Determination of Some Bacteria on Hamburger and Sushi

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Abstract

Bacteriological examination was conducted on 20 samples of fast food as chicken burger and fish sushi which were purchased from mini-shops located in Mingalar Oo, Nyaung Shwe haw gon and Myoma quarter in Taunggyi Township. Collected samples were tested by standard conventional methods for isolation. Primary isolation, secondary isolation, morphology tests, Gram staining and nine biochemical tests were employed for the identification of isolated bacteria. A total of ten isolates as *Micrococcus luteus, Bacillus coagulans, Bacillus subtilis, Staphylococcus aureus, Citrobacter freundii, Enterobacter aerogenes, Proteus morganii, Proteus vulgaris, Pseudomonas aerugenosa, Pseudomonas fluorescens* were recorded in this study. Total 60 isolates were recorded, among them *Staphylococcus aureus* was most isolated 16.67% and *Pseudomonas aerugenosa* was least isolated 5%.

Key words: Isolation, biochemical reactions

Introduction

Hamburger is one of the most popular fast foods, which carries considerable amount of meat all over the world. After production, hamburger patties are mainly being kept cold or frozen until they were used in many restaurants (Ciftcioglu *et al*., 2008). Fast foods are widely consumed in lunch boxes and its market has continued to be very important. In developing countries, the numbers of branded fast food shops have increased since the early 2010s. An increasing number of consumers tend to prefer handmade food served in a restaurant over commercially manufactured fast food because of their palatability and freshness (Jang *et al*., 2013). Sushi is a traditional Japanese food mainly consisting of cooked acidified rice combined with raw fish or other seafood. Sushi was introduced to the Western restaurant market during the 1980s and is now commonly offered as a relatively cheap, ready-to-eat product in retail stores. (Hoel *et al*., 2015)

Food-borne diseases are common in most countries of the South-East Asia region. Gastrointestinal diseases such as cholera and other forms of diarrhoea and enteric infections are common diseases. Many people, particularly in urban areas are eating outside their homes in restaurants and fast food stalls from various mini markets (Ko Ko, 1995). Poor personal hygiene, improper cleaning of storage and preparation areas and unclean utensils cause contamination of raw and cooked foods. Mishandling of raw and cooked food allows bacteria to grow. People in several developing countries such as India, Indonesia, Myanmar, Thailand and some industrialized countries have changed their life-styles gradually. Many people are eating outsides their homes in food service establishments where the food is prepared in large quantities and where food-handlers are unaware of the special precautions required under such conditions (WHO, 1992). Hamburger and sushi are also popular fast foods. Food safety is essential because most people like to eat these two items of popular fast food. So, this is one reason for choice of these items to detect the microbial value. And then another reason is to detect and observe distribution and occurrence of bacteria from these food items. With these facts this research has been chosen to conduct with the following objectives;

- to determine microbiological quality of the fast food,
- to identify some common bacteria isolated from the chicken hamburger and fish sushi.

Materials and methods

Study sites

In this study three main study sites were chosen in Taunggyi: Site I- Mingalar Oo quarter, Site II- Ngaung Shwe Haw Gon quarter and Site III- Myoma quarter in Taunggyi.

Study period

This study was carried out from July 2017 to November 2017. Not only field survey but also laboratory works were done during this study.

Sample collection

The research material consisted of a total of 20 samples of the fast food as chicken burger and fish sushi. All samples were purchased from some mini-shops located in selected study sites. Sample collections were carried out twice a month during five months study. Purchased samples were wrapped with sterilized aluminium foil and were carried to the biotechnology laboratory of the Taunggyi University.

Culture media

Nutrient agar was used as ordinary media for primary isolation, MacConkey agar was used as selective media for secondary isolation, Triple Sugar Iron, Lysine Iron Agar, SIM medium, MR VP medium, Simmon Citrate agar, Urea Agar Agar Base,

40% Urea Solution were used for biochemical reactions of bacteria recorded. (Plate1. B)

Preparation of samples

Each sample was purchased from mini shops located in three study sites. And then 50 grams of sample soaked in Bufferfield's Phosphate solution was blended with homogenizer to get a solution for 3 minutes. After blending, this soluble solution was examined by 10 fold serial dilution. And then prepared 90 ml of buffer solution in five conical flasks and 1ml of relevant dilutions were inoculated onto nutrient agar plates of the already prepared media using the four plate technique according to Andrews, 1992, method. At the same time Nutrient agar and MacConkey agar plate were inoculated by streak plate method for detection of Enterobacteriaceae and others. The plates were then incubated at 37° C for 24-48 hours and examined for colony formation. (Plate1. A)

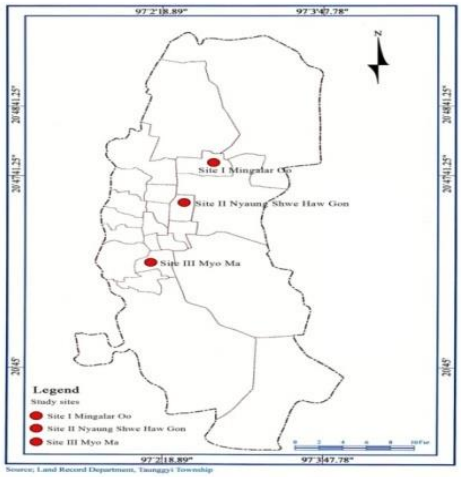
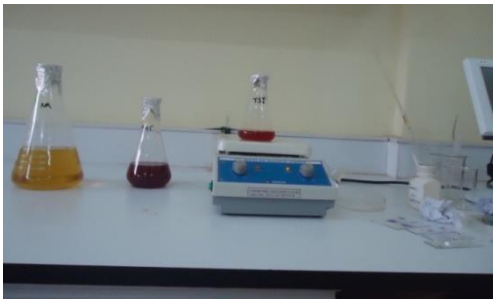
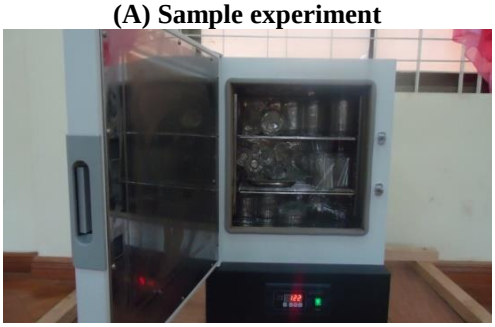


Figure 1. Location map of study sites in Taunggyi Township

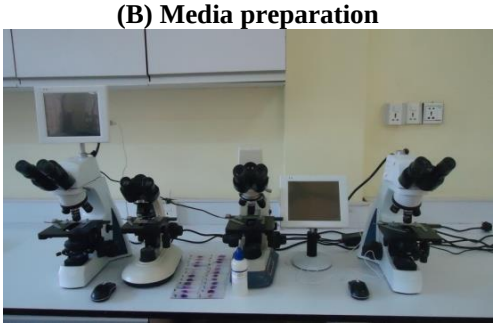
(A) Sample experiment



(B) Media preparation



(C) Sterilization on glass wears



(D) Stereomicroscope

Plate 1. Samples and materials used for observation

Results

In the present study, total of seven genera and ten species belonging to five families of four orders under class Actinobacteria, Bacilli and Gammaproteobacteria of Phylum Actinobacteria, Firmicutes and Proteobacteria and ten species were recorded.

1. General description of bacteria recorded

1.1 *Micrococcus luteus* (Schroeter, 1872)

Micrococcus luteus is a Gram-positive, obligate aerobe, nonmotile, coccus, tetrad arranging, pigmented, saparotrophic bacterium that belongs to the family Micrococcaceae.

1.2 *Bacillus coagulans* Hammer, 1915

Bacillus coagulans is a lactic acid-forming bacterial. It can be used similarly to Lactobacillus and other probiotics as beneficial bacteria.

1.3 *Bacillus subtilis* (Ehrenberg, 1835)

Bacillus subtilis is a Gram-positive, rod-shaped, catalase positive bacterium. It can form a tough, protective endospore, allowing it to tolerate extreme environmental conditions.

1.4 *Staphylococcus aureus* Rosenbach, 1884

Staphylococcus aureus also known as golden staph and is a Gram-positive, coccus, which appears as grape-like clusters when viewed through a microscope, and round, usually golden-yellow colonies. (Plate 2. E)

1.5 *Citrobacter freundii* (Braak, 1928)

Citrobacter freundii is a species of facultative anaerobic Gram-negative bacteria of the Enterobacteriaceae family. These bacteria have a long rod-shaped. Cells have flagella used for locomotion; some do not and are non-motile.

1.6 *Enterobacter aerogenes* Hormaeche, 1960

Enterobacter aerogenes is a Gram-negative, oxidase negative, catalase positive, citrate positive, indole negative, rod-shaped bacterium and is capable of motility via peritrichous flagella.

1.7 *Proteus morganii* Winslow, 1919

Proteus morganii is a Gram-negative, bacillus, motile, non-lactose fermenting. Catalase-positive, oxidase-negative and facutatively anaerobic. Attack sugars fermentatively, usually with gas production, urease positive.

1.8 *Proteus vulgaris* Hauser, 1885

Proteus vulgaris is a rod-shaped, nitrate-reducing, indole and catalase positive, Gram-negative bacterium, facultative anaerobic. It can be tested positive or negative for citrate utilization.

1.9 *Pseudomonas aeruginosa* (Schroter, 1872)

Pseudomonas aeruginosa is a Gram-negative, aerobic rod, motile, aerobic or and at times facultative anaerobic, non-spore forming, capsulated, oxidase and catalase positive. (Plate 2. F)

1.10 *Pseudomonas fluorescens* (Flugge, 1886)

Pseudomonas fluorescens is Gram-negative, bacilli, motile, catalase and oxidase positive, aerobic. Attack sugars by oxidation; do not produce gas. Diffusible yellow or green pigments are produced.

2. Isolated bacteria from collected chicken burger

A total of ten samples were examined, five isolates of *Bacillus coagulans* 14.29%, four isolates of *Bacillus subtilis* 11.43%, Five isolates of *Staphylococcus aureus* 14.29%, four isolates of *Citrobacter freundii* 11.43%, eight isolates of *Enterobacter aerogenes* 22.86%, five isolates of *Proteus morganii*14.29%, one isolates of *Proteus vulgaris* 2.86% and three isolates of *Pseudomonas aeruginosa* 8.57% were found. Among them, most isolation was found in *Enterobacter aerogenes* 22.86% and least isolation was found to be *Proteus vulgaris* 2.86 %. (Table 1)

3. Isolated bacteria from collected fish sushi

According to observation on ten samples of fish Sushi tested, it was found that five isolates of *Staphylococus aureus* was found 20% as highest. The lowest were each four isolates of *Micrococcus luteus, Bacillus coagulans, Bacillis subtilis, Proteus vulgaris* and *Pseudomonas fluorescens* encountered as 16%. (Table 2)

4. Distribution of bacteria from chicken burger and fish sushi

Between two items of samples and ten times for each, a total of 60 isolates were found during the study period. It was found that *Staphylococcus aureus* was most isolated 16.67%, second followed by *Bacillus coagulans* 15%, third followed by *Bacillus subtilis, Enterobacter aerogenes* 13.33%, fouth by *Proteus morganii* and *Proteus vulgaris* 8.33%, fifth by *Micrococcus luteus, Citrobacter freundii* and *Pseudomonas fluorescens* 6.67%. The lowest occurrence was found to be *Ps. aeruginosa* 5%. On the other hand, out of total 60 isolates, isolation of bacteria on chicken hamburger 58.33% was greater than fish sushi 41.67% during the study period. (Table.3

Table1. Isolated bacteria from collected chicken burger

Samples / Bacteria	S_1	S_2	S_3	S_4	S_5	S_6	S_7	S_8	S_9	S_{10}	Total (n) (%)
Bacillus coagulans	1 (20)	nil	nil	nil	1 (20)	nil	nil	1 (20)	1 (20)	1 (20)	5 (14.29)
Bacillus subtilis	nil	nil	1 (25)	nil	nil	nil	1 (25)	1 (25)	nil	1 (25)	4 (11.43)
Staphylococcus aureus	nil	nil	1 (20)	nil	1 (20)	nil	1 (20)	1 (20)	1 (20)	nil	5 (14.29)
Citrobacter freundii	nil	1 (25)	nil	1 (25)	nil	1 (25)	nil	nil	1 (25)	nil	4 (11.43)
Enterobacter aerogenes	1 (12.5)	1 (12.5)	1 (12.5)	1 (12.5)	nil	1 (12.5)	1 (12.5)	1 (12.5)	nil	1 (12.5)	8 (22.86)
Proteus morganii	nil	1 (20)	nil	1 (20)	nil	1 (20)	nil	nil	1 (20)	1 (20)	5 (14.29)
Proteus vulgaris	1 (100)	nil	nil	nil	nil	nil	nil	nil	nil	nil	1 (2.86)
Pseudomonas aeruginosa	nil	nil	nil	nil	1 (33.33)	nil	nil	1 (33.33)	1 (33.33)	nil	3 (8.57)
Total (n) (%)	3 (8.57)	3 (8.57)	3 (8.57)	3 (8.57)	3 (8.57)	3 (8.57)	3 (8.57)	5 (14.29)	5 (14.29)	4 (11.42)	35

(Parenthesis denoted percentage) S = Sample

Table 2.Isolated bacteria from collected fish sushi

Bacteria \ Sample	S_1	S_2	S_3	S_4	S_5	S_6	S_7	S_8	S_9	S_{10}	Total (n) (%)
Micrococcus luteus	nil	nil	1 (25)	nil	1 (25)	1 (25)	nil	nil	nil	1 (25)	4 (16)
Bacillus coagulans	nil	1 (25)	nil	1 (25)	nil	1 (25)	nil	nil	1 (25)	nil	4 (16)
Bacillus subtilis	1 (25)	nil	1 (25)	nil	1 (25)	nil	1 (25)	nil	nil	nil	4 (16)
Staphylococcus aureus	1 (20)	nil	nil	1 (20)	nil	nil	1 (20)	nil	1 (20)	1 (20)	5 (20)
Proteus vulgaris	1 (25)	1 (25)	nil	1 (25)	nil	1 (25)	nil	nil	nil	nil	4 (16)
Pseudomonas fluorescens	nil	1 (25)	nil	nil	1 (25)	1 (25)	nil	1 (25)	nil	nil	4 (16)
Total (n) (%)	3 (12)	3 (12)	2 (8)	3 (12)	3 (12)	4 (16)	2 (8)	1 (4)	2 (8)	2 (8)	25

(Parenthesis denoted percentage) S = Sample

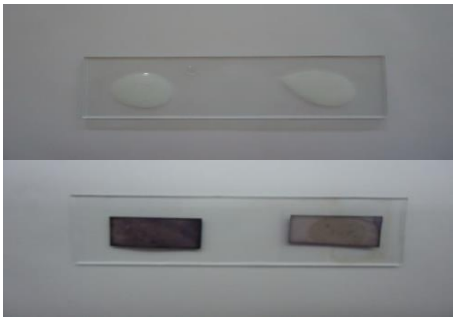
Table 3.Distribution of bacteria isolated on chicken burger and fish sushi

Bacteria	Chicken Hamburger	Fish Sushi	Total (n) (%)
Micrococcus luteus	nil	4 (100)	4 (6.67)
Bacillus coagulans	5 (55.56)	4 (44.44)	9 (15)
Bacillus subtilis	4 (50)	4 (50)	8 (13.33)
Staphylococcus aureus	5 (50)	5 (50)	10 (16.67)
Citrobacter freundii	4 (100)	nil	4 (6.67)
Enterobacter aerogenes	8 (100)	nil	8 (13.33)
Proteus morganii	5 (100)	nil	5 (8.33)
Proteus vulgaris	1 (20)	4 (80)	5 (8.33)
Pseudomonas aeruginosa	3 (100)	nil	3 (5)
Pseudomonas fluorescens	nil	4 (100)	4 (6.67)
Total (n) (%)	35 (58.33)	25 (41.67)	60

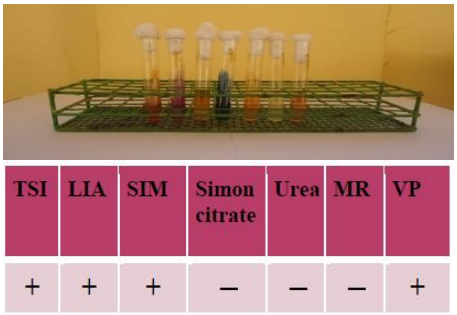
(Parenthesis denoted percentage)

(A) Subculture

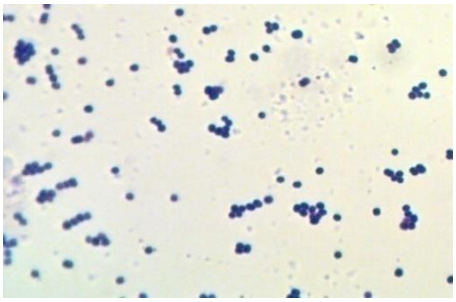
(B) Lactose ferment bacteria colonies



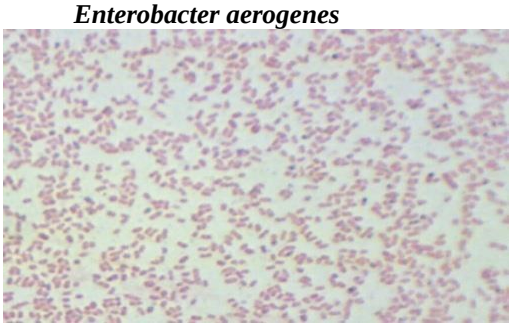
(C) Catalase and Oxidase reactions



(D) Biochemical reactions of *Enterobacter aerogenes*



(E) *Staphylococcus aureus*



(F) *Pseudomonas aeruginosa*

Plate 2. Bacteria colony, biochemical reactions and cell morphology

Discussion

Food is chemically complex matrix, and microorganisms can grow well in any food. Most food contains sufficient nutrients to support microbial growth. Several factors encourage, prevent and limit the growth of microorganisms in foods. The most important factors are water availability, pH and temperature. Food-borne infectious disease is a common, distressing and sometimes life threatening problems for millions of people around the world. Different kinds of many microbes can contain in food. These are ingested daily from a diversity of products with apparently no ill effects. But if they are present as large number or serious serotypes, the consumers can be suffered disease.

In the present study, a total of twenty foods samples were purchased from three quarters of Taunggyi for bacteriological examination. The foods tested were ten samples of chicken burger and fish sushi. Different bacteria from each sample were variable and there are differences among results from the other research papers. These differences may be due to geographical locations and weather conditions.

Some aerobes die in the absence of air. Similarly, some anaerobes die in the presence of air. Some aerobes and anaerobes are facultative. There are two groups of bacteria as aerobic and anaerobic bacteria. The occurrence and distribution of bacteria species may be due to food preparation and food handling.

It is apparent that recorded bacteria types are not reliable in guide to safety, nevertheless, a product showing excessive bacterial contamination may reasonably be assumed to be a potential health hazard in the absence of demonstration of potential pathogens (Silliker, 1963). The food bacteria of greatest importance to human pathology are the most common cause of human infection and extensively widespread in the environment using fast food. The most common infections causing food poisoning and other diseases are those associated with bacteria isolated and contaminations due to fast foods (Kay *et al.*, 1994).

Tamminga *et al*. (1982) stated that *Staphylococcus aureus* was isolated on hamburger. This result is consistent with the present study. This isolate was found in this study. Edward *et al*. (2013) showed that *Staphylococcus aureus, Bacillus spp*, and *Pseudomonas spp* were found in the tests on bacteriological quality of hamburger from fast food restaurant in Umuahia, Nigeria. These findings are consistent with the results from this study that revealed the prevalence of same bacteria species.

Escherichia coli were isolated in 49 samples out of 60 samples giving 81.66 % positive results in the study of Theingi Win Myat, (2005). This finding is not in agreement with the present study in which *Staphylococcus aureus* is the most common organisms isolated from all two food items. This may be due to sample taking from different localities and different shop.

Tamminga *et al*. (1982) emphasized that the adequate cooking time was necessary to grill meat patties for elimination microorganisms. Other studies reported that a correlation between survival rate of bacteria in the meat and cooking time.

The serving utensils used at the vending sites are often contaminated with *Micrococcus sp* which may have originated from the vendors' hands when they touch the food preparation areas, dish, cloth or water during dish washing or hand washing indicates cross

contamination between dishwasher, food preparation surface and itself (Mensah *et al*., 2002).

The degree of personal cleanliness of hands and fingers can affect the bacterial quality of the food. The findings of socioeconomic study of street food vendors were that 51% used their bare hands to touch the food without paying any sufficient attention to cleanliness or washing (Rita, 1996).

The highest percentages of handlers with contaminated hands were similar to that found by the analysis of hand and fingernail cleanliness of snack vendors. Food manufactures play an important role in monitoring the manufacturing process and conduct a periodical surveillance on microbiological quality assessment on the processing plans. Besides, it is required to increase awareness of consumers and food handlers to practice proper cooking of the food before the point of consumption, to reduce the risk of bacterial infection.

This study highlights the importance of contamination in food available in study area. Due to the isolation of pathogenic bacteria strains from foods, risk of infection causing gastroenteritis can be possible because majority of the people consumed fast food. Thus, personal hygiene, environmental sanitation and educational awareness should be streaked to prevent food contamination. Thus, every food must be prepared safely not to be contaminated by any bacteria. Everybody must be aware of the contamination of food. Storage of leftover foods is also one of the important factors allowing the multiplication of organisms to reach the infective dose.

Conclusion

The critical control points to preventing food borne illness are the preventing cross-contamination from the raw products to fast food. Avoiding recontamination after cooking by surfaces previously contaminated with the raw meat, and properly chilling and storing meat after mincing should be emphasized.

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