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Isolation of food poisoning bacteria from contaminated foods

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Abstract
Food borne pathogens are perceived as a major health problem in all over world. Contamination arises from unclean raw food materials, use of polluted water, unhygienic preparations process & use of contaminated utensils. Here in, prevalence of food borne pathogens in raw, processes food & ready to eat from five different locations were studied. Microbiological analysis as per I.P. (Indian Pharmacopeia).of 5 specimen samples were done which included dairy products, leafy vegetable salad, poultry products & meat products. The isolates were enriched, isolated & identified using suitable broth, selective agar medium & biochemical tests. Bacterial growth was found in all food samples. Microorganisms isolated includes *Staphylococcus aureus, E. coli*, *Salmonella spp* & *Clostridium* spp. This result indicated that most of the raw food, processes food & ready to eat food samples examined in the study did not meet any bacteriological quality standard as recommended by Food Authority, it poses potential risks to consumers. Raw food, processes food Ready- to- eat fast foods must be cooked and served to the consumers with all hygienic measures.

Keywords: food pathogens**,** *staphylococcus aureus, e. coli*, *salmonella spp* & *clostridium* spp.

Introduction

Food poisoning is a term used to express any type of disease or illness after consuming food. Food poisoning, food intoxication are a disease resulting from ingestion of food containing preformed microbial toxin. The microorganisms that produce these toxins are not grown in host cell and are often not alive at the time of preformed bioactive toxin. Foodborne illness, more commonly referred to as food poisoning, is the result of eating contaminated, spoiled, or toxic food. The most common symptoms of food poisoning includenausea, vomiting, and diarrhea.

Common cases of food poisoning will typically include at least three of the following symptoms: abdominal cramps, diarrhea, vomiting, loss of appetite, mild fever, weakness, nausea, headaches. Symptoms of potentially life-threatening food poisoning include: diarrhea persisting for more than three days, a fever higher than 101.5°F, difficulty seeing or speaking, symptoms of severe dehydration, which may include dry mouth, passing little to no urine, and difficulty keeping fluids down, bloody urine.

Bacteria causing food poisoning

Bacteria are by far the most prevalent cause of food poisoning. When thinking of dangerous bacteria, names like *E. coli, Staphylococcus, and Salmonella* come to mind for good reason. Salmonella is by far the biggest culprit of serious food poisoning cases in the United States. According to the CDC Trusted Source, an estimated 1,000,000 cases of food poisoning, including nearly 20,000 hospitalizations, can be traced to salmonella infection annually. *Campylobacter* and *C. botulinum (*botulism) are two lesser-known and potentially lethal bacteria that can lurk in our food.

Escherichia coli: *Escherichia coli* (*E. coli*) is a bacterium that is commonly found in the gut of humans and warm-blooded animals. Most strains of *E. coli* are harmless. Some strains however, such as Shiga toxin-producing *E. coli* (STEC), can cause severe foodborne disease. It is transmitted to humans primarily through consumption of contaminated foods, such as raw or undercooked ground meat products, raw milk, and contaminated raw vegetables and sprouts.

Staphylococcus aureus: Staph food poisoning is a gastrointestinal illness caused by eating foods contaminated with toxins produced by the bacterium *Staphylococcus aureus* (Staph) bacteria. Staphylococcal food poisoning is characterized by a sudden start of nausea, vomiting, and stomach cramps. Most people also have diarrhea. Symptoms usually develop within 30 minutes to 8 hours after eating or drinking an item containing Staph toxin, and last no longer than 1 day. Severe illness is rare. The illness cannot be passed from one person to another.

Salmonella species: Salmonella Food Poisoning is a pretty disabling and annoying pathological condition that normally occurs due to eating contaminated food. The food that the affected individual eats is usually contaminated by animal or human feces which tend to carry the bacteria called as Salmonella. Salmonella Food Poisoning are normally found when people eat foods made of poultry items like eggs or meat, although this bacteria may also contaminate the normal fruits and vegetables that we take on a daily basis. Some of the common food items that can be infected by the bacteria Salmonella and cause Salmonella Food Poisoning are undercooked eggs, raw milk, contaminated water, and raw meat. The bacteria is divided into 2 type, one is the non-

typhoidal type which is quite common and the other one is the typhoidal type of bacteria which may cause Typhoid which is potentially dangerous and may require extensive antibiotic treatment. The specific bacterial form causing this condition is called the Salmonella Typhi bacteria. The most common symptoms of Salmonella Food Poisoning are Diarrhea, Abdominal cramping, Fever ranging from 102 to 104 degrees.

Clostridium species: *C. perfringens* food poisoning is usually a mild Clostridial infection. *C. perfringens* is widely distributed in feces, soil, air, and water. Contaminated meat has caused many outbreaks. *C. perfringens* spores sometimes survive cooking; they can germinate and multiply, resulting in large numbers of bacteria when cooked meat that is contaminated with *C. perfringens* is left at room temperature or even up to 60° C (140° F, as on a warming table) for a period of time. Outbreaks typically occur in commercial establishments and rarely at home. Once inside the gastrointestinal tract, *C. perfringens* produces an enterotoxin that acts on the small bowel. Only *C. perfringens* type A has been definitively linked to this food poisoning syndrome. Mild gastro enteritisis most common, with onset of symptoms 6 to 24 hours after ingestion of contaminated food. The most common symptoms are watery diarrhea and abdominal cramps. Vomiting and fever are unusual. Symptoms typically resolve within 24 hours; severe or fatal cases rarely occur.

Material and Methods

Collection of sample

1. Vegetables were collected from the market.
2. Processed food were collected from domestic kitchens
3. Poultry products were collected from poultry yard
4. Meat and meat products were collected from slaughter house
5. Dairy products were collected from dairy

Enrichment of sample

For enrichment of pathogens from specimen samples & positive controls, each sample & pure cultures were inoculated in Soyabean- casein digest broth (SCDM-100ml) and incubated at 37ºC for 24-48 hrs. Examine the medium for growth & if growth is present use an inoculating loop to streak a portion of the on surface of selective agar medium.
Composition of SCDM (M011): Ingredients Gms / Litre Tryptone 17.000 Soya peptone 3.000 Sodium chloride 5.000 Dextrose (Glucose) 2.500 Dipotassium hydrogen phosphate 2.500 Final pH (at 25°C) 7.3±0.2

Isolation and Identification of food poisoning Bacteria

I) ***Staphylococcus aureus****:* After enrichment a loopful sample & culture of dairy product samples were streaked on Mannital Salt Agar along with positive control (*S.aureus*ATCC6538). After incubation at 30-35ºC for 24-48 hours. Further identification was done by using coagulase test.
Coagulase Test: With the aid of an inoculating loop transfer representative suspected colonies from agar surface of Mannitol Salt Agar medium to individual tubes, each containing 0.5ml of mammalian preferably rabbit or hourse, plasma with or without suitable intervals upto 24 hours. If no coagulation in any degree is observed, the sample meets the requirements of the test for absence of *Staphylococcus aureus.*
Composition of Mannital salt agar MH118:Components Item g/L Proteose Peptone 10.00, Beef Extract 1.00 Sodium Chloride 75.00, D-Mannitol 10.00, Phenol Red 0.025, Agar 15.00, Final pH (at 25°C) 7.4 ± 0.2

II) Escherichia coli: After enrichment by means of inoculating loop, sample & culture were inoculated on surface EMB agar medium plates and incubated at 37ºC for 24 hours.
Identification: A pink or rose red colonies with metalic sheen were observed on surface of EMB agar medium. Peptone water tubes were inoculated with these colonies of metalic sheen and incubated at 44ºC for 18 hours. Then observed for cherry red colour ring formation in upper 1/3rd portion after addition of Kovac's reagent and confirming with positive control (*Escherichia coli* NCTC9002).
Composition: 1) EMB agar M317: Ingredients Gms / Litre Peptone 10.000, Dipotassium hydrogen phosphate 2.000, Lactose 5.000, Saccha rose (Sucrose) 5.000, Eosin - Y 0.400, Methylene blue 0.065 Agar 13.500 Final pH (at 25°C) 7.2±0.2
2) Peptone water (M028): Ingredients Gms / Litre Peptone 10.000 Sodium chloride 5.000 Final pH (at 25°C) 7.2±0.2
3) Kovac's reagent (R008): p-dimethy lamino benzaldehyde 5.000 Amyl alcohol 75.000 Hydrochloric acid, concentrated 25.000

III) ***Salmonella species***: After enrichment the sample & positive culture were streaked on surface of Brilliant Green Agar medium. Upon examination if colonies conform to description as given in table. Proceed to further identification by transferring representative suspect colonies individually by means of inoculating loop to butt slant tube of triple sugar agar slant by means of an inoculating wire, to a slant tube of triple sugar iron agar by first streaking surface of slants & then stabbing the wire well beneath surface and incubating it at 35-37ºC for 24 hours. After incubation the tubes were observed for change in colour of the slant from acidic (yellow) to alkaline (red) with or without blackening due to H2S production.
Composition: 1) Brilliant Green agar (MM016): Ingredients Gms / Litre Peptone 10.000 Yeast extract 3.000 Lactose 10.000 Sucrose 10.000 Sodium chloride 5.000 Phenol red 0.080 Brilliant green 0.0125 Agar 12.000 pH after sterilization 6.9±0.2
2) Triple sugar agar (M021): Ingredients Gms / Litre Peptone 20.000 HM extract # 3.000 Yeast extract 3.000 Lactose 10.000 Sucrose 10.000 Glucose(Dextrose) 1.000 Iron(III) citrate 0.300 Sodium chloride 5.000 Sodium thiosulphate 0.300 Phenol red 0.024 Agar 12.000 Final pH (at 25°C) 7.4±0.2

IV) ***Clostridium species***: Enrichment: sample was inoculated separately into RCMM and incubated at 37ºC for 40-48 hours. Growth of *Clostridium* was visualized as gas bubbles. Blackening and disintegration of meat particle indicates proteolytic activity in the media. The medium further tested for presence of *Clostridium.*
Isolation and identification: after confirming enriched growth

a loop ful culture was inoculated on Clostridium agar by streak plate method. Plates were incubated at 35-37ºC for 24-48 hours and observed growth with typical colony characteristics when compared with standard.

Composition: 1) RCMM (M149): Ingredients Gms / Litre HMH peptone B # 98.000 Proteose peptone 20.000 Dextrose (Glucose) 2.000 Sodium chloride 5.000 Final pH (at 25°C) 7.2±0.2

2) Clostridium agar (M497): Ingredients Gms / Litre Tryptone 17.000 Soya peptone 3.000 Dextrose 6.000 Sodium chloride 2.500 Sodium thioglycollate 1.800 L-Cystine 0.250 Sodium formaldehyde sulphoxylate 1.000 Neomycin sulphate 0.150 Sodium azide 0.200 Agar 14.500 Final pH (at 25°C) 7.0±0.2

Results and Discussion

Food samples collected from different outlets like Dairy, kitchen, Market place, Poultry yard & Slaughter house Food house had been used for isolation & identification of food pathogens. Microbiological analysis (Microbial Limit Test as per I.P.) results revealed that the specimen samples contains as *Escherichia coli w*as the most highly abundant pathogen in Market & kitchen. *Salmonella* was most common in poultry yard where as *Staphylococcus aureus* is common in dairy & Clostridium is common in slaughter house.

Table 1: Morphologic Characteristics of *Staphylococcus aureus* on Selective Agar media

Selective Media	Characteristic colonial morphology	Gram Stain
Mannitol Salt Agar medium	Yellow colonies with yellow zones	Positive cocci in Clusters

Fig 1: Isolation of Specimen sample-1 on Mannitol Salt Agar

Table 2: Morphologic Characteristics of *Escherichia coli* on Selective Agar media

Selective Media	Characteristic colonial morphology	Gram Stain
EMB Agar	Blue-black with Green metallic sheen colonies	Negative short rods

Fig 2: Isolation of Specimen sample-2 on Eosine Methylene Blue Agar

Table 3 Morphologic Characteristics of Salmonella Spp. on Selective Agar media

Selective Media	Characteristic colonial morphology	Gram Stain
Brilliant Green Agar	Pinkish white or red colonies surrounded by red halo	Negative short rods

Table 4: Morphologic Characteristics of Clostidium Spp. on Selective Agar media

Selective Media	Characteristic colonial morphology	Gram Stain
Clostridium Agar	Pale White Colonies appeared	Positive Rods

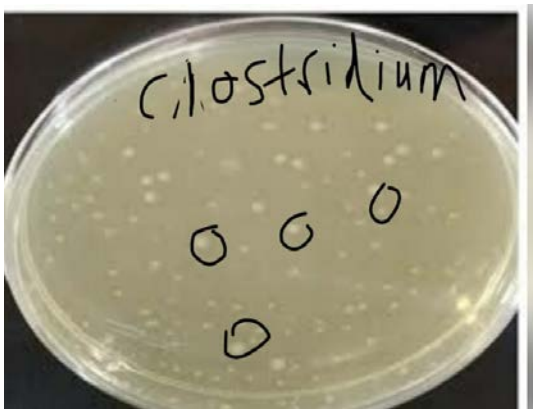

Fig 3: Isolation of Specimen sample-5 on Clostridium Agar

Conclusions

The different types of bacteria present in the food samples and in some sites in the kitchens, dairy, Market, Poultry yard & Slaughter house. *E. coli* and *Salmonella spp.* were the most abundant bacteria in the kitchen, Market yard & poultry farm which reveals the poor hygiene in these areas. *Staphylococcus aureus* & Clostridium spp. were the most abundant bacteria in dairy & slaughter house. To prevent cross-contamination, surfaces and utensils that are used to make and prepare raw food, particularly poultry and meat, should be thoroughly cleaned with an antibacterial cleanser or disinfectant after each use. When using sponges and dish towels, it is imperative that they be disinfected.

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