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ORIGINAL ARTICLE

Isolation and Identification of Pathogenic Microorganisms from Ready-To-Eat Food

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ABSTRACT
Human food is any substance which helps the body to grow well and also provide heat and energy. Ready to eat foods are sold in streets or in public places they are inexpensive and also easily accessible. The presence of any pathogenic organisms in ready to eat food can cause serious health consequences. This study was conducted to isolate and identify microbial growth in various ready-to-eat food samples taken from different areas of Abbottabad city. Microbiological analysis was conducted on five types of ready-to-eat food such as channa chat, burger, shawarma, kabab and samosa. The microorganisms were isolated and identified by various techniques such as culturing, Gram staining and biochemical tests. For culturing, MacConkey agar, sabouraud dextrose agar, nutrient agar and salmonella shigella agar were used. The presence of different pathogenic bacteria was confirmed by biochemical test such as Voges–Proskauer test, indole, Methyl red test, Simon citrate, Triple sugar iron test, catalase and coagulase test. Total 80 samples were tested out of which 64 samples were found contaminated with pathogenic bacteria. Pathogenic organisms isolated and identified were Salmonella typhi (15.62%), Salmonella Para typhi (3.12%), Proteus mirabilis (4.68%), Clostridium perfringens (6.25%), Pseudomonas aeruginosa (7.81%), Staph. aureus (10.93%), E.coli (34.37%), Streptococcus(7.81%), Shigella (6.25%), Bacillus cereus(3.12 %). This study indicates that pathogenic bacteria present in many ready-to-eat food which possesses high health problems to consumers. Food safety and public awareness are necessary to avoid any food borne illnesses due to contaminated ready to eat food.
Keywords: Voges–Proskauer test, Isolation, Pathogenic Microorganisms, ready-to-eat food

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INTRODUCTION

Ready-to-eat foods are defined as foods easily purchased from the food vendors such as restaurants or food huts. Ready to eat food can also be described as the level of food which can be eaten immediately at the site where it is sold [21]. The variety of Ready-to-eat foods are available everywhere. From one country to another according to the cultural and geographical environment, the kinds of Ready-to-eat foods are varied. As for salads, beef, chicken and gravy etc. are similar all over the world [23]. There are many kinds of Ready to eat foods available in Pakistan such as samosa, shawarma , kabab, biryani, burgers, channa chat, dahi bhally and paani puri etc are mostly purchased at food vendors. Consumption of contaminated ready to eat foods including red meat, eggs, cheese & vegetables has been documented to serve as vehicles for transmission of several bacterial pathogens and food-borne outbreaks [7]. Based on the relatively cheap and easily available of these ready-to-eat foods its consumption has increased over a few years. The street foods offer food at a low price. Furthermore, the food it provides is not easy to prepare at home and also time-consuming.

The food plays an important role in human survival. The human beings not only obtaining nutritional requirements from food but also consume undesirable agents which could be hazardous for them [3]. Most foods contain sufficient nutrients to support microbial growth. Several factors encourage, prevent or limit the growth of microorganisms in foods, the most important factor is water availability, pH and temperature [8]. Millions of people get sick by contaminated food all over the world. Food borne illnesses

harm all human life. These diseases may cause huge social, economic and cultural problems also affect the health systems [26].

The ingestion of bacterial toxins and microbial agents found in food causes food borne illness. There is much data on the problems of food borne infections which is well documented in all the countries [12]. One of the major problems in the health sector is food borne diseases by this the economy of the country became reduced [8]. Chemicals used in food products are also responsible to make people sick. The pathogenic microorganisms cause contamination in street foods has been recorded and also it is found that many diseases are caused due to ingestion of contaminated street foods. More than 250 food borne illnesses have been identified. It is difficult to recognize which disease is food borne because different microorganisms spread in many ways. Public health authorities must know the pathogenesis and causative agents of diseases hence appropriate steps should be applied to eradicate the diseases [24].

The main food borne pathogens that can contaminate food are *Salmonella species, Staphylococcus aureus, Clostridium botulinum, Bacillus species., Acinetobacter species, Escherichia coli, Pseudomonas species and the hepatitis A virus*. Intestinal parasites include *Giardia lamblia, Entamoeba histolytica, Ascaris lumbricoides and Hookworm* [2].

Aims and Objective

1. To isolate and identify the pathogenic microorganisms.
2. To determine the microbial load of isolated bacteria.
3. To establish the public health and to provide consumer protection.
4. To ensure that foods during handling, storage, processing and
5. Distribution is safe for human consumption.

MATERIAL AND METHODS

The study was carried out in the Microbiology laboratory of Veterinary Disease and Research Investigation centre (VD & RIC) Abbottabad.

Sample collection and processing

A total 80 random samples of ready to eat food including (samosa, burger, shwarma, channa chatt and kabab) each weighting 5gm were collected from different restaurants of Abbottabad city between February and March 2019. Without any delay collected samples were transferred to the laboratory under strictly hygienic measures for bacterial examination. Clean polythene bags were used for the collection of each sample.

Tested Organisms

Escherichia coli, Staphylococcus species, Streptococcus species, Salmonella species, Proteus, Pseudomonas, Klebsiella, Shigella and *Bacillus* species.

Culture media

In this study different types of agar and broth media were used for selective growth including Nutrient agar, MacConkey agar, Salmonella Shigella agar, Saboraud dextrose agar, Nutrient broth and Buffered peptone water broth.

Pouring

The different types of prepared media were poured into each of the five Petri dishes in a way so that each Petri dish gets 12-15 ml media. Culture media was dispensed into each Petri dish. Media pouring will be carried out aseptically under laminar flow hood. UV light was switched on for one hour before working in the Laminar flow hood. After pouring the medium, all Petri dishes were kept in room temperature so that the culture medium can become properly solidified.

Inoculation of sample

The sample was inoculated into the tubes of buffer peptone water broth and nutrients broth. In 9ml of nutrient broth full loop of the sample was inoculated and mix well and in 45ml of buffer peptone water (BPW) broth 5gm solid sample was thoroughly mixed. After 24 hours of incubation observe growth.

Culturing

From enrichment broths, samples were streaked on Petri dishes with the help of loops. The loop were dipped into flask which contains a sample and then streaked on petri dishes which contain media (Mackoney agar, salmonella Shigella agar, nutrient agar and Sabaroud dextrose agar). The plates were labeled as 1,2,3,4,5 according to sample. Plates were incubated at 37°C for 24- 48hours. Sabouraud-Dextrose agar plates were incubated at 28°C for 72 hrs.

Identification of organisms

After whole night incubation, the isolated organisms were identified by three methods,

1. Interpreting culture plates
2. Gram staining

3. Biochemical test

Interpreting culture plates

Culture plates were carefully examined under intense light for size of colonies, pigmentation, consistency, odor and elevation of colonies, including growth and their features on different agar plates(Table:1 and Figure:1)

Table:1. Morphological characteristics of the isolated organism

Media used	**Culture character of *Salmonella***	**Culture character of *Staphylococcus***	**Culture character of *E.coli***
Nutrient Agar	White and few yellow colonies	Yellow colonies	White colonies
MacConkey Agar	Colorless and transparent	Yellow colonies	Pink colonies with few Yellow
SS agar	Black centre colonies with few pink colonies	Yellow colonies	Pink colonies

Figure1: Growth of Bacteria on different media

Gram staining

Gram staining was done to distinguish between two groups of bacteria , Gram positive and Gram negative. For gram staining a glass slide were labeled with led pencil with sample code. With the help of dropper distilled water was put on slide. A wire loop was red hot over Bunsen burner flame. By using wire loop picked up desire colonies to make a suspension on glass slide. Slide were air dry and heat fixed by passing slide over heat flame. Slide were flooded with primary stain crystal violet dye for 1 minute and then washed with distilled water. Then flooded with iodine solution for 1 minute and washed with distilled water. Then use alcohol as a decolorizer for 30 second it decolorize bacterial cell wall and then washed off with distilled water. Then slides were flooded with counter stain which is safranin for 2 minutes and washed with distilled water. Then slides were dried at 27°C.

Microscopy

A drop of cidar wood oil was added onto each slide and observed under high power lens (100 X) of a compound light microscope. The gram negative and gram positive bacteria were separated through microscopic examination. In which *E.coli* appear as gram negative pink rod, *staphylococcus* appear as gram positive violet cocci present in cluster form, *streptococcus* appear as gram positive cocci in chains while *salmonella and Shigella* appear as gram negative rod shaped bacilli, *klebsiella* appear as gram negative rod shaped while *proteus* is gram negative rod shaped bacterium.

Biochemical tests

Biochemical test carried out to identify the isolated bacteria includes: catalase, coagulase, oxidase, urease, TSI, indole, Voges– Proskaue, Simmon Citrate and Methyl Red. It was done according to the method [20].

RESULTS

A total of 80 samples of ready-to-eat food were collected from different areas of Abbottabad city. These sample were collected from restaurant, fast food outlets to small kiosks and street vendors. Samples include equal number of samosa, burger, channa chatt ,kabab and shawarma (sixteen each). Results revealed that 64 samples out of 80 were found contaminated. Results showed that a total of 10 species of bacteria isolated and identified from these ready to eat food sample while all these samples had no growth of fungus.

Bacteria isolated from samosa

A total of 16 samples of samosa were collected and examined. Results showed that almost all the samosa samples were contaminated with bacteria. Microorganisms isolated and identified through gram staining and biochemical test were *Salmonella typhi, Salmonella paratyphi, Ecoli*, *Pseudomonas aurigenosa.* and *staphylococcus aureus* (Table 2 and 2.1). Biochemical Test and microscopic examination are illustrated in Figure 2 and Figure 3 respectively.

Table2:Bacteria isolated from samosa.

Sample	Bacteria isolated from samosa
Samosa	*Salmonella typhi, Salmonella paratyphi, E.coli*, *Pseudomonas aurigenosa. Staphylococcus aureus.*

Table 2.1:Biochemical test results

Organisms	**TSI**	**Citrate**	**VP**	**MR**	**Indole**	**Oxidase**	**Catalase**	**Gram staining**
Salmonella typhi	+	+	-	+	-	-	+	GNR
Salmonella paratyphi	+	+	-	+	-			GNR
E.coli	+	-	-	+	+			GNR
Pseudomonas aeruginosa		+	-	-	-		-	GNR
Staph. Aureus		+	+	+	-		+	GPC

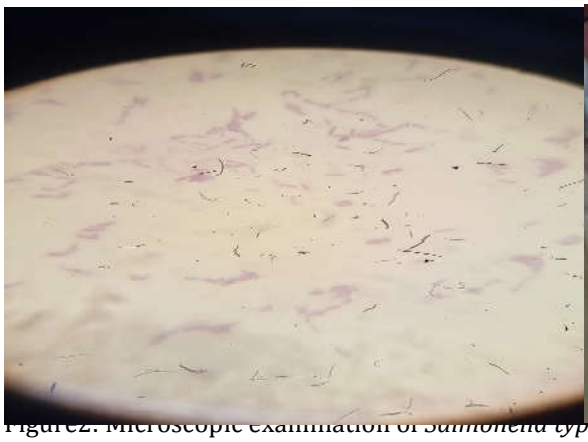

Figure2: Microscopic examination of *Salmonella typhi*

Figure3: Biochemical tests

Bacteria isolated from kabab.

A total of 16 samples of kabab were examined for bacterial and fungal contamination. Results revealed that all samples were contaminated with bacterial contamination. Bacterial species detected from kabab are *streptococcus, Sheigella, Clostridium perfringens, Pseudomonas aurigenosa*, *Proteus marbilus*, *E.coli* (Table: 3 and 3.1)

Table 3: Bacteria isolated from kabab

Sample	Bacteria isolated from kabab
Kabab	*Streptococcus, sheigella, clostridium perfringens, Pseudomonas aurigenosa*, *Proteus marbilus*, *E.coli*.

Table 3.1:Biochemical test results

organisms	TSI	Citrate	VP	MR	Indole	Oxidase	catalase	Gram staining
Streptococcus		+	-	+	-		-	GNR
Shigella	+	-	-	+			+	GNR
E.coli	+	-	-	+	+			GNR
Pseudomonas aeruginosa		+	-	-	-		-	GNR
Clostridium perfringens					-		-	GPR

Bacteria isolated from channa chat

A total of 16 samples of channa chart were cultured. 8 out of 16 sample were found contaminated. Microorganisms isolated and identified are *Bacillus cereus*, *Salmonella typhi* and *E.coli (Table 4 and 4.1).* Biochemical Test and microscopic examination are elaborated on Figure 4 and 5.

Table 4: Bacteria isolated from channa chatt

Sample	Bacteria isolated from channa chatt
Channa chat	*Bacillus cereus*, *Salmonella typhi* and *E.coli*

Table4.1: Biochemical test results

Organisms	TSI	Citrate	VP	MR	Indole	Oxidase	catalase	Gram staining
Salmonella typhi	+	+	-	+	-	-	+	GNR
E.coli	+	-	-	+	+			GNR
Bacillus cereus	+	+	-	+	-	+	+	GPR

Figure 4 Biochemical test result

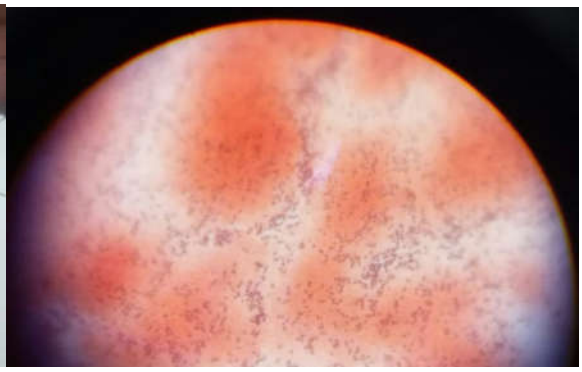

Figure 5 Microscopic examination of *Bacillius cereus*

Bacteria isolated from burger

A total of 16 samples of burger were examined. 14 out 16 samples shows positive growth. Microorganisms detected from these sample were *E.coli, Salmonella typhi, Pseudomonas aurigenosa, Streptococcus, Proteus marbilus, (Table:5 and5.1).* Biochemical test and microscopic examination are shown in figure 6 and 7 respectively.

Table 5: Bacteria isolated from burger.

Sample	Bacteria isolated from burger
Burger	*Salmonella typhi,Pseudomonas aurigenosa, Streptococcus, Proteus marbilus, E.coli.*

Table 5.1: Biochemical test results

Organisms	TSI	Citrate	VP	MR	Indole	Oxidase	catalase	Gram staining
Salmonella typhi	+	+	-	+	-	-	+	GNR
Streptococcus		+	-	+	-		-	GP cocci
E.coli	+	-	-	+	+			GNR
Pseudomonas aeruginosa		+	-	-	-			GNR
Proteus marbillus	+	+	-	+				GNR

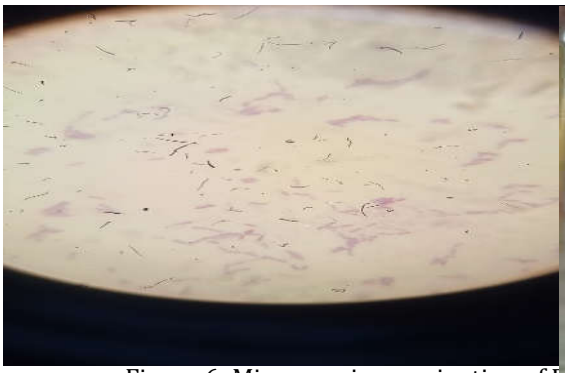

Figure 6: Microscopic examination of E.coli

Figure 7: Biochemical tests

Bacteria isolated from shawarma

16 samples were examined for bacterial contamination. 10 out of 16 samples were found contaminated. Microorganisms isolated and detected were *staphylococcus aureus, Salmonella typhi, Shigella, E.coli* and *Clostridium perfringens*. (Table 6 and 6.1). Biochemical Test and microscopic examination ate illustrated in figure 8 and 9 respectivetly.

Table 6: Bacteria isolated from shawrma

Sample	Bacteria isolated from shawarma
Shawarma	*staphylococcus aureus, Salmonella typhi, Shigella, E.coli* and *Clostridium perfringens.*

Table 6.1:Biochemical test results

Organisms	***TSI***	***Citrate***	***VP***	***MR***	***Indole***	***Oxidase***	***catalase***	***Gram staining***
Salmonella typhi	+	+	-	+	-	-	+	*GNR*
Staph. aureus		+	-	+	-		+	*GP cocci*
E.coli	+	-	-	+	+			*GNR*
Clostridium perfringens					-		-	*GPR*
Shigella	+	-	-	+				*GNR*

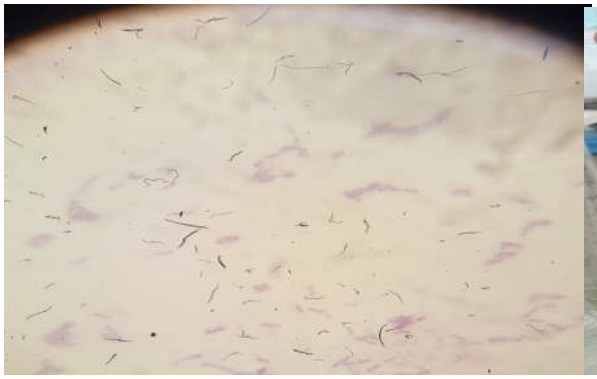

Figure 8: Microscopic examination of *Shigella*

Figure 9: Biochemical tests

Total 80 samples were tested out of which 64 samples were found contaminated with pathogenic bacteria. Pathogenic organisms isolated and identified were *Salmonella typhi (* 15.62%), *Salmonella papatyphi (*3.12% *), Proteus marbilus (*4.68% *), Clostridium perfrigens (*6.25%*), Pseudomaons aurigenosa (*7.81%*), Staph. aureus (10.93%), E.coli (34.37%), Streptococcus(7.81%), Shigella (6.25%), Baccilus cereus(3.12 %)*.

DISCUSSION

Microbial contamination is an indicator of the degree of safe handling of food which is a globally recognized vehicle for transmission of pathogens. Food hygiene has been largely neglected in the study area despite the fact that it is the most important component essential to health and productivity of individuals and the community at large. In this study bacterial load was adopted as a measure of the microbial quality of food vended or served to the general populace [18].

The main objectives of this project were isolation and identification of pathogens from ready to eat food and the characterization of the identified pathogen. Various techniques used for the isolation and the identification were morphological characterization, culturing and the biochemical tests.

During this study 50 samples of food including (samosa, shwarma, burger, channa chatt and kabab) were examined. Nine different types of organisms were identified from the selected 5 foods. They were *Klebsiella, Escherichia coli, Salmonella, Shigella, Bacillus , streptococcus , proteus, pseudomonas and Staphylococcus* .

The samples collected has high level of contamination this is due to the fact that the ready to eat food were exposed in an open air, food handlers hygiene and also places were unhygienic where these foods were sold.

Recent studies indicated that *E. coli, Staphylococcus aureus, B. cereus, Shigella, Salmonella* and *Pseudomonas spp* were isolated from the ready to eat foods indicating poor sanitary control and practices. These organisms are known food borne pathogens and opportunistic pathogens that have been implicated in food borne disease outbreaks [16, 20, 25, 27]. Our results are also in agreement with the previous studies.

Aycicek *et al.,*[6] examined the rate of *Staphylococcus aureus* contamination of ready-to-eat salads and foods in restaurants at military centers in Ankara Turkey. They tested 512 samples of salads, pizzas, and a variety of meat foods. Their results showed that 48 samples were contaminated with *Staphylococcus aureus* in meat foods and salads. *Staphylococcus aureus* contamination was significantly higher in their study which is consistent with the our results.

Feng *et al*., [10] reported that some microorganisms are harmful and cause disease while others are benevolent neutral or even helpful. Some help us to produce certain foods e.g. *Streptococcus lactis* to make butter milk break down toxins in our environment while others can sick us (for example contaminants in food like *Escherichia coli or Salmonella)* fungi cause athlete's food and ringworm, bacteria cause pneumonia, legionmaire's disease, tetanus and other diseases. Our results are also in agreement with the previous studies.

Bichai *et al*.,[7] showed that, the presence of *Escherichia coli* can be related to use of polluted irrigation waters during growth. Contamination through human handling the use of contaminated containers or washing after harvested with polluted water. It was suggested that it could increase the incidence of enteric pathogens [5, 14]. These findings are consistent with the interpretation of our results.

Richard *et al*.,[22] showed that gastrointestinal diseases has been reported by eating raw or inadequately cooked meat containing bacillus spores. B. cereus causes food poisoning by means of enterotoxins. Bacillus species were also isolated from fast food samples during our study.

P. aeruginosa widely distributed in nature (soil, water, plants, animals). *P. aeruginosa* can grow in distilled water, laboratory hot water baths, hot tubes, wet IV tubing and other water containing vessels. This explains why the organism is responsible for so many nosocomial infections [13]. *P. aeruginosa* was isolated from traditional food samples and was isolated from fast food samples.

To protect against *Salmonella* infection it is recommended that food be heated for at least ten minutes at 75^0C ($167\ ^0$F) so that the center of the food reaches this temperature. *Salmonella* is not destroyed by freezing. It can survive several weeks in a dry environment and several months in water thus they are frequently found in polluted water contaminated from the excrement of carrier animals being particularly important [9, 11]. *Salmonella* was isolated from ready to eat food (samosa, burger and shawarma) in our study.

Salmonella typhi is transmitted generally through food or water contaminated by human feces. Public food handlers or health care deliverers who are carriers can present a serious public health problem. *Salmonella spp* was isolated from fast food samples. Our results similar with the previous studies as FDA-Center for Food Safety [9].

However, since Bacillus cereus is pathogenic and also spoilage organisms whose presence may be as a result of well water for cleaning of ingredients, plates, pans or even used for mixing during production [4]. They showed that certain organisms like Staphylococcus species, Bacillus cereus, Escherichia coli and other enteric bacteria are present in well water [19]. Our results parallel with the previous study.

CONCLUSION

The main purpose of this study was to isolate and identify pathogenic bacteria from five different kinds of ready to eat food from different areas of Abbottabad city, Pakistan. We isolated and identified pathogenic microorganisms were *Salmonella typhi (*15.62%), *Salmonella papatyphi* (3.12%*), Proteus marbilus* (4.68%*), Clostridium perfrigens (*6.25%*), Pseudomaons aurigenosa (*7.81%*), Staph. aureus (10.93%), E.coli*

(34.37%), Streptococcus(7.81%), Shigella (6.25%) and *Baccilus cereus (3.12 %)*. The most contaminated sample was samosa.

It is therefore very necessary and important that training for personnel working in fast food preparation centers should be on the proper observance of health issues. The staff must be ensured that foods during handling, storage, processing and distribution are safe for human consumption and food must be hygienic to prevent the transmission of microbial contamination. The amount of contamination in food must be reduced by taking appropriate actions for the better health of the community. Food borne illness can be prevented by good hygiene practices such as the use of Good Manufacturing Practices (GMP) and Hazard Critical Control Point (HACCP).

It is recommended that regular microbiological quality control programs and education of the food handlers/ food vendors on food safety practices should be encourage. Strict supervision must be applied on ready-to-eat foods that are sold in restaurants and streets. Also strict hygienic measures should be applied during preparation of ready to eat food to improve the quality of the product and to safeguard the consumers.

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