

Bacteriological Analysis of Different Foods to Determine the Fitness for Human Consumption

Pages with reference to book, From 79 To 84

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Abstract

A total of 230 food samples were collected over a period of 18 months from different shops, vendors and canteens of Rawalpindi/Islamabad region. The sample included cooked, uncooked, frozen and non frozen (pre-packed) food. One hundred and five samples were found contaminated with different bacterial strains, which were grown at two different temperatures i.e. 35-37°C and at room temperature. The pathogenic microorganisms isolated were identified as Coliforms group, 79 (75.23%), *Escherichia coli*, 49 (46.66%), *Staphylococcus aureus*, 11(10.47%), *Clostridium perfringens*, 5 (4.85%) and *Salmonella* spp, 1 (0.9%). Other pathogens namely, *Shigella*, *Vibrio cholerae* and *Vibrio parahaemolyticus* were not isolated (JPMA 35: 79, 1985).

Introduction

It is an established fact that once food is subjected to the attack of microorganisms the microbial population increases within a few hours time at normal temperature, because food is an ideal culture medium for multiplication of a variety of microorganisms. The types and number of bacteria which cause spoilage, indicates the quality of food. Therefore, it is necessary to employ routine bacteriological testing for recommending effective sanitary control of such food. It is essential for every catering service to carry out the bacteriological quality control of its food routinely in order to protect health of the consumer and to assess its nutritive qualities.¹

Raw foods like sea food are the vehicles of food borne diseases,² since contaminating pathogens in it are virulent and able to survive the competition of other microbes and multiply during conditions of storage of food. The largest proportion of food-borne diseases in our country is probably caused not by commercially processed food like, jams, jellies and beverages, but by food prepared in institutions, markets and hotels. Processing of food by smoking at such places is an ineffective method to suppress the growth of pathogenic microorganisms. Spore bearing microorganisms in processed foods may survive even

cooking for shorter period or may even multiply if proper hygienic precautions are not taken.

Clostridium spp. produced exo-toxins in canned or vacuum-packed foods which are not processed properly by heating and hermetically sealing. In most instances *staphylococcus aureus* and *Salmonella* Spp. are introduced by insects, skin, soil, and human carriers who are involved in processing the food. Any instance of mishandling of food under unhygienic conditions can cause risk of health to hundred of persons.

Pakistan being a developing country, its food industries are few and do not meet the international standards. Processed foods like infants formula and dry milk are imported from different countries. If proper care during handling and processing is not exercised then presence of different types of bacterial contaminants, could be of high degree in such foods. To assess the keeping quality of food and its fitness for human consumption, it becomes essential to evaluate the total bacterial counts present in cooked, uncooked, frozen and non frozen (pre-packed) foods. There are Standard Bacteriological methods for food testing.^{3,4}

The Food and Agricultural Organisation (FAO) had drawn the attention of the Government of Pakistan

in 1979 to develop a project on "Food contamination study and control in Asia".

It is hoped that such a study would help to initiate the formulation of food standards in Pakistan to ensure both safety and quality of food and food stuffs as per our national requirement.

Material and Methods

1. Material

a) Food Samples

Two hundred and thirty food samples, cooked, uncooked, frozen, non frozen, (prepacked) were purchased from 50 food shops, vendors and canteens of Rawalpindi/Islamabad region. Fifty grams of each food sample was aseptically transferred to sterilized wide mouth glass stoppered bottles which were immediately transported to the Laboratory for quantitative and qualitative bacteriological analysis.

b) Biological reagents, Cultural media.

Physiological saline (0.85% NaCl solution)

Alkaline Peptone water (Difco)

Nutrient broth (“)

Selenite-F-enrichment medium (“)

Carbohydrate fermentation media (“)

Nutrient agar (“)

MacConkey agar (Oxoid)

Brilliant green agar (“)

Desoxycholate citrate agar (“)

Thiosulphate citrate bile (“)

lalts agar

Triple sugar Iron agar (“)

Urea agar (“)

Cooked meat medium (“)

Blood agar 10% defibrinated sheep

blood plus nutrient agar

c) Diagnostic sera (Difco):-

For identification of different species of organisms.

d) Rabbit Serum: -

Normal rabbits Serum for coagulase test.

Methods

A serial dilution of homogenized samples were prepared from 1:10 to 1:10,000 dilution. In the case of butter sample a portion of sample was put in a sterile beaker and kept in a water bath at 45 °C until butter became fluid enough for pipetting.⁵

Total viable bacterial counts were determined from nutrient agar plates of the serial dilutions, using a modification of the surface drop technique.⁶ Separate drops of each dilution with a calibrated pipette which deliver 40 drops/ ml were placed upon the agar surface of the relevant plate which were marked on the bottom into four equal parts. After the drying of drops the plates were incubated at 35-37°C, and also at room temperature (20-25°C) for 24 hours. Dilutions showing sufficient numbers of colonies were counted using a colony counter.

The colony count per ml was made by multiplying average number of colonies per dilution and also by multiplying with 40 (the No. of drops). Total number of organisms per ml = Average No. of colonies X Dilution X 40.

The colonies from the countable plates of each sample were picked up and examined microscopically

by gram-staining. Typical Colonies from the plates were inoculated into triple sugar Iron agar (TSI) tubes and incubated at 37°C for 24-48 hours. After incubation, tubes showing specific reactions were noted and identification of different bacterial species were confirmed by performing serological and biochemical tests.

Selenite-F-enrichment broth was used for the isolation and identification of Salmonella and Shigella spp.⁷ Attempts to isolate Clostridium species (strictly anaerobes) were carried out.⁸ Coagulase positive Staphylococcus aureus were isolated on blood agar and identified by coagulase test.⁹ Alkaline peptone water and thiosulphate citrate bile salts agar (TCBS) were used for the primary isolation of Vibrio cholerae and Vibrio parahaemolyticus and identified.¹⁰

Results

One hundred and five of 230 food samples collected were found contaminated with different bacterial strains, whereas one hundred and twenty five (125) food samples did not yield any bacterial growth. The ability of these bacteria to grow at different temperatures is of interest. Mean viable bacterial count/gm of food samples was higher at room temperature as compared to 35-37°C. The total bacterial count ranged from 15.726X10² to 49.407X 10⁴ per gm of food when incubated at room temperature and from 10.220X10² to 42.487X10⁴ when incubated at 35-37°C (Table-I).

Table – I

Mean Viable Bacterial Counts/gm of Food Samples.

Types of food	Total No. of samples analysed	No. of samples showing no growth	No. of samples showing growth	Mean viable bacterial counts/gm of food incubated at:	
				35-37°C 24 hours	Room temperature 24 hours.(20-25°C)
Cooked meal (Rice, vegetables, Eggs, Kabab, Cutlets, Chips, Mutton, Fish, Beef, Chicken)	40	16	24	30.233X10 ³	35.570X10 ³
Cooked meat (Chicken, Mutton, Fish, Beef, Roasted, Curry)	30	13	17	20.070X10 ²	25.092X10 ²
Raw meat (Chicken, Mutton, Fish, Beef)	20	–	20	42.487X10 ⁴	49.407X10 ⁴
Raw Vegetables, Fruits (Salads, Chatt)	10	–	10	44.810X10 ⁴	47.460X10 ⁴
Squashes, Juices, Jams, Pickles (Lemon, Mango, Orange)	10	7	3	15.233X10 ²	20.050X10 ²
Snacks (Sandwiches, Bread Cakes)	13	7	6	10.220X10 ²	15.870X10 ²
Ice-Cream, Butter, Creams (different varieties)	40	26	14	15.642X10 ³	17.145X10 ³
Milk samples (Fresh & Dry)	20	16	4	12.882X10 ²	15.726X10 ²
Water samples (Tap & Well)	20	13	7	22.532X10 ⁴	25.092X10 ⁴
Food products (Farex, Farlac, Complian)	, 9	9	–	–	–
Soft drinks (Cock, 7up, malt extract)	18	18	–	–	–

As shown in table-II,

Table II

The Incidence of Pathogenic Organisms in Food Samples.

Types of food	Total No of samples analysed	No. of samples found contaminated	No. of isolates from contaminated food samples with percentage.							
			Coliforms gp.	Escherichia coli	Coagulase positive staphylococcus aureus	Clostridium perfringer	Salmonella spp.	Shigella spp.	Vibrio Cholerae	Vibrio Parahaemolyticus
Raw meat (Chicken, Mutton, Fish, Beef)	20	20	20(100.00)	15(75.00)	5(25.00)	3(15.00)	-	-	-	-
Cooked meat (Rice, Vegetables, Eggs, Kabab, Cutlets, Chips, Mutton, Fish, Beef, Chicken)	40	24	13(54.17)	7(29.17)	3(12.50)	1(4.16)	-	-	-	-
Raw Vegetables, Fruits (Salads, Chutney)	10	10	10(100.00)	8(80.00)	1(10.00)	-	-	-	-	-
Ice-cream, Butter, Creams (Different varieties)	40	14	10(71.43)	4(28.57)	-	-	-	-	-	-
Cooked meat (Roasted Curry, Chicken, Mutton, Fish, Beef)	30	17	9(52.94)	6(35.29)	1(5.9)	1(5.9)	-	-	-	-
Water samples (Tap & Well)	20	7	6(85.71)	1(14.29)	-	-	-	-	-	-
Snacks (Sandwiches, bread, Cakes etc.)	13	6	5(83.33)	4(66.66)	-	-	-	-	-	-
Squashes, Juices, Jams, pickles (Lemon, Mango, Orange)	10	3	1(33.33)	2(66.66)	-	-	-	-	-	-
Milk samples (Fresh & Dry)	20	4	-	2(50.00)	1(25.00)	-	1(25.00)	-	-	-
Food products (Fares, Farlac, Complan)	9	-	-	-	-	-	-	-	-	-
Soft drinks (Coke, Tea, Malt extract)	18	0	-	-	-	-	-	-	-	-

one hundred and forty five bacterial strains were isolated from contaminated food samples. On the basis of morphological, biochemical and serological characteristics the organisms identified were, Coliform group, 79 (45.23%), Escherichia coli, 49 (46.66%), Staphylococcus aureus, 4(10.47%), Clostridium perfringens, 5 (4.85%) and Salmonella spp. 1 (0.90%) Other food poisoning bacteria namely, Shigella spp., Vibrio cholerae, Vibrio parahaemolyticus were not isolated from any of food samples tested in this study.

Discussion

Safe food is considered to be free from pathogenic microorganisms and after ingestion should not cause any illness. Safe foods may have low bacterial counts which should not hinder shelf life. Foods suspected of causing food poisoning give higher counts ranging from one million to 10 million per gm of food.¹¹

Out of 230 food samples examined in this study only 105 (42%) samples were found contaminated with different bacterial strains. The ability of these bacteria to grow at different temperatures is of interest. Mean viable bacterial count/gm of food was higher when incubated at room temperature as compared to 35-37°C for 24 hours. These findings are consistent with other studies.¹²⁻¹³

High bacterial counts are often due to mishandling, distribution and improper storage. In a study on frozen and non frozen meat and gravy, it was observed that the bacterial counts were increased significantly in cooked meat while being sliced handled under unhygienic conditions.^{1,14,15}

In this study, it was found that the highest incidence of Coliforms gp. (100%) in raw foods like, raw meats, raw vegetables, fruits salad and chutney as well. The highest recovery of *Escherichia coli* was found in raw meats (75%) and raw vegetables and fruits salad (80%). These findings, together with those obtained on Coliforms gp. suggest a possible faecal contamination and insanitary conditions under which these raw foods were being handled. All strains of *Escherichia coli* in food do not cause symptoms^{2,16,17} only certain strains are pathogenic and may cause enteritis and urinary tract infections in man and animals.

The occurrence of outbreaks of enteritis due to coagulase positive *Staphylococcus aureus* has been widely recognised.¹⁸ In this study only 11 food samples (10.47%) were found to harbour this strain which is apparently a low incidence. This finding is in agreement with another study¹⁹ where the staphylococci counts for the market cheese were not particularly high, but the presence of coagulase positive *Staphylococci* in a food supply is undesirable, because its rapid multiplication in food becomes uncontrollable if proper handling and storage is not observed.

Clostridium perfringens as contaminant in raw foods is now considered to be one of the most important anaerobes which causes food poisoning. Spores of these organisms survive cooking and are heat activated to germinate when a suitable temperature is available to foods. Isolation of high percentage of *Clostridium perfringens* from foods by various workers in different parts of the world indicates ubiquitous prevalence of these organisms as food contaminants.⁹

The pathogenicity of *Salmonella*, *Shigella* spp., *V. Cholerae*, and *V. parahaemolyticus* is beyond any doubt. In these infections, the infective doses are insignificant and thus their isolation pose a great public health problem. It is difficult to isolate these organisms because they are present in small numbers. It is believed that a minimum of 10^4 organisms of *Salmonella* spp., 10^5 organisms of *Shigella* spp. and *V. Cholerae* and 10^7 organisms of *V. parahaemolyticus* is required to cause symptoms of food-borne diseases.²⁰ In this study, *Salmonella* spp. were isolated from only one sample (25.0%) of fresh milk. *Shigella* spp., *V. Cholerae*, and *V. parahaemolyticus* were not found from any of the food samples analysed (Table - H).

Bacterial flora identified in the present study is quite high and it was observed from the results that conditions prevailing in the country are concerned, we have acute problems and deficiencies in sanitation, processing and handling of foods. The only point lies in the fact that in all cases the foods are to be cooked to a point where most of the vegetative cells die and consumption of food could be made safe if not stored for a longer time at ambient temperature. However persons handling the food in raw state are most likely to contact the infection.

In order to establish bacteriological standards of food, it becomes necessary to conduct a random survey of food samples obtained directly from all types of food and food products. However, safety of food should be that it is palatable, safe for health and good in appearance, taste and flavour as well as to meet the bacteriological standards.

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