UNIVERSITI PUTRA MALAYSIA

PREVALENCE,ISOLATION TECHNIQUES, ANTIBIOTIC PATTERN AND PLASMID PROFILE OF SALMONELLA IN BROILER CHICKEN DURING PRODUCTION, PROCESSING AND RETAIL OUTLETS.

JAMAL KHAIR BIN HASHIM

PREVALENCE, ISOLATION TECHNIQUES, ANTIBIOTIC PATTERN AND PLASMID PROFILE OF *SALMONELLA* IN BROILER CHICKEN DURING PRODUCTION, PROCESSING AND RETAIL OUTLETS.

by
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A Thesis submitted in Fulfilment of the Requirements for
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To my family, Norlia, Sara, Diaya, Muhamad, Fatin , Yusuf and Adilah.

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LIST OF ABBREVIATIONS

AOAC- Official Methods of Analysis of the Association of Official Analytical Chemists.
BPW- Buffered Paptone Water

BSA- Bismuth Sulphite Agar

FAO -Food an Agriculture Organisation, United Nation. Rome.

FDA -Food and Drug Administration, USA

IAMFES-International Association of milk, food and Environmental Sanitarians.

ICMSF- International Commission on Microbiological Specifications for food.

MBPW- Modified Buffered Paptone Water

MSCB- Modified Selanite Cystine Broth

RV- Rapaport-Vasiliadis Broth

SC- Selanite Cystine Broth

TSA- Tryptone Soya Agar

TT- Tetrationate Broth

XLD- Xylose Lysine Deoxycholate Agar

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January 1998

Chairman: Professor Dr. Gulam Rusul Rahmat Ali

Falculty : Food Science and Biotechnology

Five hundred and forty nine (549) of carcasses and intestinals content and 73 samples of chicken litter and feed were examined for *Salmonella.* Two hundred and thirty (237) *Salmonella* isolates belonging to 15 different serotypes were isolated. The predominant serotypes were: *S. enteritidis* (35.2%), *S. muenchen* (20%), *S. kentucky* (14.3%), *S. blockley* (10.4%) and *S. chincol* (5.2%). *S. enteritidis* were detected throughout the broiler production chain.

The detection rate of *Salmonella* in poultry carcasses under different conditions were as follows: 83% (126/232), direct enrichment of pelleted (DEP) rinse fluid in Rapaport Vasiliadis broth (RV) incubated at 42°C; 28% (43/232) ,for both DEP

enriched in RV and Manitol Selanite Cystine Broth (MSCB) incubated at 37°C ; and 9% (14/232), streaking the pellet directly onto the plating media. In a parallel analysis, the conventional method detected *Salmonella* in 43.5% (115/264) of carcasses, litter and feed samples compared to 18.2% (48/264) detected by rapid method TECRA UNIQUE Salmonella test kit.

Two hundrad and thirty seven (237) isolates of *Salmonella* belonging to 15 different serovars were susceptible to gentamicin, advosin and enrofloxacin except for one isolate of *S. agona*, which was resistant to enrofloxacin. *S. kentucky* isolates displayed 11 discrete antibiotic resistant patterns, followed by *S. blockley* (7), *S. agona* (4) and *S. muenchen* (3).

Plasmids were detected in 80% of the isolates with molecular weights ranging from <1 to 50 MDa. The frequency of plasmids in different serovars are as follows: *S. blockely* (96%), *S. chnicol* (92%), *S. muenchen* (90%); *S. kentuckey* (88%) and *S. enteritidis* (75%). Different variations in plasmid profile pattern were exhibited by *S. muenchen* (24 patterns), *S. kentucky* (11 patterns), *S. blockley* (14 patterns), *S. enteritidis* (7 patterns), *S.chincol* (4 patterns) and *S. newport* (5 patterns).

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oleh
JAMAL KHAIR BIN HASHIM
Januari 1998

Pengerusi: Professor Dr. Gulam Rusul Rahmat Ali

Falkulti : Sains Makanan dan Bioteknologi

Lima ratus empat puluh sembilan (549) sampel daging ayam dan usus ayam dan 73 sampel jerami dalam remban ayam dan makanan ayam telah diperiksa untuk *Salmonella.* Dua ratus tiga puluh tujuh (237) mencilan *Salmonella* yang terdiri dari 15 jenis serotip telah dapat dipencilkan. Serotip yang paling banyak ialah *S. enteritidis* (35.2%), *S. muenchen* (20%), dan *S. chincol* (5.2%).

Kadar pengesanan *Salmonella* dalam sampel ayam mengikut keadaan pemencilan adalah seperti berikut: kaedah pengkayan gentil (PG) air basuhan dalam kaldu Rapaport Vasiliadis (RV) dieramkan pada suhu 42° C ialah 83% (126/232),; kedua-dua kaedah PG diperkaya dalam RV dan kaldu Manitol Selenite Cystine (MSC) yang dieramkan pada suhu 37 ^{0}C, 28% (43/232); dan gentil basuhan dicoret terus ke atas plat media ada 9% (14/232).

Kaedah konventional telah dapat mengesan *Salmonella* dalam 43.5% (115/264) sampel ayam, jerami reban ayam dan makanan ayam berbanding dengan 18.2% (48/264) menggunakan kaedah kit pantas TECRA UNIQUE.

Dua ratus tigapuluh tujuh (237) mencilan *Salmonella* terdiri dari 15 serotip yang berbeza adalah rentan kepada gentamisin, advosin dan enrofloksasin, kecuali satu mencilan *S. agona* yang meringtang kepada enrofloksasin. Mencilan *S. kentucky* menunjukkan 11 corak meringtang antibiotik yang ketara, diikuti oleh *S. blockley* (7), *S. agona* (4) dan *S. muenchan* (3).

Plasmid telah dikesan dalam 80% dari keseluruhan mencilan dengan berat molekul diantara <1 ke 50MDa. Kekerapan terdapat plasmid dalam berbagai serotip adalah seperti berikut: *S. blockley* (96%), *S. chincol* (92%), *S. muenchen* (90%), *S. kentucky* (88%), dan *S. enteritidis* (75%). Perbezaan variasi corak profil plasmid telah ditunjukkan oleh *S. muenchen* (24 corak), *S. kentucky* (11 corak), *S. blockley* (14 corak), *S. enteritidis* (7 corak), *S. chincol* (4 corak) dan *S. newport* (5 corak).

CHAPTER I

INTRODUCTION

Foodborne hazards of microbial origin continue to be as important agenda on the list of many regulatory agencies (Archer, 1990). Although the food safety control programmes have been strengthen, the incidence of foodborne infections including Salmonellosis have increased significantly in many countries. (Todd, 1978, 1989; Roberts, 1982 and Bryan 1988).

Salmonella has been a public health concern over the past 100 years since it was discovered in 1885 and continues to be a major foodborne pathogen affecting man (Taylor, 1967; Todd 1978;1989; Silliker, 1980; Roberts, 1982; Bryan 1988; Tauxe, 1991) . The major source of *Salmonella* are food of animal origin, especially poultry, beef and pork (Bryan, 1980 and 1988; Siliker, 1980).

Out of the 2000 distinct serovars of *Salmonella* that have been identified by man, one particular serovars, *S. enteritidis*, is causing global concern. The most common type of foods implicated with the outbreak of Salmonellosis caused by *S. enteritidis* are poultry eggs and poultry products (Coyle, *et al.*, 1988; Rampling *et al.*,

1989; Humphrey *et al.*, 1988). The outbreaks of *S. enteritidis* have increased in an unprecedented rate all over the world and reached such level that some have described it as a new pandemic (Rodrigue, 1990).

Poultry has been known to be a significant source of foodborne pathogens such as *Salmonella, Clostridium perfringens*, *Stapylococcus aureus* and *Listeria* (Todd, 1989; Genigeorgis *et al.*, 1989). There are many opportunities that exist for bacterial contamination at any stage of the production and processing of poultry (McMeekin and Thomas, 1979; Mead, 1976; Kampelmacher, 1987). Therefore, a significant percentage of food poisoning outbreaks have been associated with poultry and hence poultry may pose a public health hazard if no preventive measures are taken (Todd, 1978; Silliker, 1980; Bryan, 1980) .

Salmonella problem in poultry and poultry products being extensively research and this is reflected in the large number of epidemiological reports and publication that are publised annually. In Malaysia, there are not many reports on the incidence of Salmonella in poultry except those publisehed by Lim (1984), Jagathesan (1984; 1993) dan Arumgarsuamy (1994).

In Malaysia, poultry meat is the main source of protein poultry and the consumption of poultry has increased over the years. In 1990, the consumption of poultry meat was 297,000 tonnes, repersenting 59% of total meat consumption and is expected to be 570,000 tonnes in 1995 (van der Sluis, 1995). It is estimated the poultry industry is expected to produce 1,140,000 tonnes of poultry meat to meet local needs and for export. Currently, poultry production have have been dominated by commercial dan large-scale integrated producers. Thus, information on the prevalence of *Salmonella* in the local poultry industry is indeed very vital and needs immediate investigation.

The need for a rapid detection methods of pathogens in food have lead to proliferation of many new commercial kits. In many, these methods benefit the food industry in term of saved time and money. Most of the rapid method do pose problems such as high rates of false negative and low degree of sensitivity and / or specificity (Bailey *et al*, 1991; D'Aoust and Sewell, 1988; Eckner *et al*., 1992; Eckner *et al*., 1994; Entis and Boleszczuk, 1991; Holdbrook, *et al*., 1989a; Nath *et al*., 1989; St.Clair *et al*., 1990 and Ward *et al*., 1988). It is important to evaluate any new method before it is adopted in the local poultry industry for screening and monitoring of pathogen.

The information gained from the present study might be useful to the poultry farmers, processing plants, retails and regulatory agencies such as local authorities, Veterinary Department and the medical community

To the public health authority, the imformation will be of assistance in formulating preventive and control measures in dealing with the problems of *Salmonella* particularly related to poultry and poultry products. In addition, the imformation gained form antibiotic and plasmid profiling will be useful in understanding the epidemiology of *Salmonella* especially *S. enteritidis*.

The objectives of this study are:

(1) to investigate the prevalence of *Salmonella* in broiler production and processing system and at the retail outlets;

(2) to compare the effectiveness of a rapid commercial screening method TECRA UNIQUE (Bioenterprises Pty Ltd) with the conventional method of detecting *Salmonella*.

(3) to assess different enrichment conditions, temperature and plating media used for the isolation of *Salmonella* from broiler chicken rinses.

(4) to characterize and assess the relationship of *Salmonella* isolates with regard to their drug resistance pattern and the plasmid profile pattern.

CHAPTER II

REVIEW OF LITERATURE

Salmonella

The first report on *Salmonella* was in 1885 by Dr. D.E Salmon and since than more than 2000 different serovars of the genus *Salmonella* have been isolated from man and animals. All member of the genus *Salmonella* are pathogenic to man, animal or both. Salmonellosis is a food-borne illness caused by *Salmonellae*, which when ingested are able to grow in the intestinal tract. Salmonellosis can be divided into two main groups based on clinical symptoms, mode of transmission, and pathogenesis; (1) typhoid and paratyphoid fever, caused by *S. typhi* and *S. paratyphi* A, B, and C, and (2) enteric infection caused by other *Salmonellae* serovars (ICMSF, 1978).

S. typhi and *S. paratyphi* infections are characterized by continuous fever and absence of gastroenteritis. Salmonellosis caused by the other group of *Salmonella*, categorised under food poisoning, is characterized by an abrupt onset of diarrhea, nausea, abdominal pain, prostration, chills, fever and vomiting (ICMSF, 1978; Lennette, *et al*., 1985 ; IAMFES, 1987). Clinical examinations of adult humans shows

that dosage of 10^4 to 10^5 viable cells are required to cause enteric fever (Bryan, 1978). Both types of diseases spreads easily, creating a continuous infecting cycle from animal to man, man to man, man to animal.

Salmonella belong to the family *Enterobacteriaceae*. They are gram-negative, non-sporeforming rods, and ferment glucose with or without gas production. They are oxidase negative, reduce nitrates to nitrites and do not require NaCl. They may be either motile with pertrichous flagella or non-motile. All members of the genus *Salmonella* possess two antigenic components, the somatic antigen, O, and flagella antigen, H. The 'O' unagglutinable cultures also possessed a special antigen called virulence or 'Vi' antigen and belong to the group of K antigens (Kauffmann, 1965).

Source

Salmonella is widely distributed in nature including, plants, soils and intestines of humans and animals (Lennette, *et al*., 1985). Any food of animal origin can be a vehicle for transmission of *Salmonella* to man (ICMSF, 1978). This organism have been isolated from all types of marine products , beef, milk powders, bean sprouts beef, turkey, pork, ice cream , eggs, milk including pasteurized milk , Mexican foods, baked goods, cheese and macaroni (Natarajan, 1982; Bryan, 1988; Christian, 1989).

Salmonella in poultry

Poultry has been known to be a significant source of food infection, particularly in the outbreaks of salmonellosis (Silliker, 1980; Roberts, 1982; Kampelmacher, 1987; Humphery *et al.*, 1988; Bryan, 1980; Todd, 1989). Surveillance data in the US and Canada indicates that over 50% of the outbreaks of food poisoning have been linked to meat and poultry and poultry product (Todd, 1978; Bryan, 1980). In England and Wales, (1970-1979) it was reported that 36% of food poisoning cases were linked to poultry, only second to meat in frequency (Roberts, 1982).

Occurrence in life birds

Live poultry have been known to carry a variety of *Salmonella* serotypes such *S. pullorum, S.gallinarum, S. typhimurium , S thompson, S.enteritidis* and *S. newport* (Bisseru, 1968; Mackenzie and Bains, 1976; Bhatia *et al.*, 1979; Higgins *et al.*, 1981; Lahellec *et al.*,1986; Poppe *et al.*, 1991a; Poppe *et al.*, 1991b; Jones, *et al.*, 1991; Irwin *et al.*, 1994). The findings from these studies are summarized in Table 4. It can be observed from these studies, that live birds carries from 4 to 50 different *Salmonella* serovars. A wide variation in the incidence of *Salmonella* in poultry flocks exists. The most prominent serotypes encountered were *S. typhimurium*, *S. infantis*, *S. heidelberg, S. hadar, S. schwenzegrund, S. saintpaul* and *S. agona.*

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