ejpmr, 2017,4(7), 626-631

SJIF Impact Factor 4.161

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 2394-3211

EJPMR

ISOLATION AND IDENTIFICATION OF MICROORGANISMS FROM JUNK FOODS

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Article Received on 09/05/2017 **Article Revised on 30/05/2017** **Article Accepted on 20/06/2017**

1. ABSTRACT

The junk food is a pejorative term for food containing high levels of calories from sugar or fat with little protein, vitamins or minerals. The main objectives of this project are Isolation and identification of pathogens from junk and fast food and the characterization of the identified pathogen. Various techniques used for the isolation and the identification used for this project are enumeration method, morphological characterization, culturing and the biochemical test. In this study, 9 junk food samples are selected, they are characterized and infectious agents are isolated from them. It is observed that Pani poori and Bhel poori have higher microbial content about 104 and 99 in number. Six different types of organisms were identified from the selected 9 samples. They are *Klebsiella sp, Escherichia coli sp, Salmonella sp, Shigella, sp, Bacillus sp* and *Staphylococcus sp.*

KEYWORDS: Junk food, pathogens and infectious diseases.

1. INTRODUCTION

The junk food is a pejorative term for food containing high levels of calories from sugar or fat with little protein, vitamins or minerals. The term implies that a particular food has little "nutritional value" and contains excessive fat, sugar, salt, and calories. Junk food can also refer to high protein food like meat prepared with saturated fat -which some believe may be unhealthy many hamburger outlets, fried chicken outlets and the like supply food is considered as junk food.

Junk food contains high levels of refined sugar, white flour, trans fat and polyunsaturated fat, salt, and numerous food additives such as monosodium glutamate and tartrazine; at the same time, it is lacking in proteins, vitamins, essential minerals, fiber, among other healthy attributes. These foods have little enzyme producing vitamins and minerals and but contain high level of calories in their place. A food that is high in fat, sodium, and/or sugar and provides high calories yet useless in value is generally known as a junk food. On the contrary, junk food is easy to carry, purchase and consume. Generally, a junk food is given a very attractive appearance by adding food additives and colors to enhance flavor, texture and for increasing long shelf life.

When junk food is consumed very often, the excess fat, carbohydrates, and processed sugar found in junk food contributes to an increased risk of cardiovascular disease, diabetes, weight gain, and many other chronic health conditions. Also consumers tend to eat too much at one sitting and consumers who have satisfied their appetite with junk food are less likely to eat healthy foods like fruit, vegetables or dairy product.

Keeping a diverse community of microbes in our guts is a key way our bodies defend off illnesses like the common cold, diabetes, obesity, and also low energy or mood swings. Some experts say Spector's study is yet another block of evidence showing what you eat is just as important as how much you eat. "Junk food, including most fast food and processed food, is bad for us.

2. OBJECTIVES

- Isolation and identification of pathogens from junk and fast food.
- Characterization of the identified pathogen.

4. MATERIALS AND METHODS

4.1 Sample collection

Junk food and fast food were collected from bakery, hotels and street venders to the laboratory under sterile condition.

4.2 Samples taken

The samples were collected from different areas like Palakkad, Chavadi, Coimbatore and Kanjikode. These are the samples that are collected.

1. Pepsi.
2. Sprite.
3. Alfam chicken.
4. French fries.
5. Imily jelly.
6. Kalan.

7. Pani puri
8. Bale puri.
9. Vegetable nuggets.

4.3 Processing of sample

4.3.1 Serial dilution

For the bacteriological examination of the sample serial dilution technique was performed. The food sample was mixed by using mortar and pestle into a powdery mass. 1gm of the sample was mixed with 9ml of distilled water and was incubated for 24hrs at room temperature with periodic shaking. The suspension was then serially diluted up to 10^{-4} to make tenfold dilution.

4.3.2 Spread plate technique

Spread plate technique was performed by transferring 1ml of the liquid form respective dilution onto sterile plate. These plates were incubated at 37^0c for 24hrs.

4.3.3 Isolation on selective media

Random cultures from the spread plated was inoculated on to selective media such as nutrient agar, *Salmonella - Shigella* agar, Mannitol Salt agar, Eosin Methylene Blue agar, Bismuth sulfite agar Mac Conkey agar medium to study the colony characteristics.

4.3.4 Media used for isolation

Nutrient agar, Salmonella Shigella agar, Eosin Methylene Blue agar, Mannitol salt agar,
Bismuth sulfite agar and Mac Conkey agar.

4.3.5 Morphological and biochemical characterization of the isolates in the sample

Isolated colonies from the plates were characterized by various morphological and biochemical tests.

3. RESULT AND DISCUSSION

5.1 Enumeration of the bacteria

The isolation of microorganism and the number of colonies counted are tabulated in table no 5.1.1.

Table No: 5.1.1

SL No	Sample	Dilution		
		10^{-3}	10^{-4}	10^{-5}
1	Pepsi	101	66	41
2	Sprite	73	54	49
3	Alfam chicken	TNTC	TNTC	85
4	French fries	NG	NG	NG
5	Imily jelly	NG	NG	NG
6	Kalan	TNTC	TNTC	92
7	Pani poori	TNTC	TNTC	104
8	Bel poori	TNTC	TNTC	99
9	Vegetable nuggets	88	59	46

*TNTC- too numerous to count
* NG - No growth.

5.2 Morphological characterization

The selected bacterial strains from the food sample were identified by Gram staining, motility and spore staining and the results are presented in table number 5.2.1 to 5.2.15.

Table No: 5.2.1

	Organism	Morphology		Motility
		Gram staining	Spore staining	
Sample 1 Pepsi	Isolate 1	Gram negative rod	Non spore forming	Non motile
	Isolate 2	Gram negative rod	Non spore forming	motile

Table No.5.2.2

	Organism	Morphology		Motility
		Gram staining	Spore staining	
Sample 2 Sprite	Isolate 3	Gram negative rod	Non spore forming	Non motile
	Isolate 4	Gram negative rod	Non spore forming	motile

Table No.5.2.3

	Organism	Morphology		Motility
		Gram staining	Spore staining	
Sample 3 Alfam chicken	Isolate 5	Gram negative rod	Non spore forming	Motile
	Isolate 6	Gram negative rod	Non spore forming	Motile
	Isolate 7	Gram negative rod	Non spore forming	Non motile

Table No.5.2.4.

Sample 4 French fries	Organism
	No growth

Table No.5.2.5

Sample 5 Imily jelly	Organism
	No growth

Table No.5.2.6

	Organism	Morphology		Motility
		Gram staining	Spore staining	
Sample 6 Kalan	Isolate 8	Gram negative rod	Non spore forming	Motile
	Isolate 9	Gram negative rod	Non spore forming	Motile
	Isolate 10	Gram negative rod	Non spore forming	Non motile

Table No.5.2.7

	Organism	Morphology		motility
		Gram staining	Spore staining	
Sample 7 Pani puri	Isolate 11	Gram negative rod	Non spore forming	Motile

Table No.5.2.8

	Organism	Morphology		Motility
		Gram staining	Spore staining	
Sample 8 Bel puri	Isolate 12	Gram negative rod	Non spore forming	Motile
	Isolate 13	Gram negative rod	Non spore forming	Non motile
	Isolate 14	Gram positive rods	Spore forming	Motile

Table No.5.2.9

	Organism	Morphology		motility
		Gram staining	Spore staining	
Sample 9 Vegetables nuggets	Isolate 15	Gram negative rod	Non spore forming	Motile
	Isolate 16	Gram negative rod	Non spore forming	Non motile

5.3 Cultural characterization

The colony morphology of the isolated organism from the junk foods on different selective media like Mannitol salt agar, MacConkey agar, eosin Methylene blue agar, Salmonella- Shigella agar, Bismuth sulfite agar, Sabouraud dextrose agar are studied and that are present in following table number 5.3.

Table No: 5.3

Sl. No	Organism	Selective media						
		Nutrient agar	EMB	MAC	MSA	SSA	BSA	SDA
1	Isolate 1	Large, white dome shaped mucoid colonies.	good growth of brown, dark-centered, mucoid colonies	Bright pink color colonies.	No growth	No growth.	No growth.	No growth
2	Isolate 2	Large, thick, grayish, white, moist colonies.	Green metallic sheen colonies.	Bright pink colonies.	No growth	Partial to complete inhibition; pink to rose red colonies with precipitate	Partial to complete inhibition; brown-green colonies.	No growth
3	Isolate 3	Large, white dome shaped mucoid colonies.	good growth of brown, dark-centered, mucoid colonies	Bright pink color colonies.	No growth.	No growth.	No growth.	No growth
4	Isolate 4	Large, thick, grayish, white, moist colonies.	Green metallic sheen colonies.	Bright pink colonies.	No growth.	Partial to complete inhibition; pink to rose red colonies with precipitate	Partial to complete inhibition; brown-green colonies.	No growth
5	Isolate 5	Large, thick, grayish, white, moist colonies.	Green metallic sheen colonies.	Bright pink colonies.	No growth.	Partial to complete inhibition; pink to rose red colonies with precipitate	Partial to complete inhibition; brown-green colonies.	No growth

6	Isolate 6	Small, circular, translucent, colorless colonies.	good growth of grey mucoid colonies	Small, circular, translucent, colorless non lactose fermenting colonies.	No growth.	Growth; colorless colonies with or without black centers.	Jet black with metallic sheen colonies.	No growth
7	Isolate 7	Circular, convex, smooth and translucent colonies.	Large, colorless colonies.	Small colorless colonies.	No growth.	Growth; colorless colonies.	No growth.	No growth
8	Isolate 8	Large, thick, grayish, white, moist colonies.	Green metallic sheen colonies.	Bright pink colonies.	No growth.	Partial to complete inhibition; pink to rose red colonies with precipitate	Partial to complete inhibition; brown-green colonies.	No growth
9	Isolate 9	Small, circular, translucent, colorless colonies.	good growth of grey mucoid colonies	Small, circular, translucent, colorless non lactose fermenting colonies.	No growth.	Growth; colorless colonies with or without black centers.	Jet black with metallic sheen colonies.	No growth
10	Isolate 10	Circular, convex, smooth and translucent colonies.	Large, colorless colonies.	Small colorless colonies.	No growth.	Growth; colorless colonies.	No growth.	No growth
11	Isolate 11	Large, thick, grayish, white, moist colonies.	Green metallic sheen colonies.	Bright pink colonies.	No growth.	Partial to complete inhibition; pink to rose red colonies with precipitate	Partial to complete inhibition; brown-green colonies.	No growth
12	Isolate 12	Small, circular, translucent, colorless colonies.	good growth of grey mucoid colonies	Small, circular, translucent, colorless non lactose fermenting colonies.	No growth.	Growth; colorless colonies with or without black centers.	Jet black with metallic sheen colonies.	No growth
13	Isolate 13	Circular, convex, smooth and translucent colonies.	Large, colorless colonies.	Small colorless colonies.	No growth.	Growth; colorless colonies.	No growth.	No growth
14	Isolate 14	Large, white, irregular colonies.	Poor growth.	No growth.	No growth.	No growth.	No growth.	No growth
15	Isolate 15	Small, circular, translucent, colorless colonies.	good growth of grey mucoid colonies	Small, circular, translucent, colorless non lactose fermenting colonies.	No growth.	Growth; colorless colonies with or without black centers.	Jet black with metallic sheen colonies.	No growth

5.4 Biochemical characterization

The microorganisms isolated were subjected to biochemical test to identify the specific microorganism and the result is given in the table number 5.4.1.

Table No: 5.4.1

Sl. No	Organism	Indole	MR	VP	Citrate	TSI	H_2S	Catalase	Coagulase	Gelatin	Urease	starch
1	Isolate 1	-	-	+	+	A/A	-	+	-	-	+	-
2	Isolate 2	+	+	-	-	A/AK	-	-	-	-	-	+
3	Isolate 3	-	-	+	+	A/A	-	+	-	-	+	-
4	Isolate 4	+	+	-	-	A/AK	-	-	-	-	-	+
5	Isolate 5	+	+	-	-	A/AK	-	-	-	-	-	+
6	Isolate 6	-	+	-	+	A/AK	+	+	-	+	-	+
7	Isolate 7	-	+	-	-	A/AK	-	+	-	-	-	-
8	Isolate 8	+	+	-	-	A/AK	-	-	-	-	-	+
9	Isolate 9	-	+	-	+	A/AK	+	+	-	+	-	+
10	Isolate 10	-	+	-	-	A/AK	-	+	-	-	-	-
11	Isolate 11	+	+	-	-	A/AK	-	-	-	-	-	+
12	Isolate 12	-	+	-	+	A/AK	+	+	-	+	-	+
13	Isolate 13	-	+	-	-	A/AK	-	+	-	-	-	-
14	Isolate 14	-	+	-	-	A/AK	-	+	-	+	-	+
15	Isolate 15	-	+	-	+	A/AK	+	+	-	+	-	+

5.5 Isolated organisms are

Table No: 5.5.1

Sl. No	Isolate	organism
1	isolate 1 and 3	*Klebsiella sp*
2	isolate 2, 4, 5, 8 and 11	*E.coli*
3	isolate 6, 9 and 12, 15	*Salmonella sp*
4	isolate 7, 10 and 13	*Shigella sp*
5	Isolate 14	*Bacillus sp*
6	Isolate 17	*Staphylococcus sp*

4. DISCUSSION AND SUMMARY

The main objectives of this project are isolation and identification of pathogens from junk and fast food and the characterization of the identified pathogen. Various techniques used for the isolation and the identification used for this project are enumeration method, morphological characterization, culturing and the biochemical test.

It is observed that Pani poori and Bhel poori have higher microbial content about 104 and 99 in number. Six different types of organisms were identified from the selected 9 samples. They are *Klebsiella, Escherichia coli, Salmonella, Shigella, Bacillus and Staphylococcus.*

Infectious disease along with food can be a source of danger, involving multiple agents, mainly bacterial (*Salmonella, Campylobacter* are verotoxin producing *Escherichia coli, Listeria sp*), but also parasitic (*Toxoplasma gondii, Cyclospora cayetanensis, Trichinella* sp.), and viral (*Norovirus, Hepatitis A virus*), as well as non-conventional communicable agents and mycotoxins.

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