A PRELIMINARY STUDY OF BACTERIOLOGICAL QUALITY OF MARKET BEEF OF TRICHUR WITH SPECIAL REFERENCE TO **SALMONELLA AND STAPHYLOCOCCI**

BY

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THESIS

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DECLARATION

I hereby declare that this thesis entitled "A PRELIMINARY STUDY OF BACTERIOLOGICAL QUALITY OF MARKET BEEF OF TRICHUR WITH SPECIAL REFERENCE TO SALMONELLA AND STAPHYLOCOCCI" is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship, or other similar title of any other University or Society.

E. Nanu

Mannuthy,

25-8-1978.

CERTIFICATE

Certified that this thesis, entitled "A PRELIMINARY STUDY OF BACTERIOLOGICAL QUALITY OF MARKET BEEF OF TRICHUR WITH SPECIAL REFERENCE TO SALMONELLA AND STAPHYLOCOCCI" is a record of research work done independently by Sri.E.Nanu, under my guidance and supervision and that it has not previously formed the basis for the award of any degree, fellowship or associateship to him.

25.8.78

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CONTENTS

INTRODUCTION

INTRODUCTION

The importance of meat in human diet is well known for its richness in protein, fat, vitamins and minerals. The nutritive value of meat is high. This quality of meat is helping not only the human consumers but also the micro-organisms which get access to the meat during its preparation, preservation and handling. The micro-organisms thus present in the meat are either saprophytic or pathogenic. The presence of saprophytic organisms in meat results in the deterioration of the quality and its spoilage whereas that of pathogenic type causes food-borne infections and intoxications among the consumers. It is noteworthy that in certain countries which have relatively high hygienic standard and efficient reporting services, more than half of all outbreaks of food-borne diseases are caused by meat and meat products (Joint FAO/WHO expert Committee on meat hygiene, 1962). In developing countries where the standard of hygiene is low, the chances of food-borne infection through meat is greater than that of developed countries.

In India, one of the developing countries, meat is being prepared from different species of animals. It is estimated that the total annual production of meat in

India is 7,20,000 tonnes of which 54% is from sheep and goat, 26% from buffaloes, 13% from poultry and 7% from pigs (Chattergee, 1976). The picture of meat production in Kerala is different from the rest of the country. The number of bovines slaughtered in Kerala in 1975 was 1.8 lakhs and that of sheep and goat was 3.25 lakhs (statistical Bulletin Animal Husbandry Department). This accounts for only about 58% of local bodies in the State. A survey conducted by the Central Research Institute, Madras, regarding the availability of hides and skin in Kerala, seven lakhs cattle and buffaloe hides were produced in Kerala during 1970, with an annual increase of 3%. The number of hides from fallen animals in Kerala will definitely be small as there is no ban on cattle slaughter in the State. It is therefore to be concluded that the availability of hides and skin seems to be more logical to assess the number of animals slaughtered.

Out of the total quantity of meat produced in Kerala, beef forms the bulk and is being freely marketed without preservation or processing. There is no religions taboo for beef consumption in this State. The standard of hygiene in the slaughter and marketing operations is unsatisfactory. By examining meat samples sold in the market, it is possible to assess the bacteriological quality of meat. Salmonella and Staphylococcus

important bacteria of public health importance encountered in raw meat. Other organisms like Clostridium welchii Bacillus cereus, Escherichia coli and Streptococcus are also met with. On perusal of literature, it is found that not much work has been done on the bacteriological quality of market beef in India except by a few like Jayaraman (1973); Janakiraman and Rajendran (1974); Manickam and Victor (1975) and by Rao (1977) who worked on buffalo meat. Therefore a project "A preliminary study of bacteriological quality of market beef of Trichur with special reference to Salmonella and Staphylococci" was taken up with the objective of gathering information on the extent and magnitude of prevalence of bacterial contamination of market beef in and around Trichur which would facilitate further investigation on the problem.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

One of the sources of bacteria causing food-borne infection is the animal from which the products are obtained. Other sources are the food handlers, utensils and environment.

There are many reports on the occurence of food-borne infections by consumption of foods of animal origin especially milk and meat (Munch-Petersen, 1963). Staphylococci, Salmonella and Cl. perfringens were found often associated with food-borne infections from unprocessed food, whereas Cl. botulinum occur in canned food stuffs.

Staphylococcus

The Staphylococcus, as one of the causative organisms of food poisoning, was first reported in 1884 by Vaugham. Coagulase positive S.aureus was found responsible for food borne intoxications. They were isolated frequently from milk and milk products like cheese, butter, cream, dried milk powder etc. (Allen and Stovall, 1960; Foltz et al., 1960; Hausler et al., 1960; Mickelsen et al., 1961; Post, Bliss and Okeffe, 1961; Mickelsen et al., 1963; Malaki, Kohneshahri and Gharagozloo, 1973). Rao (1977) examined 234 milk samples

and reported that 8.12% samples contained coagulase positive Staphylococci, of which 36.64% were found to be enterotoxigenic.

Human infection with Staphylococci is right from birth, particularly in maternity hospitals of which a very high percentage were antibiotic resistant (Eliasjones, Gordon and Whittaker, 1961; Choudhuri and Basu Mallick, 1968; Malaki, Kohneshahri and Gharagozloo, 1973 and Sharma and Yadava, 1973). Ravenholt et al.(1961) isolated coagulase positive Staphylococci from the nostrils of 102 of the 318 meat workers in fifteen establishments. A variety of Staphylococci were obtained from lesions of meat animals and meat workers. Similar work has been carried out by Munch Petersen (1963), Ashapasricha, Bhujwala and Shriniwas (1972), and Seligmann and Rosenbluth (1975).

Coagulase positive Staphylococci were isolated from samples of Shrimp (Lee, Orth and Anderson, 1970 and Virgilio et al., 1970). Christiansen and King (1971) isolated coagulase positive Staphylococci from salads and sandwiches.

Graham and Blumer (1971) isolated coagulase positive Staphylococci from pre-frozen dry cured ham.

Ostovar and Bremier (1975) examined a total of 480 commercially produced frozen food items including beef, poultry, fish and ready-to-eat dessert products and reported S.aureus in 18.3% beef and poultry products and 8.3% in ready-to-eat frozen desserts. Of the isolates, most of them produced enterotoxins. Guthertz et al., (1976) isolated S.aureus from 80% of the fresh ground turkey meat purchased from retail market. Guthertz et al. (1977) examined frozen comminuted turkey meat and reported that 50% of the samples contained S.aureus. Pathogenic Staphylococci were isolated from dressed chicken (Panda, 1971 and Rao, 1977)

Jay (1961) and Vanderzant and Nickelson (1969) isolated coagulase positive Staphylococci from market pork. Singha, Roy and Narayana (1970), isolated coagulase positive Staphylococci from pig carcass, of which 90% fermented mannitol. Ray et al., (1976) isolated coagulase positive Staphylococci from 11.54% pork samples. Rao (1977) examined 55 pork meat samples collected from market and reported that 25.45% pork samples revealed coagulase positive Staphylococci of which two were entero-toxigenic.

Mukerji and Lahri (1960) found that Staphylococci were the most predominant organisms present in apparently normal udders of healthy goats collected from slaughter houses. Coagulase positive Staphylococci were isolated from goat meat and mutton samples obtained from the market by many workers (Vanderzant and Nickelson, 1969; Rao, 1976; Rao, 1977; Sinha and Mandal, 1977; Nair and Sengupta, 1977). Some of the isolates were found to be enterotoxigenic.

Mukerji and Lahri (1960) also reported that Staphylococcus was the most predominant organism present in apparently normal udder of healthy cows collected from slaughter houses. Jay (1961) isolated coagulase positive Staphylococci from hamburger, beef liver and round steak obtained from retail stores. The isolates were found after phage typing to be similar to that obtained from handlers. Over 70% of the isolates produced haemolysins characteristic of human strains of S.aureus. Vanderzant and Nickelson (1969) examined muscle tissue of beef carcass and stated that Staphylococci were predominant among the isolates. Of the isolates 45.9% were coagulase positive. Duitschaever et al.(1973) examined a total of 213 samples of raw refrigerated ground beef obtained from different retail stores and reported that all samples yielded Staphylococci of which 17% were coagulase positive.

Rao (1977) examined sixty six buffaloe meat samples collected from market and found 27.27% of them coagulase positive Staphylococci. Of the isolate five were found to be enterotoxigenic.

Quie et al.(1960) found that 20.8% of 1248 isolates of coagulase positive Staphylococci from 144 patients to be resistant to neomycin. The isolates showed cross resistance between neomycin and Kanamycin. Rolinson et al. (1960) found that all the cultures of Staphylococcus pyogenes tested were sensitive to BRL 1241 in concentration of 1.25 to 2.5 microgram/ml regardless of their resistance to penicillin G and other antibiotics. Jackson and Rao (1961) made a comparative in vitro study of five Penicillin preparations against Penicillinase producing Staphylococci, found that seven strains of Staphylococci were inhibited by 125 microgram penicillin G per ml; five by 250 microgram/ml and one was not inhibited even at this level. PA 248 was consistently more inhibitory than Penicillin G. Jay (1961) reported that non-human strains of coagulase positive S.aureus were sensitive to Penicillin and three other antibiotics of which 6% were resistant to Streptomycin, 3% to Chlorotetramycline.

Knox and Smith (1961), made laboratory studies of methicillin resistance in cultures of Staphylococcus pyogenus and observed that varients resistant to methicellin, whether naturally occuring or artificially trained in the laboratory were also resistant to all the other Penicillins tested. The resistance occured independently of Penicillinase activity since it was shown both by Penicillinase positive and Penicillinase negative strains. Rao, Khera and Sharma (1966) reported the in vitro sensitivity of coagulase positive S.aureus strain of bovine mammary origin to different antibiotics. Of the 137 strains examined, 87.59% were sensitive to Penicillin, 86.86% sensitive to Streptomycin and all to Aureomycin, Chloromycin and Terramycin. Choudhuri and Basumallick (1968) reported that 94.9% and 36.2% of the coagulase positive Staphylococci isolated from human patients were Penicillin and Streptomycin resistant respectively as compared to 78.9 and 47.3% of coagulase negative strains. The percentage of strains showing sensitivity to all the antibiotics was higher with coagulase negative than with coagulase positive strains.

Salmonella:

Salmonellae are widely dispersed and occur frequently among the various species of lower animals and man as is evident from numerous reports. The first report on the

organism later named as Salmonella, associated with a fatal case of gastroenteritis, appeared as early as 1888 (Merchant and Parker, 1967). Anderson et al.(1961) found that infection in man and lower animals occur with the same type of Salmonella with a definite correlation between such infections.

Salmonellae are found to be present in the Gastro-intestinal tract of most of the lower vertibrates and man. More than 1400 serotypes of Salmonellae has been identified which are widely distributed. Among domestic animals, cattle and pigs are important reservoirs, though duck, poultry, and turkey harbour various types of Salmonellae. In cattle S.dublin and S.typhimurium were associated with various clinical illness among the young stock and in pigs S.choleraesuis and S.typhimurium has been commonly encountered (Jayaraman and John, 1969; Sodhi and Singh, 1970; Pramanik and Khanna, 1976; and Sharma and Pathak, 1977). Rokey and Erling, (1960) and Anderson et al.(1961) reported the isolation of S.typhimurium from calves. Kapur et al.(1973) isolated Salmonella from goat faeces. Singh and Kaura (1976) isolated various species of Salmonellae from pigs and man.

Meat from various species was found to contain Salmonella to a considerable extent. Poultry and Turkey meat are well known for contamination with Salmonella. A number of isolations from these were made (Bryan et al. 1968); Timoney et al., (1970) Guthertz et al., (1977). Isolation of Salmonella from mutton and goat meat has been made by many workers like Randhawa and Kalra (1970); Childers et al.,(1973); Janakiraman and Rajendran, (1974); Manickam and Victor, (1975). Nair and Sengupta (1977) isolated S.paratyphi A and S.paratyphi B from goat meat obtained from the market. Buxton, (1957) and Childers et al. (1973) isolated Salmonella from pork whereas Patterson (1969), Prior and Badenhorst, (1974) and Muralidhara Rao and Nandy (1977) reported isolation of Salmonella from pork sausage. Muralidhara Rao and Nany (1976) isolated fifteen different serotypes of Salmonellae from frog leg and fish meat. Isolations of Salmonella from chopping block in butcher shops (Randhawa and Kalra, 1970) and from slaughter house drains (Nath et al., 1970) were made.

Anderson et al., (1961) while investigating a food-borne infection, incriminated to calves' meat isolated same serotypes of Salmonella typhimurium from the patients as well as calves suffering from enteritis. They also isolated similar organisms from calf plates in a butcher

shop. Patterson (1969) could isolate S.dublin from one out of 125 frozen beef samples. Prior and Badenhorst, (1974) found 64% of fresh minced beef to contain; Salmonella, mostly S.typhimurium and S.thompson. Janakiraman and Rajendran (1974) examined 65 beef samples of which two were positive for Salmonella. Manickam and Victor (1975) could isolate S.dublin from four out of 67 beef samples examined. Childers et al.,(1973) could not isolate Salmonella from beef though he examined carcass of 93 cattle. They found, out of 218 carcases including 85 swine and 80 sheep, isolation rate of Salmonella was 10%. Pramanik and Khanna (1976) examined entrails from 120 cattle and isolated Salmonella from 10% of them but they could not isolate the organism from the meat. James (1977) thoug isolated 56 strains of Salmonella from 873 samples of materials comprising of rectal swab, faeces, mesentric lymphnodes, liver, bile, spleen, lung and drinage water could not isolate Salmonella from 25 beef and 20 goat meat samples.

Sharma and Agarwal (1970) found that most of the Salmonella strains isolated from human and animal sources were sensitive to Ampicillin or Tetracycline. Three

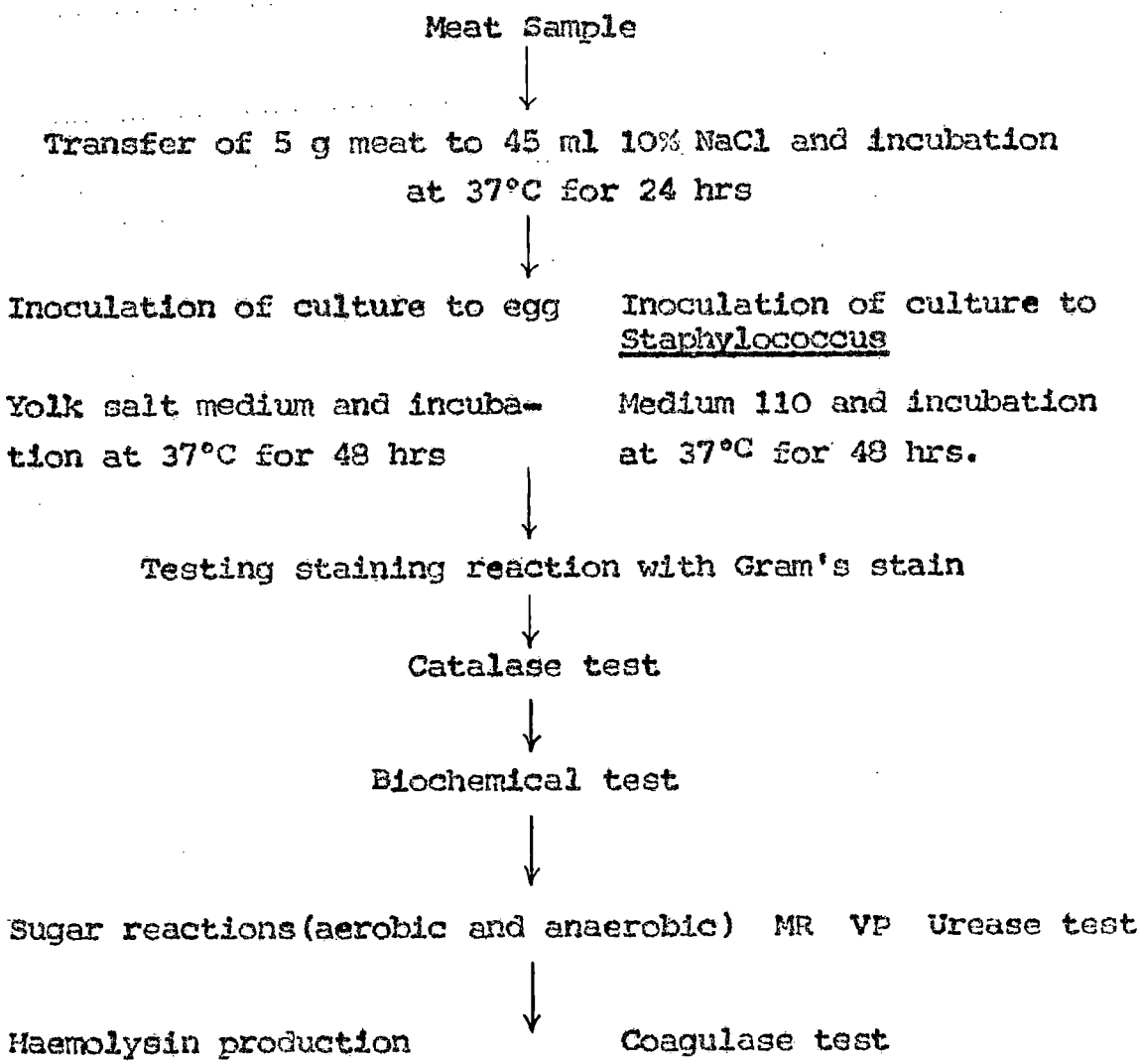
strains of S.typhi and one strain each of S.typhimurium and S.bovis morbificans were resistant to Ampicillin, Tetracycline and Chloramphenicol.

Dasgupta and Chandra Choudhury (1976) reported that among 60 Salmonella isolates comprising 13 serotypes from beef, pork and goat meat 56.7% were resistant to Bacitracin, 40% to Tetracycline, 30% to Streptomycin and 18.3% to Chloramphenicol. All strains were sensitive to Neomycin.

Upadhyay and Misra (1978) reported that all the 29 strains of S.welteverden isolated from buffalo, goat, pig and poultry were found sensitive to Ampicillin, Tetracycline, Oxytetracycline, Streptomycin, Chloramphenicol and Kanamycin. All the strains were resistant to Bacitracin. One strain from pig and three strains from poultry were resistant to Erythromycin and one each to Polymyxin.

MATERIALS AND METHODS

FLOW CHART FOR THE ISOLATION OF STAPHYLOCOCCI



MATERIALS AND METHODS

Beef samples for the present investigation were collected from retail shops in and around Trichur Town. Seventy beef samples were collected at random from five different meat stalls located in different areas. The samples were collected between November 1977 and April '78.

All samples were transported to the laboratory in separated clean polythene bags. The time for transportation was less than half an hour in all cases. Meat was processed immediately on arrival in the laboratory. All instruments and glasswares used for the work were suitably sterilized.

Initially, about 15 g of meat was separated from the surface of the meat sample with the aid of scissors and forceps, and placed in petridishes.

ISOLATION PROCEDURE FOR STAPHYLOCOCCI

Enrichment procedure

About 5 g of meat was transferred to 45 ml of 10% sodium chloride, and was incubated at 37°C for 24 hrs (Nair, Sengupta and Ghosh, 1975; and Rao, 1976).

Plating on selective medium

A loopful of inoculum from the enrichment media was streaked on two plates of Egg yolk salt medium and Staphylococcus medium 110 (S110) and incubated at 37°C

for 48 hrs (Jay, 1961; Mickelsen et al., 1963; Nordic Committee on Food Analysis, 1968; and Chou and Marth, 1969).

Selection of suspected colony

Suspected colonies from S110 (round, smooth, glistening opaque, convex, amorphous, edge entire and of yellow to golden yellow) and from Egg yolk salt medium (intense yellow surrounded by an opaque whitish grey precipitation zone) were transferred to nutrient agar slant by means of a sterilized loop and incubated at 37°C for 18-24 hrs.

Morphology and staining reaction

The morphology and staining reaction of the colony were studied by Gram's method of staining (Cruickshank et al., 1970). Gram positive cocci in clusters were selected.

Catalase test (Cowan, 1974)

Organism was grown in nutrient broth. After incubation overnight, one ml of 3% H_2O_2 was added and observed immediately and after five minutes, for evolution of gas. The gas production indicated catalase activity.

Acid production from sugars - Aerobic and anaerobic

Aerobic and anaerobic fermentation of mannitol and glucose were carried out by standard procedure (Cowan,1974).

Addition of liquid paraffin over the surface of the media in the tube was done for testing anaerobic activity. An over night broth culture was inoculated into tubes containing basal medium and 1% mannitol and to another set of tubes containing basal medium and 1% glucose and incubated at 37°C. It was examined daily for seven days for the production of acid evidenced by an yellow colour. The tubes which did not develop acid were retained for 30 days observing them daily for colour change.

Methyl red test (MR test)

Young agar slope culture was inoculated into glucose phosphate peptone water and incubated at 37°C for 48 hrs. Five drops of methyl red reagent was added and mixed. Immediate development of bright red colour indicated positive reaction and an yellow colour negative. If the results after 48 hrs were equvocal, the test was repeated with cultures that has been incubated for five days.

Voges-Proskauer test (V.P.test)

The agar slope culture was inoculated into glucose phosphate peptone water and incubated at 37°C for 48 hrs. To this 0.5 ml O'Meara reagent was added. The tubes were placed in water bath at 37°C for 4 hrs. Aeration was done

by shaking at intervals. A positive reaction was denoted by the development of an eosin pink colour, in 2-5 min.

Urease activity (Cowan, 1974)

Selected colonies were tested for urease activity by inoculating on slope of Christeneen's urea medium and incubated at 37°C. They were examined after 4 hrs incubation and then daily for five days for urease activity. Development of red colour indicates positive reaction.

Haemolysis

Culture was streaked on 5% blood agar plates using rabbit blood or sheep-blood, and incubated at 37°C for 24-48 hrs. Further, the sheep-blood agar plates were kept overnight in the refrigerator (Harrigan and McCance, 1976). The plates were observed for the occurance of haemolysis.

Coagulase test

Coagulase test was carried out by tube and slide methods (Cowan, 1974).

a) Tube method

To 0.5 ml of one in ten dilution of rabbit plasma in saline, 0.1 ml of an 18-24 hrs broth culture of the organism was added and incubated at 37°C and examined after 1, 3 and 6 hrs for a coagulam. Negative tubes were left at room temperature overnight and then re-examined.

b) Slide test

A colony was emulsified in a drop of distilled water on a microslide to produce a thick suspension was stirred with a straight platinum loop which has been dipped in rabbit plasma. A positive result was indicated by macroscopic clumping within 5 sec. Delayed clumping was not considered as a positive reaction.

Antibiotic sensitivity test (Cruickshank et al, 1975)

Nutrient agar plates were dried in the incubator. A loopful of an overnight broth culture was placed and spreaded evenly over the whole plate with a sterile bent glass-rod. The antibiotics used in the test were Ampicillin, Chloromycetin, Erythromycin, Kanamycin, Penicillin G, Streptomycin and Terramycin. The antibiotics discs were prepared as described in appendix. The antibiotic discs were placed two or more centimeters apart on the surface of the agar plate with sterile fine pointed forceps and pressed gently to ensure full contact with the medium and moistening of the disc, and incubated at 37°C for 24 hrs. The zone of inhibition of growth was measured by the use of caliper and was recorded. Interpretation was made as per standard prescribed (Anderson, 1973).

FLOW CHART FOR THE ISOLATION OF SALMONELLAE

Meat sample

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Transfer of 5 g meat to tetrathionate broth (45 ml) and incubation at 37°C for 24 hrs (enrichment)

Transfer of 5 g meat to seleni broth (45 ml) and incubation at 37°C for 24 hrs (enrichment)

↓

Inoculation of culture on brilliant green agar (BGA) and incubation at 37°C for 24 hrs

↓

Inoculation of selected colonies on Mac Conkey's agar and incubation for 24 hrs

↓

Staining reaction by Gram's stain

↓

Inoculation of selected colonies on TSI slant and incubation at 37°C for 24 hrs

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Motility test of colonies producing acid, and H_2S in TSI slant by hanging drop method

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Biochemical tests

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Indole test
MR test
Decarboxylase reaction
Nitrate reduction test
Catalase test
Sugar fermentation test

(glucose, lactose, sucrose, salicin, dulcitol, mannitol, raffinose and arabinose)

Citrate utilization test
VP test
Gelatin hydrolysis
Growth in KCN
Urease test

ISOLATION OF SALMONELLA

Enrichment procedure

From the meat sample, about 5 g of meat was transferred to two different enrichment media, namely 45 ml of tetrathionate broth and 45 ml of selenite broth and incubated at 37°C for 24 hrs, for the isolation of Salmonella (Nair, Sengupta and Ghosh, 1975).

Plating on selective medium-brilliant green agar (BGA) (Edwards and Ewing, 1972)

Using a platinum loop, cultures from tetrathionate broth and selenite broth were streaked on two plates each of BGA, and incubated at 37°C for 24 hrs and observed for growth of organism. In case where there were no growth after 24 hrs, the plates were kept in the incubator for another 24 hrs. (Randhawa and Kalra, 1970; Manickam and Victor, 1975; Singh and Kaura, 1976 and Shrivastava and Subramaniam, 1977).

Selection of suspected colonies

Colonies which were pinkish white, pinkish, colourless translucent to opaque with surrounding medium pink to red and transparent green colonies, on BGA plates were regarded as suspicious, (Manickam and Victor, 1975; Singh and Kaura, 1976 and Shrivastava and Subramaniam, 1977).

The colony was transferred to nutrient agar (NA) slant with a sterile loop. The NA slant was incubated at 37°C for 18-24 hrs and observed for growth. It was kept in the refrigerator for further processing.

Purification of the suspected colonies of Salmonella

The suspected colonies from NA slant were transferred to MacConkey's agar plates (Cruickshank et al., 1975). The plates were incubated at 37°C for 24 hrs (Singh and Kaura, 1976; and Shrivastava and Subramaniam, 1977). The moderately large, thick, greyish White, moist, smooth and circular pale or colourless non-lactose fermenting colonies in MacConkey's medium (Cruickshank et al. 1970) were transferred to NA slant and incubated for 18-24 hrs at 37°C.

Morphology and staining reaction

Smears prepared from colonies grown on NA slant were stained by Gram's method (Cruickshank et al. 1970). The colonies which had gram negative, rod shaped organisms were selected for further tests.

Triple sugar iron agar (TSI) reaction (Cowan, 1974).

The selected colonies were stabbed at the deep butt using a sterile straight wire and streaked on the slope

of TSI agar and incubated at 37°C for 24 hrs (Manickam and Victor, 1975; Singh and Kaura, 1976 and Shrivastava and Subramanian, 1977). The isolates which showed in the TSI agar tubes an alkaline slant (red), acidic butt (yellow) and black stab due to production of H_2S were selected and others were discarded.

Motility test

The selected colonies from TSI agar were tested for motility by hanging drop method (Cruickshank et al., 1970).

Biochemical test

IMVIC reactions (Cruickshank et al. 1975)

Among the IMVIC reactions all tests except the test for inocitol production were carried out.

Indole test

The colonies were inoculated into the basal medium and incubated at 37°C for 48 hrs. 0.5 ml Kovac's reagent was added and shook gently. A red colour in the alcoholic layer indicated a positive reaction (indole negative colonies were further incubated for 96 hrs, for optimum accumulation of indole).

Citrate utilisation test

The culture was inoculated into Simmon's citrate medium from saline suspension and incubated at 37°C

for 96 hrs. Blue colour and streak of growth indicated positive reaction and original green colour with no growth indicated negative reaction.

MR - VP tests

The tests were conducted similar to that for Staphylococcus.

The organisms which were indole negative, MR positive, VP negative and citrate positive were presumed to be Salmonellae and were subjected to further processing.

Decarboxylase reaction (Cowan, 1974)

The culture was inoculated into the tubes containing decarboxylase media, with and without 0.5% L-lysine hydrochloride and incubated at 37°C. The incubated tubes were examined daily for four days. Decarboxylation was indicated by a purple colour, whereas the control and negative tubes were yellow.

Gelatin hydrolysis (Liquefaction) (Cowan, 1974).

Culture was inoculated on gelatin agar plate and incubated at 37°C for three days. The surface of the plate was flooded with 5-10 ml 30% trichlor acetic acid solution; clear zones indicated areas of gelatin hydrolysis.

Nitrate reduction test (Cowan, 1974)

Fresh culture was inoculated into the nitrate broth and incubated at 37°C for five days. To this one ml of

nitrite reagent A (0.8% sulphanilic acid) followed by one ml of nitrite reagent B (0.05% alpha-naphthalamine) were added. Development of red colour within five minutes indicated the presence of nitrite by reduction of nitrate.

KCN test (Cowan, 1974)

A loopful of an over night broth culture was inoculated into KCN broth in screw capped bottles and incubated at 37°C for 48 hrs. The bottles were examined after 24 and 48 hrs for turbidity indicating growth of organism which denote positive reaction.

Catalase and urease tests were conducted in the same way as for Staphylococci.

Fermentation of sugars

Tests for fermentation of sugars such as glucose, lactose, mannitol, sucrose, salicin, dulcitol raffinose and arabinose by the organism were carried out, following procedure, prescribed by Cowan (1974). One percent solutions of the above sugars in peptone water were used. After inoculation, the sugar solutions were incubated at 37°C and examined daily for seven days for the production of acid or acid and gas. The tubes showing negative reactions

were retained for 30 days under incubation and daily observation. Fermentation of glucose and arabinose was tested under anaerobic condition by adding melted paraffin over the sugar media immediately after inoculation of the organism.

Antibiotic sensitivity tests were conducted in the same way as for Staphylococcus.

RESULTS

RESULTS

The results of the present study on a total of 70 beef samples collected from retail shops in and around Trichur town for the presence of *Staphylococci* and *Salmonella* are tabulated (Table I).

Staphylococci

Out of the 70 beef samples tested, *Staphylococci* could be isolated from 62 (88.57%). The isolates were identified as *Staphylococci* by the various tests (Table II). All the 62 isolates were gram positive cocci in clusters. They produced acid from glucose aerobically and anaerobically. Among the isolates, 17 (27.42%) produced haemolysis in five per cent rabbit blood agar (RBA) and 24 (38.71%) in five per cent sheep blood agar (SBA). Of the isolates, six (9.68%) produced haemolysis in both RBA and SBA (Table III). Acid was produced from mannitol by 55 (88.71%) isolates aerobically and by 51 (82.26%) anaerobically.

Among the 62 *Staphylococci* isolates, 49 (79.03%) were coagulase positive. Of the 62 isolates, 47 (75.81%) were coagulase positive by tube method, whereas 49 (79.03%) were coagulase positive by slide method (Table IV).

In voges proskauer (VP) and methyl red (MR) tests, 27 (43.55%) were VP positive and 35 (56.95%) were MR positive. Thirty seven (59.68%) were urease positive. All the isolates were catalase positive (Table V).

The result of the above tests revealed that 49 (79.03%) of the isolates were coagulase positive Staphylococci (S.aureus), and 13 (20.97%) were other Staphylococci.

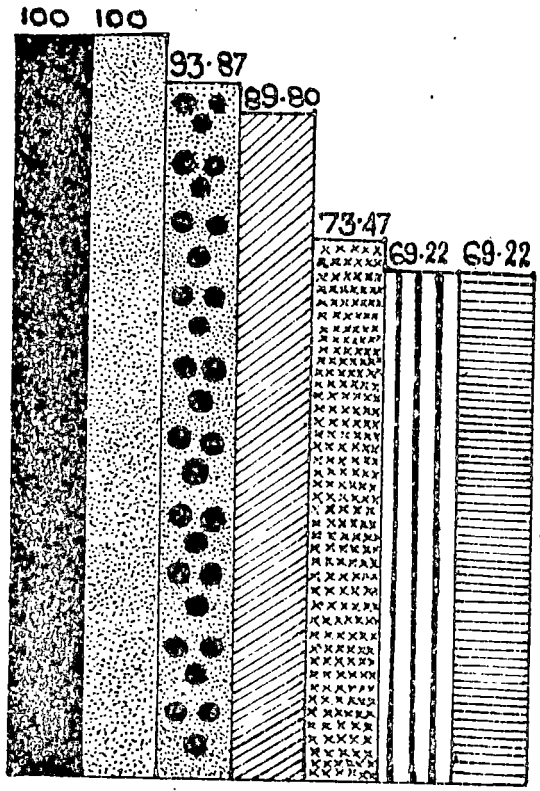
In the antibiotic sensitivity test all the 62 isolates were resistant to Penicillin G and Ampicillin (Table VI). Regarding the other antibiotic resistance of isolates, 58 (93.55%) for Erythromycin, 49 (79.03%) for Terramycin, 40 (64.52%) for Chloromycetin, 34 (54.84%) for Kanamycin and 36 (58.07%) for Streptomycin were observed. Twenty seven (43.55%) of the isolates were resistant to all the seven antibiotics.

Regarding the antibiotic resistance of S.aureus and other Staphylococci isolates (Table VII) all of them were resistant to Ampicillin and Penicillin G. Of the other antibiotics tested 46, 44, 36, 30 and 30 Staphylococcus isolates were found to be resistant to Erythromycin, Terramycin, Chloromycetin, Kanamycin and Streptomycin respectively. For the other Staphylococci (13) the respective antibiotic resistance was found in 12, 5, 4, 4 and 6

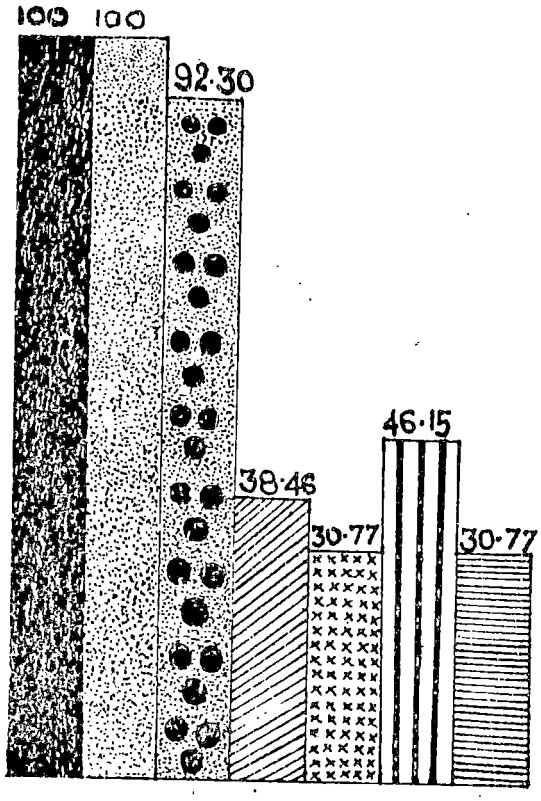
ANTIBIOTIC RESISTANCE OF ISOLATES

STAPHYLOCOCCUS

COAGULASE POSITIVE



COAGULASE NEGATIVE



PENICILLIN G

AMPICILLIN

ERYTHROMYCIN

TERRAMYCIN

CHLOROMYCETIN

STREPTOMYCIN

KANAMYCIN

Salmonella

A total of 70 beef samples collected from five different meat stalls in and around Trichur Town were processed for isolation of Salmonella.

Growth of suspected Salmonella colonies were noticed in 55 (85.94%) samples by their non-lactose fermenting character on MacConkey's agar and characteristic growth on Brilliant green agar (BGA).

All the 55 non-lactose fermenters were gram negative rods. Only four of the isolates produced acid and hydrogen sulphide in TSI agar and were urease negative (Table VIII & IX).

The four isolates which produced acid, and H_2S in TSI agar were subjected to further detailed test. Only one of these (K6) showed characteristics of Salmonella (Table X). The other three had shown negative results in lysine decarboxylase test. On the basis of cultural, morphological and biochemical findings it is presumed that one of the isolates (K6) belonged to the genus Salmonella.

Antibiotic sensitivity test of the isolate revealed that it was resistant to all except Kanamycin of the seven antibiotics namely Penicillin G, Ampicellin, Erythromycin, Terramycin, Chloromycetin, Kanamycin and Streptomycin.

DISCUSSION

DISCUSSION

Salmonellae and Staphylococci have been isolated from fresh meat, prepared meat and other animal products by a number of workers. The present investigation conducted on 70 samples of market beef in and around Trichur revealed the presence of Staphylococci in 62 (88.57%) of the samples and in only one, Salmonella could be detected.

STAPHYLOCOCCUS

Staphylococcus has been isolated from market beef of various species and meat products by many workers in India (Mukerji and Lahiri, 1960; Panda, 1961; Rao, 1977; and Sinha and Mandal, 1977). But report on isolation of Staphylococci from market beef in this country is rare.

Identification of Staphylococci were based on their colony characters, viz; Yellow or golden yellow colour, convex and round shape which are smooth and glistening and characteristic growth on egg yolk salt medium, morphological and staining reactions on the organisms and biochemical characters such as coagulase activity, fermentation of mannitol and glucose and haemolysis in rabbit and sheep blood agar.

Seventy nine point zero three per cent of isolates were found to be coagulase positive which is indicative of their pathogenic status. The presence of coagulase positive Staphylococci has been reported from beef by Vanderzant

and Nickelson (1969) and Duitchaever et al.(1973). Rao (1977); has isolated coagulase positive Staphylococci from market buffalo meat. These reports show the incidence vary from 17% to 45.9%. The presence of coagulase positive Staphylococci in the market beef in such high percentage (79.03) of samples may be from either the meat handlers or from slaughtered animals or possibly from the environment. Staphylococci being widely distributed in nature are encountered on the skin and upper respiratory tract of man and animals. It is more frequently the etiological agent of human infection than in lower animals. The carrier rate of S.aureus range between 20 and 30% among healthy persons (Cruickshank et al. 1976). In animals Staphylococci are present in inflammatory lesions especially in the udder as well as normal udder tissue (Mukerji and Lahiri, 1960). The source of Staphylococci in meat could be the meat handlers, or the animals or the environment. Staphylococci which are present in the skin, respiratory tract and other sites like udder of animal slaughtered can contaminate the meat heavily during the process of slaughter and dressing of carcasses. The meat stalls from where the samples were collected are located very close to the roads having frequent traffic movement and are not protected from dust. The dust which is lifted during the movement of vehicles

can stick to the surface of exposed meat and in that process add Staphylococci which are generally present in the dust. The carcass is being suspended in the meat stall for indefinite period till the last piece of meat is sold out. The atmospheric temperature in this part of the country can favour the growth of bacteria in the meat. All these factors could have contributed to the high percentage of pathogenic and toxin producing Staphylococci in the market beef.

Seventeen of the 49 coagulase positive Staphylococci produced haemolysis in rabbit blood agar, 24 in sheep blood agar and six of the above showed haemolysis in both. The haemolysis in rabbit blood agar and sheep blood agar is indicative of Staphylococci of human and animal origin respectively (Cowan, 1974; and Cruickshank et al., 1975). This character is indicative of the source of contamination in meat. It requires phage typing of isolates for confirmation.

The antibiotic sensitivity of the isolates revealed that all of them were resistant to Penicillin G and Ampicillin. Choudhury and Basu Malick (1968) has reported 94.9% of coagulase positive Staphylococci resistant to Penicillin and Sharma (1966) found 87.59% of coagulase positive S.aureus of bovine origin sensitive to Penicillin.

Jay (1961) reported that none of the non-human coagulase positive S.aureus isolated from meat was resistant to Penicillin. Cruickshank et al.(1970) observed 80% of S.aureus from hospital are Penicillin resistant. The present observation of total resistance to Penicillin G and Ampicillin may be due to the resistance acquired by the organism in nature by their previous exposure to the antibiotics. With regard to other antibiotics, varying degrees of resistance has been observed. 93.87% of S.aureus and 92.30% of coagulase negative Staphylococci were resistant to Erythromycin. Individual isolates have shown varying degrees of multiple resistance to different antibiotics. The drug resistance has taken the form of multiple resistance of individual strains of Staphylococc and strains resistant to more than one antibiotic these days is general than exception (Burrows, 1968). Further, the study reveals a definite difference in the sensitivity of isolates to antibiotics based on their coagulase activity This difference may be due to the difference in the pathogenicity of the organism and its exposure to the antibiotics during treatment of cases.

The presence of Staphylococcus in market beef has public health importance. The large percentage of coagulase positive isolates indicate the potential danger of

food-borne intoxication to the consumers if there is a chance of multiplication and toxin production in meat. It is well known Staphylococcal enterotoxin is thermostable and cooking will not destroy it normally. To reduce the load of Staphylococci in meat, the meat handlers should be screened and protective clothing, gloves, aprons and masks should be provided to break the channel of contamination. The animal with inflammatory lesion should not be slaughtered and strict slaughter house hygiene is to be followed. Meat should be transported in clean covered vehicles to prevent contamination en route. The meat stalls should be designed and constructed in such a way that the dust from outside is prevented from entry inside. Wherever the meat is to be kept for a long time before it is sold off, it should be under refrigeration.

SALMONELLA

Salmonellosis is the most frequently reported food-borne disease of man and Salmonellae are found as contaminants of food more than any other organism (WHO, 1968). The incidence of gastero-enteritis and food poisoning are reported to occur mostly through symptomless animals and animal products like meat. For lack of adequate public health awareness, the actual incidence of salmonellosis

in India is grossly under estimated. There are few reports of isolation of Salmonella from meat, abattoir equipments and drains. (Jayaraman and John, 1969; Randhawa and Kalra, 1970; Janakiraman and Rajendhran, 1974; Manickam and Victor, 1975; and Nair and Sengupta, 1977). In the present study only one isolate of Salmonella showing typical characters, could be isolated from 70 beef samples tested.

Though Manickam and Victor (1975) made four isolates of Salmonella from 64 samples of buffaloe meat. A detailed survey on the incidence of Salmonellae in meat animals conducted in Kerala did not reveal presence of Salmonella in beef (James, 1977).

Considering the wide distribution of Salmonella in nature especially among the animals, the rate of isolation from meat appears small. The reason for this cannot be attributed to a high standard of slaughter house hygiene or low carrier rate among the slaughtered animals, both of which may not be true in this area. In slaughter houses, contamination of meat can be either from the slaughtered animals, meat handlers or from environment, particularly the water used. The former, two factors could not be assessed, but the water is from protected supply. The low percentage of isolation cannot be attributed to any reason, and this is not much different from the results

of work done by others like Pramanick and Khanna,(1976) and James (1977).

The Salmonella isolate was found to show varying degree of resistance to the antibiotics tested. Reports on antibiotic resistance of Salmonella isolated from various sources including meat indicate wide variation (Sharma and Agarwal, 1970; Dasgupta and Chandrachowdhury, 1976; Upadhyay and Misra, 1978).

The observation is very important, indicating that infection with this organism is not uniformly amenable to treatment with all usual antibiotics. Food-borne infection with meat contaminated with similar organism is a potential challange to medical and public health workers.

Isolation of drug resistant Salmonella from the market beef denotes the necessity of improvement in food hygiene practices. It is true that meat is well cooked, before consumption in India. But contaminated meat can introduce cross contamination in the kitchen and cooked food stuffs. This boils down to the necessity of maintenance of high standard of hygiene in slaughter houses and of meat till it is released for human consumption, in order to prevent multiplication of micro-organism.

SUMMARY

SUMMARY

Salmonella and Staphylococci are two of the important bacteria responsible for food-borne infections in man. Food of animal origin particularly meat and meat products are increminated for such infection. The isolation of Salmonellae and Staphylococci from animal products had been recorded by many workers. Beef which forms the bulk of meat prepared and marketed in Kerala is a peculiar feature of meat trade in India. In order to study the presence of Salmonellae and Staphylococci in market beef with a view to its public health importance, the present investigation was conducted.

Seventy samples of beef obtained from meat stalls in and around Trichur town were processed for isolation of Staphylococci and Salmonella following standard methods. The isolates were identified on the basis of cultural, morphological and biochemical characters. Wherever necessary, standard differentiation tests were also conducted for confirmation.

A total of 62 isolates of Staphylococci of which 49 coagulase positive and 13 coagulase negative were recovered. Only one isolate of Salmonella could be obtained. All the coagulase positive isolates were considered as Staphylococcus aureus. All the isolates were resistant

to Penicillin G and Ampicillin. About 93% were resistant to Erythromycin. The resistance rate against other antibiotics was 89.80% for Terramycin, 73.47% for Chloromycetin, 69.22% for Streptomycin and 69.22% for Kanamycin for S.aureus. For other Staphylococci the resistance rate was 38.46%, 30.77%, 46.15% and 30.77% respectively. The importance of coagulase positive Staphylococci in beef, in view of their ability to cause intoxication and their high resistance to the common antibiotics indicate their public health significance. The possible sources of Staphylococci in beef and suggestions for reduction of contamination are discussed.

Regarding Salmonella, the isolation rate from market beef is small (1.43%). Salmonellae are known to be widely distributed. Man and animal harbour the organism in large numbers and disseminate through the excreta. The reason for the low rate of isolation is not clear. Its antibiotic sensitivity was tested against seven common antibiotics, and found to show varying degrees of resistance. The resistance of the organism to most of the common antibiotics indicates its potential threat to food-borne infection. The importance of food-hygiene practices is stressed.

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TABLES

Table No. I. Statement of isolates from 70 samples of market beef

No. of Staphylococci	Percentage	No. of Salmonella	Percentage
62	88.57	1	1.43

Table II. Details of tests conducted for isolation of Staphylococci from market beef

Sample No.	Staining reaction (Gram's) staining	Coagulase test		Haemolysis in		Acid from mannitol		Acid from glucose		Urease activity	Catalase activity	MR	VP
		Tube	Slide	rabbit blood agar	Sheep blood agar	Aerobic	Anaerobic	Aerobic	Anaerobic				
1	2	3	4	5	6	7	8	9	10	11	12	13	14
M1	+	+	+	-	-	+	+	+	+	+	+	+	-
M2	+	+	+	-	+	+	+	+	+	+	+	-	+
M3	+	+	+	+	-	+	+	+	+	+	+	+	-
M4	+	+	+	-	-	+	+	+	+	+	+	+	-
M5	+	+	+	-	+	+	+	+	+	+	+	+	-
M6	+	+	+	-	+	+	+	+	+	-	+	+	-
M7	+	+	+	-	-	+	+	+	+	-	+	+	-
M8	+	+	+	-	-	+	+	+	+	-	+	+	-
M9	+	+	+	-	+	+	+	+	+	-	+	+	-
M10	+	+	+	+	-	+	+	+	+	-	+	+	-
M11	+	+	+	+	-	+	+	+	+	+	+	-	+
M12	+	+	+	-	-	+	-	+	+	+	+	-	+
M13	+	-	+	+	+	+	+	+	+	+	+	-	+
M14	+	-	-	-	-	+	+	+	+	+	+	-	+
M15	+	+	+	-	-	+	+	+	+	-	+	-	+
M16*													

1	2	3	4	5	6	7	8	9	10	11	12	13	14
K1	+	+	+	−	−	+	+	+	+	+	+	−	+
K2	+	−	−	−	+	−	−	+	+	+	+	−	+
K3	+	+	+	−	−	+	+	+	+	+	+	−	+
K4	+	+	+	−	−	+	+	+	+	+	+	−	+
K5	+	−	+	+	+	+	+	+	+	−	+	+	−
K6	+	+	+	+	−	+	+	+	+	+	+	+	−
K7	+	+	+	−	+	+	+	+	+	+	+	+	−
K8	+	+	+	+	−	+	+	+	+	+	+	+	−
K9	+	+	+	+	−	+	+	+	+	−	+	+	−
K10	+	+	+	−	−	+	+	+	+	−	+	+	−
K11	+	+	+	−	−	+	+	+	+	−	+	+	−
K12	+	+	+	+	+	+	+	+	+	−	+	−	+
K13	+	+	+	−	+	+	+	+	+	+	+	−	+
K14	+	−	−	−	+	+	−	+	+	−	+	−	+
K15	+	+	+	−	−	+	+	+	+	−	+	−	+
K16	+	+	+	−	+	+	+	+	+	−	+	+	−
K17	+	−	−	−	+	+	+	+	+	+	+	−	+
K18	+	−	−	−	−	+	+	+	+	+	+	−	+
K19*													
K20*													
K21	+	+	+	−	−	+	+	+	+	+	+	−	+
K22	+	+	+	−	+	+	+	+	+	−	+	+	−
K23	+	−	−	−	−	−	−	+	+	+	+	+	−
K24	+	−	−	+	−	−	−	+	+	+	+	+	−
K25*													
K26*													

1	2	3	4	5	6	7	8	9	10	11	12	13	14
K27	+	+	+	-	-	+	+	+	+	-	+	-	+
K28*													
K29*													
K30	+	+	+	+	-	+	+	+	+	+	+	+	-
N1	+	-	-	-	-	-	-	+	+	-	+	+	-
N2	+	+	+	-	+	+	+	+	+	-	+	+	-
N3	+	+	+	-	-	+	+	+	+	+	+	+	-
N4	+	+	+	+	+	+	+	+	+	+	+	+	-
N5	+	+	+	+	-	+	+	+	+	+	+	-	+
N6	+	+	+	+	-	+	+	+	+	+	+	+	-
N7	+	+	+	-	-	+	+	+	+	-	+	+	-
N8*													
N9	+	-	-	-	+	+	-	+	+	-	+	+	-
N10	+	+	+	+	+	-	-	+	+	-	+	+	-
Na1	+	-	-	+	-	+	+	+	+	-	+	-	+
Na2	+	+	+	+	+	+	+	+	+	+	+	-	+
Na3	+	+	+	-	+	+	+	+	+	+	+	+	-
Na4	+	-	-	-	-	-	-	+	+	+	+	-	+
P1	+	+	+	-	-	+	+	+	+	+	+	+	-
P2	+	+	+	-	+	+	+	+	+	+	+	-	+
P3	+	+	+	-	+	+	+	+	+	+	+	+	-
P4	+	+	+	+	+	+	+	+	+	-	+	-	+
P5	+	-	-	-	-	-	-	+	+	-	+	+	-
P6	+	+	+	-	+	+	+	+	+	+	+	-	+
P7	+	+	+	-	-	+	-	+	+	+	+	-	+
P8	+	+	+	-	-	+	+	+	+	-	+	-	+
P9	+	+	+	-	-	+	+	+	+	+	+	+	-
P10	+	+	+	-	+	+	+	+	+	+	+	+	-

Table No. III.

Haemolytic activity of isolates (Staphylococci) in rabbit blood and sheep blood agar

No. of isolates tested	Haemolytic	Percentage	Haemolysis in rabbit blood agar (RBA)	Percentage	Haemolysis in sheep blood agar (SBA)	Percentage	Haemolysis both in SBA and RBA	Percentage	No haemolysis	Percentage
62	35	56.45	17	27.42	24	38.71	6	9.68	27	43.55

Table No. IV.

Result of coagulase test on isolates (Staphylococcus)

No. of isolates tested	Coagulase positive				Coagulase negative	
	Slide	Percentage	Tube	Percentage	Total No.	Percentage
62	49	79.03	47	75.81	13	20.97

Table No. V.

Summary of tests on isolates suspected for Staphylococci

Total No.of isolates	Coagulase positive by		Haemolysin production in		Acid from glucose		Acid from mannitol		Total no.of MR positive	Total no.of VP positive	Total no.of urease positive	Total no.of catalase positive
	Tube	Slide	Rabbit blood agar	Sheep blood agar	Aerobic	Anaerobic	Aerobic	Anaerobic				
62	47	49	17	24	62	62	55	51	35	27	37	62

Table No. VI.

Result of in vitro antibiotic sensitivity of *Staphylococcus* isolated from beef

Antibiotics used	No. of strains tested	No. of sensitive strains	Percentage	No. of resistant strains	Percentage
Penicillin G	62	0	0	62	100
Ampicillin	62	0	0	62	100
Streptomycin	62	26	41.94	36	58.07
Kanamycin	62	28	45.16	34	54.84
Chloromycetin	62	22	35.48	40	64.52
Terramycin	62	13	20.97	49	79.03
Erythromycin	62	4	6.45	58	93.55

Table No. VII.

Antibiotic resistance of *Staphylococci* isolated from market beef

Antibiotics	*S.aureus*		Other *Staphylococci*	
	No.of isolates resistant	Percentage of isolates resistant	No. of isolates resistant	Percentage of isolates resistant
Ampicillin	49	100	13	100
Penicillin G	49	100	13	100
Erythromycin	46	93.87	12	92.30
Terramycin	44	89.80	5	38.46
Chloromycetin	36	73.47	4	30.77
Streptomycin	30	69.22	6	46.15
Kanamycin	30	69.22	4	30.77

Table No. VIII.

Details of tests conducted for isolation of *Salmonella* from market beef

Sample No.	Growth in BGA	Lactose fermentation in MacConkey's agar	Gram's staining reaction	TSI reaction	Urease activity
1	2	3	4	5	6
M1	+	-	-	a	
M2	+	-	-	-	
M3	+	-	-	-	
M4	-	-	-	-	
M5	-	-	-	-	
M6	+	-	-	-	
M7	+	-	-	a	
M8	+	-	-	-	
M9	+	-	-	-	
M10	+	-	-	-	
M11	+	-	-	-	
M12	+	-	-	-	
M13	+	-	-	-	
M14	+	-	-	-	
M15	+	-	-	-	
M16	+	-	-	-	
K1	+	-	-	-	
K2	+	-	-	a	
K3	+	-	-	-	
K4	+	-	-	a	
K5	+	-	-	a and H_2S	-
K6	+	-	-	a and H_2S	-
K7	+	-	-	-	
K8	+	-	-	a	

1	2	3	4	5	6
K9	+	+			
K10	-	-	-	-	
K11	+	+			
K12	+	-	-	-	
K13	+	-	-	a	
K14	+	-	-	-	
K15	+	-	-	a	
K16	+	-	-	-	
K17	+	-	-	-	
K18	+	-	-	-	
K19	+	-	-	-	
K20	+	-	-	a	
K21	+	-	-	-	
K22	+	-	-	-	
K23	+	-	-	a	
K24	+	+			
K25	+	-	-	-	
K26	+	-	-	-	
K27	+	-	-	-	
K28	+	+			
K29	+	-	-	a	
K30	+	+			
N1	-	-	-	-	
N2	+	-	-	a	
N3	+	-	-	a	
N4	+	+			
N5	+	+			
N6	+	-	-	a	
N7	+	-	-	a	
N8	+	-	-	-	
N9	+	-	-	a	
N10	+	-	-	a and H_2S	-

1	2	3	4	5	6
Na1	+	-	-	a	
Na2	+	-	-	-	
Na3	+	-	-	-	
Na4	-	-	-	-	
P1	+	-	-	a	
P2	+	+			
P3	+	-	-	-	
P4	+	-	-	a and H_2S	-
P5	-	-	-	-	
P6	+	-	-	-	
P7	+	+			
P8	+	-	-	-	
P9	+	-	-	-	
P10	+	-	-	-	

\+ = Positive reaction/growth

\- = Negative reaction/no growth

a = acid

Table No. IX.

Summary of result of processing market beef sample for isolation of *Salmonella*

No. of samples processed	Non-lactose fermentation	Grams staining Gram negative	Urease negative	Acid, and H_2S production in TSI
70	55	55	4	4

Table No. X.

Summary of the tests performed on the isolates suspected for Salmonella from beef samples

Test performed	Number of the isolates			
	K_5	K_6	N_{10}	P_4
Gram staining	-	-	-	-
Motility	+	+	+	+
Urease activity	-	-	-	-
H_2S from TSI	+	+	+	+
Growth in KCN	-	-	-	-
Gelatin liquefaction	-	-	-	-
Lyzine-decanboxylase	-	+	-	-
Catalase	+	+	+	+
Nitrate reduction	+	+	+	+
MR	+	+	+	+
VP	-	-	-	-
Indole production	-	-	-	-
Citrate utilization	+	+	+	+
Glucose	ag	ag	ag	ag
Glucose (Anaerobic)	a	a	a	a
Arabinose	ag	ag	ag	ag
Arabinose (anaerobic)	ag	ag	ag	ag
Lactose	-	-	-	-
Sucrose	-	-	-	-
Mannitol	ag	ag	ag	-
Dulcitol	-	-	-	-
Salicin	-	-	-	ag
Raffinose	-	-	-	-

+ = Positive reaction
- = Negative reaction
a = acid
ag = acid and gas

APPENDIX

APPENDIX

1. Antibiotic sensitivity test

Preparation of wet discs for antibiotic sensitivity test (Cruickshank et al.,1975)

Punched out discs of 6.25 mm in diameter from No.I Whatman filter paper, batches of 100 discs in screw capped bottles were sterilized by dry-heat at 140°C for 60 minutes. Antibiotic solutions were prepared. Every one ml of the solution contained 100 times the amount of antibiotics required in the disc. Added one ml solution to each bottle of 100 disc and the whole of this volume were absorbed. It was assumed that each disc absorbed approximately 0.01 ml antibiotic. The discs were in wet condition. They will retain the moisture and potency for at least three months if kept in screw-capped bottles with the caps screwed on tightly.

2. Biochemical test (Sugar fermentation test)

(1) Preparation of basal media (Peptone water) (Cowan, 1974)

Peptone	..	10 g
Sodium chloride	..	5 g
Dist.water	..	1000 ml

Dissolved the ingredients in warm water, adjusted the pH 8.0 to 8.4 and boiled for 10 minutes. Filtered, adjusted the pH 7.2 to 7.4 and sterilized at 115°C for 20 minutes.

(ii) Sterilization of sugars

Dissolved the sugar in distilled water and sterilized by filteration using seitz filter.

(iii) Preparation of sugar media (Cowan, 1974)

To 900 ml of peptone water already prepared added 10 ml of 0.2% phenol red indicator and sterilized at 115°C for 20 minutes. Added aseptically 90 ml of the appropriate sugar solution which was previously prepared, sterilized and mixed. About 5 ml of the mixture was distributed into sterile test tubes with inverted Durham's tubes and steamed for 30 minutes.

3. Brilliant green agar (Edwards and Ewing, 1972)

Yeast extract	..	3 g
Peptone (bacto)	..	10 g
Sodium chloride	..	5 g
Lactose	..	10 g
Sucrose	..	10 g
Phenol red	..	0.08 g
Brilliant green	..	0.0125 g
Agar	..	20 g
Distilled water	..	1000 ml

(Final pH about 6.9-7)

Sterilized at 121°C for exactly 15 minutes cooled and poured into plates.

Two ml of 0.5% brilliant green in distilled water was used for one litre of medium. One gram of phenol red was dissolved in 40 ml 0.1 N sodium hydroxide, added 460 ml distilled water, 4 ml of the solution per liter of medium was used.

4. Blood agar (Cowan, 1974)

Defibrinated blood	..	50 ml
Nutrient agar	..	950 ml

Melted the nutrient agar, cooled to 50°C and added the blood aseptically. Mixed and distributed in plates.

5. Catalase test (Cowan, 1974)

To an overnight incubated nutrient broth culture of the organism, was added one ml 3% H_2O_2 and examined immediately and after five minutes for evolution of gas, which indicated catalase activity.

6. Citrate utilization test

(i) Preparation of Simmon's Citrate (Cowan, 1974)

Koser's citrate	..	1000 ml
Agar	..	20 g
Bromthymolblue, 0.2% solution	..	40 ml

Dispensed, autoclaved at 121°C for 15 minutes and allowed to set as slopes.

(ii) Koser's citrate (Cowan, 1974)

Sodium chloride (Nacl)	..	5 g
Magnesium sulphate ($Mg\ SO_4.7H_2O$)	..	0.2 g
Ammonium hydrogen phosphate ($NH_4H_2PO_4$)	..	1 g
Secondary potasium phosphate (K_2HPO_4)	..	1 g
Distilled water	..	1000 ml
Dissolved the salts in water	..	
Citric acid	..	2 g

Added to the salt solution and adjusted to pH 6.8 with N-NaOH. Filtered through a sintered-glass funnel. The medium should be colourless. Sterilized at 115°C for 20 minutes.

7. Decarboxylase media (Cowan, 1974).

Peptone	..	5 g
Yeast extract	..	3 g
Glucose	..	1 g
Distilled water		1000 ml
Bromcresol purple 0.2% solution	..	10 ml

The solids were dissolved in the water, adjusted to pH 6.7 and added bromcresol purple solution. Sterilization was carried out at 115°C for 20 minutes. Poured about 250 ml into each bottle. To one of the bottles containing the

Another bottle with the media without addition of lysine hydrochloride was also taken.

Readjusted the pH to 6.7 if necessary and transferred 2 ml volumes in small tubes and sterilized at 115°C for 10 minutes.

8. Egg yolk salt medium (Nordic Committee on Food Analysis, 1968).

(i) Basic medium

Yeast extract	..	2.5 g
Tryptone	..	10 g
Gelatine	..	30 g
Mannitol	..	10 g
Lactose	..	2 g
Sodium chloride (Nacl)	..	75 g
Secondary potassium phosphate (K_2HPO_4)	..	5 g
Agar	..	15 g
Distilled water	..	1000 ml

Medium was adjusted to pH 7.2

Dissolved the agar by autoclaving in about 500 ml of water at 120°C for 45 minutes. Dissolved yeast extract, tryptone, gelatine, Nacl, and secondary potassium phosphate in another 500 ml of water at about 50°C.

Mixed the two solutions and filtered. Finally added mannitol and lactose. Distributed the medium in flasks inportions of 200 ml and autoclaved at 120°C for 15 minutes.

(ii) Trypsin-Ox-heart broth

Minced, fresh ox heart	..	250 g
Pancreatin	..	2.5 g
Secondary sodium phosphate ($Na_2HPO_4 . 12H_2O$)	..	3 g
Sodium chloride (Nacl)	..	2 g

Mixed ox heart with 1,000 ml of water heated to 50°C and added pancreation. Allowed the mixture to stand for digestion at 50-56°C for 2 hours with occasional stirring. Filtered through two layers of gauze, boiled the filterate for 5 minutes and filtered through a filter paper Whatman No. 1.

Filled the filterate upto 1000 ml with distilled water, and secondary sodium phosphate and sodium chloride and adjusted the pH to 7.4 with NaOH. Boiled for 30 minutes and filtered through a filter paper. Distributed the medium in bottles and autoclaved for 15 minutes at 120°C.

Poured the finished medium under aseptic conditions in portions of 12-15 ml into sterile petridishes which were allowed to stand until the agar had soldified and the surface was dry.

(iii) Working Medium

To 200 ml of basic medium melted and cooled to 45°C added immediately before use, 4 g sterile egg-yolk shaken up in 22 ml trypsin-ox-heart broth previously heated to 45°C and poured aseptically into petridishes and allowed to solidify.

9. Gram's method of staining (Cruickshank et al., 1970)

A. Solutions required

(i) Ammonium oxalate-Crystal violet solution

Crystal violet	..	20 g
Methylated spirit	..	200 ml
Ammonium oxalate 1% in water		800 ml

(ii) Iodine solution

Iodine	..	10 g
Potassium iodiade	..	20 g
Distilled water	..	1000 ml

(iii) Liquor iodi fortis (BP)

Iodine	..	10 g
Potassium iodide	..	6 g
Methylated spirit	..	90 ml
Distilled water	..	10 ml

(iv) Iodine-acetone

Liquor iodifortis	..	35 ml
Acetone	..	965 ml

(v) Dilute carbol fuchsin

Ziehl-Neelsen's (strong) carbol fuchsin	.. 50 ml
Distilledwater	.. 950 ml

(vi) Ziehl-Neelsen's (strong) carbol fuchsin

Basic fuchsin	.. 10 g
Absolute alcohol (ethanol)	.. 100 ml
Solution of phenol (5% in water)	..1000 ml

Dissolved the dye in the alcohol and added to the phenol solution.

B. Staining procedure

(i) Covered the slide with ammonium oxalate-Crystal violet and allowed to act for about 30 seconds.

(ii) Poured off crystal violet stain and washed freely with iodine solution, covered with fresh iodine. solution and allowed to act for about 30 seconds.

(iii) Poured off iodine solution and washed freely with iodine-acetone. Covered with fresh iodine-acetone and allowed to act for about 30 seconds.

(iv) Washed thoroughly with water.

(v) Counterstained with dilute carbol fuchsin for about 30 seconds.

(vi) Washed with water, blotted and dried.

It was essential that the whole slide was flooded with each reagent in turn, and that the previous reagent was thoroughly removed at each step.

10. Gelatin media (Cowan, 1974).

Gelatin agar

Gelatin	..	4 g
Distilled water	..	50 ml
Nutrient agar	..	1000 ml

Gelatin was soaked in water and when thoroughly softened, the melted nutrient agar was added to it, mixed and sterilized at 115°C for 10 minutes and distributed into plates.

11. Indole test (Cruickshank et al., 1975).

(i) Medium

Peptone	..	20 g
Sodium chloride (Nacl)	..	5 g
Distilled water	..	1000 ml

Adjusted the pH to 7.4. Dispensed and sterilized by autoclaving at 121°C for 15 minutes.

(ii) Kovac's reagent

Isoamyl alcohol	..	150 ml
p-Dimethyl-aminobenzal-dehyde	..	10g
con. Hydrochloric acid	..	50 ml

Dissolved the aldehyde in the alcohol and slowly added the acid. Stored in the refrigerator. Shaken gently

12. KCN broth (Cowan, 1974)

Peptone	..	3 g
Nacl	..	5 g
Potassium phosphate (Monobasic) (KH_2PO_4)	..	0.225 g
Sodium phosphate (Dibasic) ($Na_2HPO_4.2H_2O$)	..	5.64 g
Distilled water	..	1000 ml

The solids were dissolved in water, filtered through a sintered glass funnel and distributed in 100 ml volumes, in screw capped containers. Sterilization was carried out at 45°C for 20 minutes.

For use, added 1.5 ml of a freshly prepared 0.5% Potassium cynide (KCN) solution in sterile water to 100 ml base. Mixed aseptically, distributed one ml amounts into sterile 5 ml screw-capped bottles.

13. Mac Conkey's agar (Cruickshank et al., 1975)

Peptone	..	20 g
Sodium taurocholate	..	5 g
Water	..	1000 ml
Agar	..	20 g
Neutral red solution 2% in 50% ethanol	..	3.5 ml
Lactose, 10% aqueous solution		100 ml

Dissolved the peptone and taurocholate in water by heating. Added the agar and dissolved it by autoclaving. Adjusted the pH to 7.5. Added lactose and neutral red, which was well shaken before use, and mixed. Heated in the autoclave with free steam (100°C) for one hour, then at 115°C for 15 minutes, poured into plates.

14. Methyl-Red test (Cruickshank et al., 1975)

(i) Medium (Glucose phosphate peptone water)

Peptone	.. 5 g
Dipotassium hygrogen phosphate (K_2HPO_4)	.. 5 g
Water	.. 1000 ml
Glucose, 10% solution (Sterilized separately)	.. 50 ml

Dissolved the peptone and phosphate, adjusted the pH to 7.6, filtered, dispensed in 5 ml amounts into the test tubes and sterilized at 121°C for 15 minutes. Added 0.25 ml glucose solution to each tube (final concentration 0.5%).

(ii) Methyl red indicator solution

Methyl red	.. 0.1 g
Ethanol	.. 300 ml
Distilled water	.. 200 ml

15. Nutrient broth (Cowan, 1974).

Beef extract	.. 10 g
Peptone	.. 10 g
Nacl	.. 5 g
Water	..1000 ml

Dissolved the ingredients by heating in the water. Adjusted to pH 8-8.4 with N-NaOH and boiled for 10 minutes. Filtered and adjusted to pH 7.2-7.4 and sterilized at 115°C for 20 minutes.

16. Nutrient agar (Cowan, 1974)

Nutrient agar was nutrient broth gelled by the addition of 2% agar.

17. Nitrate broth (Cowan, 1974)

Potassium nitrate (KNO_3)	.. 1 g
Nutrient broth	.. 1000 ml

KNO_3 was dissolved in nutrient broth, and distributed into tubes and sterilized at 115°C for 20 minutes

18. Selenite broth (Edwards and Ewing, 1972)

Tryptone	.. 5 g
Lactose	.. 4 g
Disodium phosphate	.. 10 g
Sodium acid selenite	.. 4 g
Distilled water	.. 1000 ml

(Final pH about 7)

Dispensed about 45 ml of the media into sterile tubes and sterilization of media was carried out by exposing the tubes to flowing steam for 30 minutes.

19. Staphylococcus medium 110 (Merchant and Packer, 1971)

Bacto yeast extract	.. 2.5 g
Bacto Tryptone	.. 10 g
Bacto gelatin	.. 30 g
Bacto lactose	.. 2 g
d-mannitol	.. 10 g
Sodium chloride (Nacl)	.. 75 g
Dipotassium phosphate (K_2HPO_4)	.. 5 g
Bacto agar	.. 15 g
Distilled water	..1000 ml
(Adjusted to pH 7.0)	

The ingredients were dissolved in boiling water and then sterilized in the autoclave for 15 minutes at 15 lbs pressure. Hot medium was poured into petri-dishes agitating the flask containing the medium.

20. Tetrathionate broth (Cruickshank et al. 1975)

(i) Complete media preparation

Nutrient broth, pH 7.4	..	90 ml
Calcium carbonate ($CaCO_3$)	..	5 g
Brilliant green, 1 in 1000 aqueous solution	..	1 ml
Thiosulphate solution	..	10 ml
Iodine solution	..	2 ml
Ox bile solution	..	5 ml

Added $CaCO_3$ to the broth and sterilized it by autoclaving at 121°C for 20 minutes. When cooled added the other solutions and distributed into flasks. Heated once in the steamer at 100°C for 10 minutes.

(ii) Thiosulphate solution

Sodium thiosulphate ($Na_2S_2O_3.5H_2O$)	50 g
Sterile water	100 ml

Mixed the salt and water with sterile precautions and steamed at 100°C for 30 minutes.

(iii) Iodine solution

Potassium iodine (K.I.)	..	25 g
Iodine (I)	..	20 g
Sterile water	..	100 ml

With sterile precautions dissolved the potassium iodide in water and added the iodine.

(iv) Ox bile solution

Desicated ox bile	0.5 g
Water	5 ml

Dissolved with sterile precautions

21. Triple sugar iron agar (Cowan, 1974)

Beef extract	3 g
Yeast extract	3 g
Peptone	20 g
Glucose	1 g
Lactose	10 g
Sucrose	10 g
Ferrous sulphate ($FeSO_4.7H_2O$)	0.20 g
Sodium Chloride (Nacl)	5 g
Sodium thiosulphate ($Na_2S_2O_3.5H_2O$)	0.3 g
Agar	20 g
Distilled water	1000 ml
Phenol red, 0.2% solution	12 ml

Water with the solids was heated to dissolve them in it, and the Phenol red solution was added, mixed and poured into test tube. Sterilized at 115°C for 20 minutes and cooled to form slopes with deep butts.

22. Urea medium (Cowan, 1974)

Peptone	..	1 g
Sodium chloride (Nacl)	..	5 g
Secondary Potassium phosphate (KH_2PO_4)	..	2 g
Agar	..	20 g
Distilled water	..	1000 ml

Dissolved the ingredients by heating, adjusted to pH 6.8, filtered and sterilized at 115°C for 20 minutes.

Added

Glucose	..	1 g
Phenol red, 0.2% solution		6 ml

to the molten base, steamed for one hour and cooled to 50-55°C

Added

Urea 20% aq. solution sterilized by filteration 100 ml aseptically to the base. Distributed aseptically into sterile containers and allowed to cool as slopes.

23. Voges - Proskauer test (Cruickshank et al. 1975)

i) Medium - Glucose phosphate peptone water

The composition and preparation of the media was similar to that of methyl red test.

ii) O'Meara reagent (Cruickshank et al. 1975)

Potassium hydroxide	..	40 g
Creatine	..	0.3 g
Distilled water	..	100 ml

24. Zone size interpretive chart (Anderson, 1973)

Antibiotic or chemotherapeutic agent	Disc potency	Inhibition zone diam to nearest mm		
		Resistant	Intermediate	Susceptible
Ampicillin	10 µg	20 or less	21-28	29 or more
Chloromycetin	30 µg	12 or less	13-17	18 or more
Erythromycin	15 µg	13 or less	14-17	18 or more
Kanamycin	30 µg	13 or less	14-17	18 or more
Penicillin G	10 unit	20 or less	21-28	29 or more
Streptomycin	10 µg	11 or less	12-14	15 or more
Terramycin	30 µg	14 or less	15-18	19 or more

A PRELIMINARY STUDY OF BACTERIOLOGICAL QUALITY OF MARKET BEEF OF TRICHUR WITH SPECIAL REFERENCE TO **SALMONELLA AND STAPHYLOCOCCI**

BY

E. NANU

ABSTRACT OF A THESIS

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ABSTRACT

With a view to investigate the bacteriological quality of market beef in and around Trichur Town in respect of bacteria of public health importance, with special reference to Staphylococci and Salmonellae, the present study was undertaken. Seventy samples of market beef collected from meat stalls in and around Trichur town were processed for isolation of Salmonellae and Staphylococci following standard procedures. Sixty two isolates of Staphylococci, of which 49 coagulase positive suggestive of S.aureus were obtained. All Staphylococci were resistant to Penicillin and Ampicillin. A high rate of resistance was observed against Erythromycin, Streptomycin, Kanamycin, Terramycin and Chloromycetin. Coagulase positive characters and antibiotic resistance denote its importance in public health.

Only one isolate was identified as Salmonella satisfying the characters of the genus. It showed varying degree of resistance to different antibiotics.

Importance of hygienic practices, in slaughter houses, meat stalls, transport and storage of meat is stressed.