isolated from 60 - 75% cases of specimen from healthy as well as diseased fowls. The isolation of the organism from faeces also was similar.

STAPHYLOCOCCI.

In all 113 cultures were isolated from the different regions. The distribution of the Staphylococci according to the region and source has been tabulated in Table No: 10 (Page No: 93).

On the basis of pigment production the strains of Staphylococci were identified as Staphylococcus aureus, 53 strains, Staph.albus, 37 and Staph.citreus 23 strains. Except one none of the strains of the group Staph.citreus and Staph.albus was coagulase positive. In case of Staph.aureus out of 53 strains, 23 were coagulase +ve thus were grouped as pathogenic. The alpha - beta type of haemolysis was seen in the case of 50, 4 and 3 strains of Staph. aureus, albus and citreus. 20 strains of S. aureus, 4 strains of

T A B L E NO:- 1o.

:: TABLE SHOWING THE REGIONWISE ISOLATION OF STAPHYLOCOCCI. ::

		Central Poultry Farm.	Private Poultry Farm.	Slaughter House.
Healthy Fowls.	Ant. Reg.	4	5	7
	Post. Reg.	2	5	4
Dead Fowls.	Ant. Reg.	8	10	11
	Post. Reg.	10	12	17
Cloacal Swabs.	Healthy	3	5	x
	Diseased	4	6	x
		31	43	39

T A B L E NO: 11.

TABLE SHOWING
THE ACTIVITY OF HAEMOLYSIN, COAGULASE PRODUCTION AND MANNITOL FERMENTATION BY STAPHYLOCOCCI.

Type of organism.	No: of strains isolated	Mannitol positive	Coagulase positive.	Production of Haemolysis		
				Alpha	Beta	Alpha Beta.
S.aureus	53	20	23	-	-	50
S.albus.	37	4	1	-	-	4
S.Citreus.	23	2	-	-	-	3

Staph.albus and 2 of Staph.citreus were mannitol positive. The pathogenic strains (on the basis of the coagulase production) were all except 2 strains isolated from the diseased birds (posterior region) and in 3 cases from the cloacal swabs. Only one strain which was coagulase positive was isolated from the anterior region. The two strains which were isolated from healthy birds were from the anterior region.

The results of the three tests, viz., Mannitol fermentation, coagulase production and haemolysis are illustrated in Table No: 11 (Page No: 93).

MICROCOCCI.

48 strains of Micrococci were seen from different region. The cells of Micrococci were large sized, mainly ovoid arranged in pairs, tetrads or irregular masses. Most of the strains were Gram positive but readily lost their character. All the 48 strains were catalase positive. Identification upto species level was not carried out. The genus characters were studied. The cultures with slight variation were negative for indol, Nitrate reduction, Gelatine liquefaction, milk coagulation. Lactose was not fermented whereas glucose, sucrose and mannitol were fermented. Ammonia production was positive.

The number of micrococci isolated was more from the posterior region of the diseased fowls than healthy.

T A B L E NO: 12.

-: TABLE SHOWING THE REGIONWISE DISTRIBUTION OF STREPTOCOCCI. :-

****

Region		Source of Collection		
		Central Poultry Farm.	Private Poultry Farm.	Slaughter House
1		2	3	4
Healthy Bird.	Ant. region.	12	Nil	10
	Post. region.	24	Nil	16
Carcasses of the diseased birds.	Ant. region.	20	18	Nil
	Post. region.	24	30	Nil
Cloacal swab.	Healthy	18		
	Diseased		16	
T O T A L :-		98	64	26

STREPTOCOCCI.

Out of the 180 strains isolated 65 strains were alpha haemolytic and 47 strains were beta haemolytic. The rest of the strains were found to be non- haemolytic. All the strains were negative for catalase test. Out of the 47 beta haemolytic strains on cultural and biochemical characters 20 were typed as Str. pyogens. These cultures fermented glucose, maltose, sucrose, trehalose, lactose (by 11 cultures) salicin (9 positive). These were negative for sorbitol, and arabinose sodium hippurate was

not affected. The 27 beta haemolytic strains fermented glucose, maltose (only 14 strains) lactose sulicin, sucrose and sorbitol (11 cases). These were negative for mannitol and trehalose. The strains were grouped as Str.zoocpidemicus. Growth in milk agar containing 6.5% sod chloride in case of all the Str.pyogenes and Str.zoocpidemicus strains was negative.

TABLE NO: 13.

SHOWING THE CHARACTERISTIC DISTINGUISHING CHARACTERS OF STREPTOCOCCI,PYOGENES,EPIDEMICUS,FAECALIS.

Various characters.	Species of Streptococci.		
	Str.Pyogenes.	Str.zoocpidemicus	Str.faecalis
Haemolysis	Beta	Beta	alpha
Growth in 6.5% of Sod chloride.	-	-	+
Litmus milk.	+	+	+
Hydrolysis of Sodium Hippurate.	-	-	-
Ammonia production.	+	+	+
Glucose.	+	+	+
Maltose.	+	±	+
Lactose.	±	+	+
Salicin.	±	+	+
Trehalose.	+	-	+
Sorbitol	-	±	-
Mannitol.	-	-	+
Sucrose.	+	+	±

The alpha haemolytic strains which were identified as Str.faecalis were giving all the positive reaction with the sugars used in case of two other organisms (Str.pyogenes and Str.zooepidemicus)except sorbitol which was negative in this case. Growth on milk agar medium which contained 6.5% of sodium chloride was also positive. 40 strains out of this 67 strains liquefied gelatine hence were typed on Str. faecalis Var liquefaciens. The remaining 25 strains did not liquify gelatin so they were typed as Str.faecalis out of this strain 20 were identified as Str.faecium with the help checking haemolysis on horse blood agar where the Str.faecium gave alpha haemolysis whereas Str. faecalis did not give any hemolysis. The non-haemolytic strains were not preserved as they were giving very variable characters.

The region-wise isolation of Streptococci has been represented in Table No:12.

The chief distinguishing characters of the three types of Streptococci isolated are summerised in Table No:13(Page No: 96).

PROTEUS.

In all 34 strains of Proteus were isolated from t the different regions as shown in Table No: 2 and 3(Page Nos: 72 and 73).The strains were identified and differentiated with the help of biochemical characters viz., Mannitol, maltose, gelatin liquefaction, Indole production and

citrate utilisation, urea decomposition. The results showed predominante of Pr. morganii but the strains of Proteus rettgeri and Pr. velgaris were also found. The biochemical typing was done according to the Table No:14.

TABLE NO: 14.

TABLE SHOWING THE DIFFERENTIAL CHARACTERS OF PROTEUS SPECIES.

[Cruickshank, Medical Microbiology]

Substrate.	Pr.vulgaris	Pr.morganii	Pr. rettgeri.
Mannitol	-	-	+
Maltose	+	-	-
Gelatin	+	-	-
Indole	+	+	+
Citrate	-	-	+

According to the above biochemical differentiation the isolation of the proteus strain was in the order of Proteus morganii 20 cultures, Proteus rettgeri 9 and Pr.vulgaris 5.

Two cultures selected at random from the Proteus morganii were pathogenic for mice when inoculated at the rate of 1 ml. The mice dead within 48 hours and the cultures were isolated in pure form from the inoculated mice.

DISCUSSION.

-:: DISCUSSION ::-

Association of different bacteria to the enteric infections could be put forth only when the normal flora of the gastrointestinal tract is studied in conditions of health and disease. The digestive system alongwith the respiratory system is connected with many pathological conditions. Many organisms which form the normal bacterial flora of the gastrointestinal tract are also associated with the pathological conditions directly affecting the intestinal canal. A clear demarcation between non-pathogenic and pathogenic organisms cannot be made till the pathogenicity of the organism is tested in the laboratory animals. So in the case of the present study an attempt was made to identify as far as possible the organisms on the maximum available differentiating characters. In certain cases the pathogenicity of the culture was studied in mice whereas in few cases it was also studied in fowls and addition to rabbits and guineapigs.

E.coli is widely distributed organism among varieties of animals, birds and man. It is the predominant organism in the intestinal canal gaining its entry immediately after birth. In the present study E.coli was observed in a percentage of 60 to 70 percent in healthy as well as diseased birds. In case of cloacal swabs from healthy and diseased birds the percentage was more or less same.

same. The results are in confirmation to the reports of Smith and Crabb (1961) and Smith (1965) who also pointed out the predominance of the culture in the normal intestinal flora and faeces.

During the routine isolation 38 cultures out of the 48 cultures of E.coli which were tested for pathogenicity proved pathogenic on inoculation into the white albino mice. The cultures were collected from the cases apparently showing the lesions of acute enteritis. This is in confirmation to the findings of many workers who have reported the pathogenicity of the E.coli culture(Sato-1963). Hardy (1964). In Bihar also E.coli has been associated as the cause of "Coli septicaemia" by Sarkar (1966) in one of the Private Poultry Farms owned by M/S Tisco Private Ltd. The view in the present study only differs from the findings of the above workers to the extent that the strain was found pathogenic not only for young chicks but also was equally affecting adult fowls. Davis (1938) has demonstrated the disorders due to this organism pertaining to digestive system in healthy, weak fowls. The question arises whether the fowls were weak due to the effect of the infection of organism or due to any other effect.

A case of "Coli granuloma(Hjarre's disease)has also been reported in the present study from one of the

fowls in which tuberculous lesions were found throughout the intestinal region and liver. This is in confirmation to the many reports of the similar disease like Weakwan (1948), Kohlar (1957), Joubert et al (1964). From India also Iyer et al (1965) have reported the disease. The pure culture isolated from the case proved pathogenic for fowls when intravenous, subcut and oral administration was given, yet the organism could not produce the typical lesions. The attempts to produce the typical lesions by the innoculation of culture has failed in the hands of different workers like Kohlar (1957), Joubert et al(1964). Various arguments have been put forward by different workers for the factors which initiate the organisms to assume the pathogenic role. Woloszyn et al (1964) held Vitamin A deficiency responsible for the organism to assume a pathogenic role. Minett (1948) associated grouping together of calves and the extreme climatic conditions to the causation of white scours in calves. Qureshi (1957) put forth the stress factor. Coccidial infection as a prelude to the disease was the factor advocated by Savroo (1963). In the present case the specimens were collected from the Farms which had a fixed schedule of feeding in which enough of minerals and Vitamins were added and chances of feeding errors and Vitamin deficiency was less though could not be completely ruled out. Stress factor can be more attributed in this case as the whole collection

of material was done during extreme hot weather when the mercury stood at about 102 - 110°F. The birds otherwise were also showing signs of heat stroke and mortality was more or less daily found. Parasitic infestation could also be attributed as one of the causes. Keeping all the hypothesis in view, the stress, grouping of fowls together and feeding errors along with parasitic infection could play as the predisposing causes for the E.coli assuming pathogenicity. E.coli, if, was the only cause for the formation of granulomas then there is no reason to believe why the same culture which has been isolated from a case of Coli granuloma should not cause the typical lesions when innoculated into the susceptible host -- fowl.

In the present study IMVIC reactions were ++-- which corresponded with the IMVIC reactions recorded by Charter and Taylor (1952) Ewing et al (1955) Wilson and Miles (1955) and Rees (1960). All the cultures fermented glucose with or without gas production, lactose, arabinose, and mannitol. The culture showed a great variation in the fermentation of sucrose, salicin, raffinose and dulcitol and thus the cultures could be devided into 20 different boichemical patterns as shown in Table No: 4 of the Result portion. There was no correlation between the sugar fermentation pattern of healthy or diseased fowls. A boichemical pattern shown by a non-pathogenic strain was

similar to another one from the pathogenic group.Similarly no difference was observed in the boichemical fermentation pattern of so the isolates from anterior or posterior region. More number of cultures from the diseased group fermented dulcitol specially those isolated from the posterior regions the percentage of the dulcitol fermenters in the posterior region of the diseased fowl was 55%. Out of the 38 pathogenic cultures 32 were found dulcitol positive.This confirms the result of Harry and Chubb (1964) who attributed dulcitol fermentation to the pathological activity of the strain. The fermentation of sucrose could however not be correlated with the pathogenicity. The increased number of dulcitol fermenters in the posterior region could be presumed to be due to the fact that enteritis was more pronounced in the case of the posterior region than the anterior one.

On bacterio phage typing of the organism it was found in the present study that no differentiation could be laid down as to the particular grouping of the phage sensitive,healthy or diseased fowls. Strains from the healthy as well as diseased group were equally sensitive to a particular phage similarly no phage was sensiti specific in its lysogenic activity on strains isolated from anterior or posterior region. However it was observed that more number of strains in the present study were typable by phage T_4 and T_2 than other T series phages.The

This may be due to the fact that a particular phage might have differed from the other by means of acquired phage resistance by contact with different phages in the field Smith and Chrabb(1956). Untypability by particular phage by a particular group of strains isolated from specific region is in confirmation to the findings of Smith and Chrabb (1956) who found that all the 32 phage types found in scouring calves were also found in healthy calves.

The antiboitic sensitivity of the culture was tested by six antiboities viz., achromycin, neomycin, dihydrostreptomycin sulphate, furadentin, aureomycin and ledermycin with disc method. The resistance shown by various strains to different antiboities was in the order of 16.6 percent, 19.1 percent, 20.1 percent, 22.6 percent, 24.5 percent and 25.2 percent respectively. The resistance shown by the cultures to the antiboitic is due to the fact that the fowls had formed resistance to the antiboities because of the addition of antiboitic feed supplements in the different feeds and their constant uses resulting into the resistance of the organism. Corey and Byrnes (1963) have isolated antiboitic resistant strains,commercially processed chickens were the animals under experiments. These chickens had been fed on antiboitic feeds. The presence of the resistant strains gives confirmation to the work of Smith and Crabb (1956) who isolated 11 E.coli

strains resistant to Streptomycin and two to Aureomycin and Terramycin. The point is more clear on perusal of Table No: 6 in the present study. The antiboitic resistant strain were found more in the cultures isolated from the fowls which had been given antiboitic feeding whereas the number was much less than the feed was not having any addition of antiboitic feed supplement. The ratio of resistant strains from antiboitic fed fowls and non-antiboitic fed fowls in some case was to the tune of 3 : 1. These results are in confirmation with the findings of Ingram et al (1958) who observed strains resistant to Penicillin and Aureomycin more in the cases of calves when they were fed antiboitic alongwith milk in their daily feeding schedule. The only variation was observed in that of the Dihydrostreptomycin Sulphate which was more inactive in non-antiboitic fed fowls than those of the antiboitic fed fowls. The reasons may be due to the individual resistance of the particular fowl. No correlation could be had as to the resistance of a particular culture typable by a particular phage and its sensitivity or its resistance for a particular antiboitic. The strains resistant to the same antiboitic were recovered from healthy as well as diseased birds. Similarly there was no correlation between the isolates from the anterior region or posterior region for its antiboitic sensitivity.

PSEUDOMONAS:—The association of Pseudomonas with the pathogenicity in man, animals and birds has been well established. It is no longer the old blue pus organism. Its association with many diseases in animals and birds are known world wide. and Essex et al (1930) reported its isolation from the birds dying from a flock of about 400 White Leg horn fowls.

In the present investigation Pseudomonas was isolated from 72 cases. Its isolation from healthy cases was only 12% whereas the isolation was above 38% in case of diseased fowls. Most of the isolations in the case of diseased group were done from the cases of mortality in a Private Poultry Farm (mortality was 10 - 15 birds per day in a flock of about 900 White Leg horn and Rhode Island Red). The affected fowls were showing apparently acute enteritis and congestion of the visceral organs as detailed in the Result Chapter. The very high percentage of the organism in the diseased group in comparison to the healthy group goes a long way in proving the pathogenicity of the organism. The cultures were found to be pathogenic to mice, guineapigs and fowls. The association of the organism with the pathological conditions in fowls have also been reported by Calaprice (1958) Veladao (1961). They reported the prevalence of the organism in young chicks only. The findings in the present study differ from their views as to the fact that the

fowls of all ages were affected and on testing their pathogenicity it was observed that the strains were pathogenic to the fowls more than twelve weeks old. The pathogenicity of the organism can be well assessed from the fact that the two rabbits kept in the cages which were previously used for keeping the Pseudomonas innoculated fowls died showing very characteristic symptoms of tremors and convulsions. Pure culture of the organism could be isolated from the heart blood of the rabbit on postmortem examination. The droppings and the contaminated left-overs were the probable cause of infection. This is in confirmation to the finding of Prasad et al (1966) who have shown water to be the source of infection in the calves dying of Pneumonia due to Pseudomonas infections.

The organism was isolated from the Brilliant green agar plates which were used for the isolation of Salmonella. These plates contained 16 mg.of Sodium Sulphadiazine to prevent the growth of un-necessary growth of contaminents including the Pseudomonas. Their growth in those plates is contradictory to the finding of Galton et al (1954) who have suggested the addition of 8 to 16 mg. of Sodium Sulphadiazine to avoid its growth.

The organism was found resistant to most of the antibeitic used in case of E.coli except Streptomycin and to some extent Neomycin. This confirms the results of many

workers. Thelma et al (1966) have reported Kobs serotype 2,6,11 and 1 from 80% cases of human and animal sources. Serotype 6, 3, 14 (Sandvik, 1960) were isolated from 80% cases of animal and 6,2,1 and 5 from 60% cases of human, animal, plant and water and milk. The above report shows that Kobs serotype 6 is more common in human beings,animals, plants, water and milk which necessitiates the thorough investigation of the human,animal, and even isolates.Since the organism has been associated with many human and animal infections a thorough investigation in the possible correlation of the Pseudomonas strain from animal origin with the Pseudomonas isolated from human beings and vice versa will have a long way keeping in view the zoonosis aspect of the problem.

SALMONELLA:—Out of all the samples examined for the isolation of Salmonella only three cases gave a biochemical reaction. Bihar State has yet been free from the Salmonella as far as poultry salmonellosis is concerned. In the present investigation also no Salmonella could be isolated from the specimens collected from the Central Poultry Farm.However, the cases suspected for Salmonella genus were collected from the local Slaughter House. The culture gave all the biochemical patterns but till a thorough checking by serotyping is done the strains cannot be definitely grouped as Salmonella.Though a very weak type of lysis which could

neither be designated as confluent nor semi-confluent, was the observed with 'O'-1 phage, no definite idea could be made as the phage was very old and had been received in the Laboratory quite long back. The cultures are being maintained and arrangements for their typing in a recognised Laboratory are being made. It is suggested that a thorough investigation and survey be made in the local Farms at times so that the infection, if any, any time could be immediately diagnosed and prophylactic measures taken to keep the Farms free.

STREPTOCOCCI:- During the course of present study the Streptococci were isolated from 49% of the healthy fowls whereas in case of diseased fowls the percentage of more than 57%. Similarly the Streptococci were also isolated from 65% or more of the cloacal swab(Table No: 2 and 3 of page No:72 and 73). This confirms the findings of(Lev and Briggs(1956), Barnes(1956)and Smith(1966)who showed the predominance of the organism in the normal flora of gastrointestinal tract of poultry and various other animals. The Streptococci which is a normal inhabitant of the tract has also been associated with many pathological conditions. 27 strains of Beta haemolytic strains of Streptococcus zooepidemicus were isolated from the posterior part of the alimentry canal of the fowls. This confirms the results of Agrimi (1956) and Sato (1960). They isolated this organism from young chick whereas in the present study the organism were found

adult fowls also. Their presence in the body of the fowl could not hold them responsible for the causation of indirect disorders or septicaemic infections as the isolation was also done in the case of apparently healthy fowls. Other factors must be there to excite the organism in taking up the pathogenic role. As discussed earlier the collection was done in summer months and the heat might have acted as the predisposing cause. Secondly, the organisms were isolated in combination with many other organisms like Pseudomonas, E.coli, Staphylococci and Micrococci, no definite idea could be had whether the strain was pathogenic or not. Its pathogenicity in fowls and mice was not done for want of animals. Str.faecalis var liquefaciens was also isolated from the cases apparently showing enteritis. The same was isolated from the cloacal swabs from the diseased birds. Gross (1962) also isolated Str.faecalis in combination with Staph. aureus and pasteurella. Agrini (1966) isolated the same in combination with Str.zooepidemicus in cases of mortality in fowls. The isolation of the different strains of Streptococcus in the intestinal canal though a normal inhabitant should always be looked for the pathogenicity since the clear demarcation cannot be drawn between the pathogenic strains and non-pathogenic strains. Their presence in the normal as well as pathological conditions

throws a challenge to the investigator regarding their pathogenicity which could be confirmed only by innoculation of laboratory animals.

STAPHYLOCOCCI:—The presence of this organism in the intestinal tract and cloacal swabs was much less as compaired to the Strentococci. The presence of Staphylococci was 20% in the specimens which confirms to the results of Barns (1958), Smith and Williams (1961) who have shown that their number is less as compaired to Strentococci. The Staphylococci isolated from the diseased group of fowls was more than 42% which indicates their association with pathological conditions. These isolations were also made from the cases which had apparent enteritis.The Staphylococci has been associated with the mortality in fowls by Mondini at al (1959), DalSanto (1959). It has also been associated with cases of food poisoning in men after consumption of Staphylococcal infected food. The present study twenty seven strains of coagulase positive Staphylococci were isolated. The rabbit plasma was clotted within one hour in case of most of the coagulase positive cultures only in few cases the plasma took more time to be clotted but never more than 12 hours. Since the coagulase positive Staphylococci are considered as pathogenic strain (Cruickshank -1937 and Christie and Keogh- 1940), their presence in the intestinal tract along with their increased number associate them to

the pathogenicity which has also been reported by DalSanto (1959)and Gross (1962). Its association along with other organism like Streptococci, Pasteurella in the causation of pathological conditions has already been discussed in Streptococci portion.

PROTEUS AND MICROCOCCUS:- 34 strains of Proteus and 48 strains of Micrococci were also isolated. These organisms remain as a non-pathogenic organisms in the alimentry tract of man, birds and animals and may act as the predisposing causes for the other organisms who may not be so pathogenic to cause the disease otherwise without any predisposing factor. The Proteus species have been associated with the gastroenteritis cases in animals, birds and man. The predominance of Proteus morganii associated with the gastroenteritis as it has already been proved as the cause of enteritis by Reuss (1936). The Proteus rettgeri which was originally isolated from the cases of cholera in chickens by Restigian and Stuart (1943) were also isolated in this case. Two cultures selected at random when innoculated in mice proved pathogenic. The cultures might be associated with gastroenteritis but no further work was done on it.

LACTOBACILLUS:-The Lactobacilli were isolated from more than 60% cases of the specimens. This confirms the findings of Smith and Crabb (1956) and Smith (1965) who have shown their presence in the normal flora of the intestinal tract and faecal material since they are non-pathogenic organisms of not much of importance on pathological aspect no further

work was done.

From the perusal of the literature, results and discussion it becomes clear that the organisms found in case of healthy and diseased birds were more or less the same except the difference of their total strength. All these organisms remain as a normal inhabitant of the gut but on getting some congenial factors like stress weakness, food errors or grouping together, individually or collectively, change the role of the organism non pathogenic normal inhabitant to the pathogenic one. Water, food, droppings and left-overs are presumed to be the via media of infection and the absence of a pathogenic organism may not be taken as granted for times together and a occasional check will help reducing the mortality. The pathogenicity test in laboratory animals of the representative isolates will help in the diagnosis of the pathogenic role of an organism which otherwise might not have been a common pathogen. As in the present investigation the Pseudomonas could never have been taken as the cause of mortality in the flock if the pathogenicity test was not performed. The antiboitic sensitivity of the isolates will be helpful to be tested before a particular treatment, prophylactic or curative is taken up, since the organisms show their individual variation towards their behaviour towards a particular antiboitic.

SUMMARY.

-:: S U M M A R Y ::-

During the course of present study the bacteriological examination of 370 samples from the gastrointestinal tract poultry was carried out. These included 200 samples from the cases which had died due to unknown causes, 120 samples from the healthy birds and 50 cloacal swabs from the fowls. The collection of the specimens was done from three different sources viz.,Central Poultry Farm, Patna, a Private Poultry Farm and the local Slaughter House.

Specimens were collected from the anterior region (Proventriculous and gizzard) and the posterior region (the whole of the intestinal canal upto its junction with cloaca) and the total number of organisms thus isolated was 881 from the gastrointestinal tract and 153 from the cloacal swabs.

Most predominant organism was E.coli which was isolated from 65 - 75 percent of the samples in healthy and diseased fowls. Next in order were Lactobacilli 62-65 percent, Streptococci 49.6 - 57 percent, Staphylococci 20.8 - 42.5 percent, Pseudomonas 12 percent, E.fruendii 9 - 15 percent, Micrococcus 3 - 18 percent and Proteus 1 - 18 percent. 16 percent to 29.5 percent of the cultures could not be typed. The position of the organism from the cloacal swabs was also more or less similar.

A case of Coli granuloma was also observed from the Central Poultry Farm, Patna. The strain thus isolated proved pathogenic for mice, rabbit and fowls. The efforts to reproduce the typical lesions in fowls did not meet success.

38 out of the 48 strains of E. coli isolated from the cases apparently showing the lesions in severe enteritis were found to be pathogenic for mice. No correlation between the pathogenicity of a culture, its biochemical pattern and phage sensitivity and its origin were found. 55 percent of the specimen isolated from the posterior region of the cases showing enteritis, were Dulcitol fermenters. The correlation of the pathogenicity was studied.

On Bacteriophage typing of the cultures 180 strains out of the total lot of 236 could be typed by the different 'T' series of the phages. However, no correlation between the pathogenicity of a particular strain and its sensitivity towards a specific phage was found. More number of strains could be typed with T_4 and T_2 types of phages and the least number of the strains were sensitive to the lysogenic action of T_7 phage.

The strains isolated were put to the antiboitic sensitivity against six antiboitics viz., Achromycin,

Neomycin, Streptomycin, Furadentin, Aureomycin and Ledermycin. Achromycin, Neomycin and Streptomycin were sensitive to more cultures than the other three, whereas Aureomycin and Ledermycin showed a great number of resistant strains. The resistant strains were more seen from cases which were collected from Farms incorporating antiboitic feed supplements in their rations, thus the fowls had formed a resistance to the actions of the antiboitics. The resistance of fowls to antiboitics has been discussed.

A highly pathogenic strain of Pseudomonas aeriginosa was isolated from the fowls in one of the Private Poultry Farms where it was the cause of mortality of an appreciable number of birds. The isolated strain was pathogenic to mice, guineapigs, rabbits and fowls. The strain showed resistance to most of the antiboitics except Streptomycin and to some extent Neomycin. The isolation of Pseudomonas from the causes of mortality among fowls from Bihar has been reported for the first time in the present study. Probably this is the first report of Pseudomonas infection in Poultry in India.

Salmonella was found absent from the Central Poultry Farm, Patna, however, three strains which could be identified as Salmonella on cultural, biochemical and sugar fermentation reactions was recovered from the three cases of specimens collected from the local Slaughter

House. The cultures are awaiting final confirmation on serotyping and arrangements have already been made with Dr. Joan Taylor, International Reference Laboratory, London, who has kindly agreed to type out the strains.

110 strains of Staphylococci were also isolated from the fowls and were typed as Staph.aureus, albus and citreus. 23 strains out of this were grouped as pathogenic on the basis of their coagulase production.

113 strains of Streptococci were isolated. These were typed as Str.pyogenes, Str.zooepidemicus and Str.faecalis var liquefaciens and Str.faecium.

In addition to all these organisms Lactobacilli Micrococci and Proteus were invariably found.

The type and number of organisms isolated from healthy as well as diseased birds were same. The presence of any particular organism in a diseased bird could only be ascertained unless and untill pathogenicity was tested in laboratory animals. The possible factors for the organisms to gain pathogenicity have been discussed on circumstantial hypothesis. The typing of these strains with public health point of view has also been discussed.

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