

FOOD HYGIENE WITH REFERENCE TO PUBLIC HEALTH MICROBIOLOGICAL CONTAMINANTS OF DIFFERENT FOODS IN RAWALPINDI AND ISLAMABAD

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Abstract

A total of 282 food samples of different varieties were analysed for microbiological contamination. High microbial load and presence of pathogenic organisms renders the poor hygienic standard of the foods examined. Mean viable bacterial count was found to be 1.2×10^8 /gm food and 49% of the samples were declared unfit for human consumption.

Pathogenic bacteria recovered included *E. coli* (14.9%), *Staphylococcus aureus* (1.8%), *Clostridium perfringens* (1.1%) and *Salmonella* (0.7%). *Bacillus cereus* (0.3%) was also incriminated.

Foods from street side shops and vendors were heavily contaminated (70%). Middle and upper class restaurants also indicated a high rate (40%) of microbial contamination (JPMA 36:141 1986).

INTRODUCTION

Food can be an important vehicle for transmission of a broad spectrum of diseases. The transmission of human diseases through food, water and waste water is a global problem, particularly of developing countries. Awareness of microbiological health hazards arising from the consumption of contaminated food has grown in recent years and has resulted in national and international intensification of food hygiene programmes.¹

The interaction between nutrition and infection is of fundamental importance to public health workers, especially in developing countries like Pakistan, because infections take a heavy toll 'of human life. Epidemiological studies indicate that most infections make the nutritional status worse if the individual is already consuming a deficient diet.²

Malnutrition in combination with endemic diarrheal disease is one of the most significant health problems among children in many developing countries¹. A correlation between contaminated food, water and diarrhea has also been proved.³

One thousand seven hundred and three outbreaks of foodborne origin (97,590 cases) were reported by state Health Department and other agencies during 1967 through 1971.⁴ During this period, staphylococcal intoxication, salmonellosis and *Clostridium perfringens* gastroenteritis were the diseases most frequently reported.⁵

Microorganisms contaminating food may be non pathogenic and yet may cause chemical changes that render food unfit for human consumption or may be pathogenic and cause infections or food poisoning when consumed.⁶

Keeping in view the importance of the subject, and to know the microbial status of foods in this region this study on microbiological contamination of different foods was undertaken, at National Institute of Health, Islamabad.

MATERIALS AND METHODS

1. Collection of food specimens:

Foods of public health importance were mainly analysed for microbiological contaminants. A total of two hundred and eighty two (282) samples investigated in the study were collected from different hotels, restaurants, institutional canteens, railway station and small food industries in Rawalpindi and Islamabad area.

Fifty grams of each food sample was aseptically placed in wide mouth glass stoppered flasks and immediately transported to the laboratory for microbiological examination.

2. Primary isolation media:

a. Plating media:

Viable bacterial count was performed on plate count agar. SSA (Difco) and Mac Conkey agar (Difco) were used for the isolation of members of the enterobacteriaceae and other gram negative organisms. Thiosulphate Citrate Bile Salts sucrose agar TCBS (Difco) was used for recovery of *V. cholerae* and *V. parahaemolyticus*. However, isolation of gram positive aerobic organisms was carried out on Baird parker medium (Oxoid) and anaerobic organisms on Blood agar i.e 10% defibrinated sheep blood' in nutrient agar (Difco). Egg yolk agar (Oxoid) was medium of choice for *Bacillus cereus*. Fungal species were recovered on Saborauds dextrose agar (Difco).

b. Enrichment medium:

Different enrichment media were used according to the organisms recovered. Tetrathionate broth (Oxoid) was used for *Salmonella* and *Shigella* organisms while alkaline peptone water pH 8.6 (Difco) for *Vibrio cholera*. Phosphate buffer saline pH 7.2 (Difco) was used for the enrichment of *Yersinia enterocolitica*.

For anaerobic organisms fluid thioglycollate medium and cooked meat medium were employed. Glucose broth and MacConkey's broth were also used for gram positive and gram negative organisms respectively.

PROCEDURE

Twenty five gram of food sample weighed aseptically was blended with 225 ml of PBS pH 7.2. From this homogenate serial ten fold dilutions were made from 1×10^1 to 1×10^6 .

Methodology used for isolation and identification of organisms and viable plate counts are given in flow diagrams I, II & III.⁷ Viable aerobic counts were determined by the pour plate method.⁷

Coagulase positive staphylococcus were identified by using standard methods⁸. Members of enterobacteriaceae were identified by using system No. 1 by Eiken chemical, Japan and scheme proposed by Edward and Ewing.⁹ Other organisms were isolated and identified using methods given by Topley and Wilson¹⁰

RESULTS

Two hundred and eighty two samples of different varieties of food including raw, cooked and processed foods were screened for the microbiological contamination. The detail description of foods examined and microbiological contamination from different types of food is given in table I.

TABLE-I – Incidence of Microbial Contamination in Different foods

Type of Food	No. of Samples Examined	Contaminated Food Samples No. (%)
Cooked foods (rice, veg., eggs etc)	26	10 (38.5%)
Cooked Meat. (Chicken, beef, mutton, fish)	51	25 (49%)
Raw Foods (Veg. & Fruits)	12	12 (100%)
Raw Meats (Chicken, beef, mutton, fish)	14	14 (100%)
Processed Foods. (Squashes, Veg., Fruits, Meat, Pickles etc.)	45	12 (26.7%)
Dried Foods (Spices, spaghatties, Yeast etc.)	17	17 (100%)
Snacks (Cakes, Pastries, Patties, biscuits, sandwiches, chatts etc.)	22	9 (41%)
Dessert (Cooked sweet dishes sweet meats etc.)	20	9 (45%)
Milk (Dry and Fresh)	24	10 (41.7%)
Milk Products. (Cheese, butter, cream, Yoghurt etc.)	30	19(63%)
Soft Drinks.	21	1 (5%)
TOTAL	282	138 (49%)

Each food sample yielded 0-3 microbial agents. Out of 282 foods examined, 138 (49%) were found to be contaminated with different bacterial agents.

TABLE - II Viable Aerobic counts of different kind of Foods.

	Cooked food	Cooked meat	Raw meat	Raw food	Milk	Milk products	Desserts	Snacks	Processed foods	Dried foods
Mean viable count	2.3×10^6	1.1×10^8	2.3×10^6	1.3×10^6	2.6×10^8	1.2×10^{10}	5.1×10^6	1.9×10^7	9.3×10^5	1.6×10^8
Lowest viable count	1×10^2	1×10^2	3×10^4	1×10^1	1×10^1	1×10^2	1×10^1	1×10^2	2×10^2	1.5×10^3
Highest viable count	1.2×10^7	8×10^8	2×10^7	1.4×10^7	2.8×10^9	1×10^{11}	2.5×10^7	1.2×10^8	8×10^6	8×10^8
*** (m)*	5×10^4 /gm	10^3 /gm	10^6 /gm	10/gm	5×10^4 /gm	2.5×10^4 /gm	10^4 /gm	10^4 /gm	10^3 /gm	10^4 /gm
Permissible range	(M)** 10^6 /gm	10^4 /gm	10^7 /gm	10^3 /gm	5×10^5 /gm	2.5×10^5 /gm	10^6 /gm	10^6 /gm	10^4 /gm	10^6 /gm

*(m) = Level of test organism acceptable and attainable in food (ICMSF, 1978).

** (M) = Hazardous level of contamination caused by bad sanitary practice, including improper storage (ICMSF, 1978).

*** = The International Commission of Microbiological specifications of foods (ICMSF, 1978).

Total mean viable count = 1.2×10^8 /gm of food.

Table II also indicates the percentage of microbial contaminants found in different varieties of food. The highest percentage of contamination was found in raw foods, raw meats and dried.

The total viable count in aerobic plates ranged upto 1×10^8 /gm of the food when incubated at 37°C , whereas the mean viable plate count of the examined foods was 1.2×10^8 /gm (Table II). Of the examined food samples 51% were found fit for human consumption having counts within the permissible range or were completely sterile showing no microbial growth.

This table also shows the permissible range, mean viable aerobic plate counts, the highest and the lowest viable aerobic counts per gram of different types of food examined.

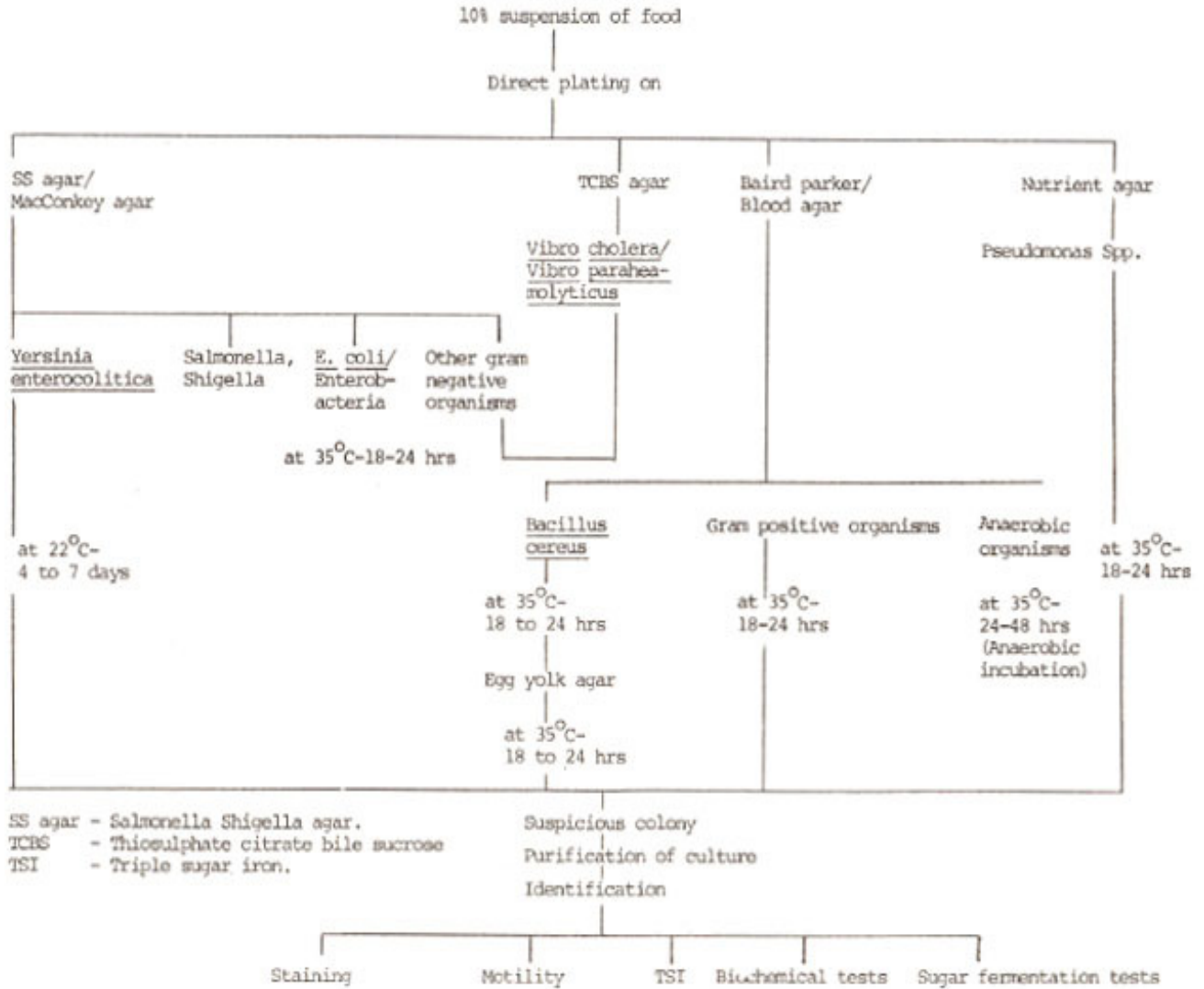
Microbial contamination with reference to the site of collection was also determined (Table III).

TABLE - III Microbial contaminants of Food related to Site of Collection.

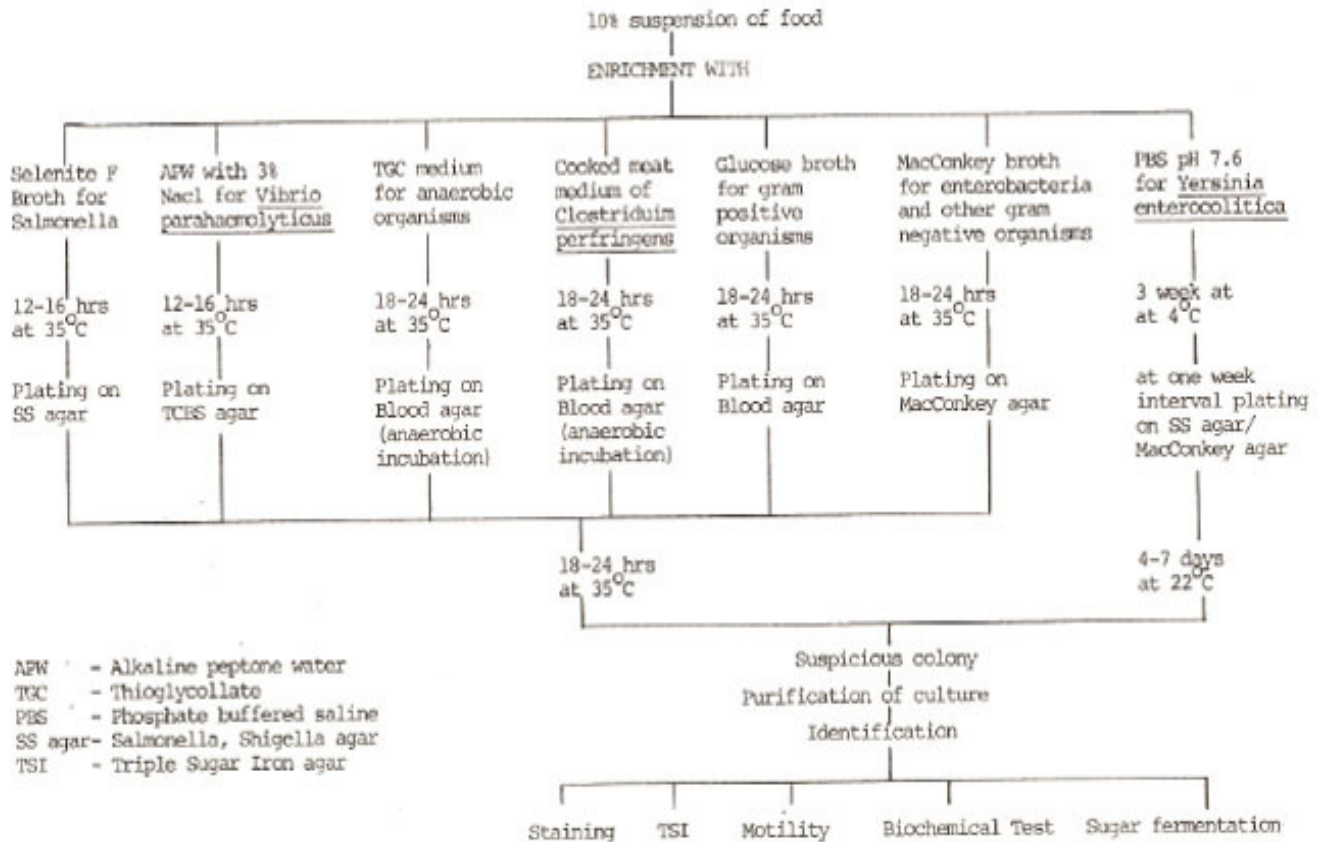
Site of Collection	Total No. of Food Samples	Contaminated Food Samples No. (%)
Upper class restaurants and institutions.	48	19 (39.6%)
Middle class hotels and institutions.	30	12 (40.0%)
Street side shops and vendors.	20	14 (70.0%)
Other sources	88	43 (49.0%)
TOTAL	186	88 (47.3%)

The highest rate of contamination in different type of foods was found in street side shops and vendors i.e. 70%, whereas in the middle and high class hotels and restaurants the rate of contamination was approximately 40%, which is again a high figure.

I FLOW DIAGRAM FOR ISOLATION OF ORGANISMS FROM FOOD

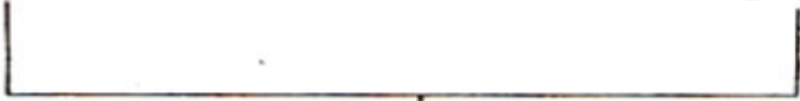


II FLOW DIAGRAM FOR ISOLATION OF ORGANISMS FROM FOOD



III FLOW DIAGRAM FOR PLATE COUNT OF AEROBIC BACTERIA

Weigh 25 gm of food sample to be tested 225 ml of phosphate buffered saline (PBS) pH 7.2



Suspension by

blending for 1 min

Serial 10-fold dilutions

with PBS, pH 7.2

Dispense one ml aliquots into duplicate petri-dishes

(aseptically)

Mix well with 15-20 ml of melted nutrient agar being kept at 50°C

Incubate at 35°C for 48 hrs in the state of upside down

Enumerate the numbers of colonies appeared, with the colony counter.

(Select a dilution which yields 30-300 colonies per plate)

The microbial concentration in the original sample is estimated from the arithmetic mean of the counts.

The microorganisms isolated from different type of foods are given in Table IV.

TABLE - IV Distribution of Microorganisms isolated from Different Foods.

Type of Food	E. Coli	Staph Aureus	Cl. Perfringens	Streptococcus Faecalis	Bacillus Cereus	Salmonella Sp	Other* Organisms	Fungus
Cooked food (n = 26)	3(11.5%)	1 (3.8%)	-	-	-	-	10 (38.5%)	-
Cooked meat (n = 51)	10(19.6%)	1 (2 %)	2 (4 %)	-	-	-	23(45 %)	-
Raw food (n = 12)	5(41.6%)	1(8.3%)	-	-	-	-	12(100%)	-
Raw meat (n = 14)	7(50%)	-	1(7.1%)	-	-	1(7.1%)	11(78.6%)	-
Processed food (n = 45)	-	-	-	-	-	-	11(24.4%)	9(20%)
Dried foods (n = 17)	-	1(5.9%)	-	-	-	-	13(76.6%)	5(29.4%)
Snacks (n = 22)	2(9.1%)	-	-	-	-	-	8(36.4%)	3(13.6%)
Dessert (n = 20)	4(20%)	1(5%)	-	-	-	-	8(40%)	-
Milk (n = 24)	-	-	-	-	1(4.2%)	1(4.2%)	8(33.3%)	-
Milk products (n = 30)	11(36.6%)	-	-	1(3.3%)	-	-	10(33.3%)	-
Soft drinks (n = 21)	-	-	-	1(4.8%)	-	-	-	-
TOTAL (282)	42(14.9%)	5(1.8%)	3(1.1%)	2(0.7%)	1(0.3%)	2(0.7%)	120(42.5%)	17(6%)

The highest percentage after non pathogenic strains was of E. coli (14.9%). The coliform group and other microorganisms were 42.5% in the examined foods, Clostridium perfringens was present to the extent of 1.1% in the raw and cooked meat products only.

Streptococcus faecalis was found in 0.7% and Staphylococcus aureus was recovered from 1.8% of the food samples.

Bacillus cereus was incriminated in 0.3% of the examined foods. Salmonella spp. was isolated only from 2 samples (0.7%) one from milk and the other from raw meat. Fungus was mostly isolated from processed or dried foods and incidence was 12.3% of the examined foods.

DISCUSSION

Presence of microorganisms like Escherichia coli, faecal coliforms, Streptococcus faecalis and Staphylococcus aureus (relatively recently accepted as an indicator organism) in food and water provides a useful indication that faecal contamination has occurred in the food¹.

Foods that are eaten raw present a health hazard if they are contaminated with pathogenic microorganisms.¹¹ Our results indicate that the highest percentage of contamination was mainly found in raw foods, raw meats and dry foods. It is an established fact that once food is subjected to the attack of microorganisms the microbial population increases within a few hours at normal temperature,

because food is an ideal culture medium for multiplication of a variety of microorganisms.¹² The international Commission on Microbiological Specifications for Foods (ICMSF) has recommended criteria for different foods (Table II). When these criteria were applied to the data of present study the bacterial numbers were very high. Viable bacterial counts suggest practice of inadequate hygienic measures, malhandling and unhygienic conditions of the retail shops. This is in agreement with another study.¹³

Comparing the foods according to the site of collection it has been found that the highest contamination was recovered from the foods of street side shops and vendored foods, indicating more unhygienic conditions. But, the rate of contamination in the middle and high class hotels was also quite alarming (Table III).

It is stated that routine microbiological testing of foods is necessary for recommending effective sanitary control of hotels, markets and institutional canteens.⁵ It was also recommended that every catering service should carry out the microbiological quality control of its food routinely, in order to protect health of the consumer.¹⁴

The percentage of *Escherichia coli* was highest among pathogenic strains (Table IV). Although the presence of *E. coli* does not necessarily mean a pathogenic strain, but implies a certain risk as some serotypes are enteropathogenic and cause diarrhoea in infants and its presence in large numbers may cause diarrhea in adults.^{3,15}

Great majority of outbreaks of food poisoning in every country throughout the world were caused by enterotoxin of *Staphylococcus aureus*.¹⁶ In this study only 1.8% foods harboured this strain which is apparently a low incidence. This finding is in agreement with other studies.^{12,17}

Clostridium perfringens was present to the extent of 1.1% in meat and meat products. This is considered to be one of the most important causes of food poisoning. Spores of typical food poisoning strains and certain type c strains are heat resistant.¹⁸

Streptococcus faecalis was found in 0.7% food samples. These are preferred as indicators of faecal pollution, since they survive longer in water than coliform bacteria.¹

Bacillus cereus was the third most common cause of bacterial food poisoning between 1960 and 1968 in Hungary.¹⁹ However in our study only 0.3% of the foods were incriminated with *Bacillus cereus*. Salmonellosis is usually food/waterborne infection and the number of organisms required to produce clinical infection depends on many factors. An extensive outbreak of *Salmonella eastbourne* food poisoning on North American Continent in 1974 involved chocolate candy containing well under one salmonella bacterium per gram of food.²⁰

Fungus was mostly isolated from processed or dried foods. The correlation between fungi and mycotoxins is, however, very poor. In many cases large number of mouldy foods have been examined for mycotoxins with a few or no positive results.¹

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