

## Microbiological Quality Analysis Along With The Drug Resistance Pattern Of The Identified Bacteria From Different Types Of Locally Produced Sauces Available In Some Popular Fast Food Shops In Dhaka Metropolis

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**Abstract:** Sauces are very popular nutritive food additives which are especially consumed with fast foods. Contamination can occur by misuse, drastic changes in storage temperatures, hygienic condition of the preparation area, quality of the raw ingredients etc. The nutritive properties present in sauces and the low pH can give enough opportunities to the acid loving microorganisms as well as some fungi to survive and multiply. Current study included four different categories of locally made sauces (five samples for each category) to study the microbial quality and the antibiotic susceptibility of the isolated bacteria as well. Almost all the samples were contaminated (with the exception of mustard sauce where no fungal count was observed) with total bacteria and fungi counting up to  $2.9 \times 10^6$  cfu/gm and  $2.1 \times 10^6$  cfu/gm respectively. Tomato sauces were free from *Salmonella* spp. and *Escherichia coli* meeting the GSO criteria. Tamarind sauces were highly contaminated with the most predominating bacteria *Pseudomonas* spp., *Escherichia coli* and *Klebsiella* spp. Mustard sauces showed the best quality under this study with no presence of *Salmonella* spp., *Staphylococcus* spp. and *Escherichia coli*. Mayonnaise samples were the least quality product. Use of raw contaminated eggs without proper hygiene might be triggered this situation. Most of the isolates found in all of these samples showed to be susceptible for most of the antibiotic drugs used in this study except only a few isolates. Ceftazidime was the antibiotic to which maximum isolates showed resistance compared to other antibiotics.

**Keywords:** Sauce, contamination, microbiological quality, hygiene, antibiotic susceptibility.

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### I. Introduction

Fast food items are getting popularity due to a variety of flavors and categories. And it is beyond imagination to eat fast foods without sauces. Students from schools to universities as well as the working people prefer fast foods with different sauces. As the popularity of sauces is increasing and consumed by the people of all ages, it is necessary to study the qualities of them as well to know about their safety upon consumption. Fresh vegetables like tomato, chili, tamarind, mustard etc. are used to prepare sauces which are very popular food additives while enjoying fast foods. The nutritional values of these vegetables can also be transferred to the sauces. Some of such components include are vitamins, minerals (calcium, phosphorus, sodium), several bioactive compounds (flavanone glycosides, hydroxycinnamic acid, antioxidants), phenolics which help to improve human health status, maintain blood lipid profile in hypercholesterolemia patients, inhibit breast cancer, urinary tract infections, congestive heart failure etc (Tasnim et al., 2010; Tambekar et al., 2009; Hyson, 2011; FDA, 1999; Basar and Rahman, 2007; Durgesh et al., 2008; Ayaji et al., 2006). Higher bacterial count in tomato, chili and higher fungal count in tamarind can result in the contamination of the final produced ketchup. Though the very low pH condition and the presence of benzoic acid, acetic acid, preservatives etc often reduce the contaminating bacterial and fungal count and increase the shelf life of the ketchups, acid tolerant bacteria and some fungi can thrive in the ketchups causing substantial product damage as well as diseases after consumption (Trias et al., 2008; Ogunbanwo et al., 2014). Mayonnaise is another spreads used often in fast food items like burgers, sandwiches, hot dogs etc. which is actually made up with raw eggs, vegetable oil, lime juice or vinegar, seasonings and so on (Jay, 1986). Pathogenic *Salmonella* spp., *Escherichia coli* O157:H7, *Staphylococcus* spp. and *Clostridium* spp. can easily get entrance in mayonnaise from raw egg collected from infected chicken and mishandling by the operator making mayonnaise (Peter and Snyder, 2008). Mustard seeds are often used to produce mustard paste which is also used as spread in several fast food items and mixed with other sauces as well. Mustard paste contains antimicrobial activity against several bacteria like gram negative and acid tolerant bacteria (Liu, 2005; Douglas, 2005). Moreover, ingredients used in preparing sauces, hygienic condition of the machines, surface of processing areas etc. affect the quality of ketchup and if not properly

maintained by transferring pathogenic microorganisms to the finished product. And if this happens, the consumers will suffer from food borne gastrointestinal disorders and even death (Victorian Government Department of Human Services 2005; Oliveira *et al.*, 2006; Nicolas *et al.*, 2007; Tambekar *et al.*, 2009; Durgesh *et al.*, 2008; Tsige Ketema *et al.*, 2008). Pathogenic microbes found in sauces include *Escherichia coli*, *Lactobacillus* spp., *Listeria* spp., *Salmonella* spp., *Shigella* spp., *Vibrio* spp., *Staphylococcus* spp., *Streptococcus* spp. etc. (Barro *et al.*, 2006). The treatment of diseases caused by these microorganisms becomes difficult if they are antibiotic resistant and the resistance property can come due to various reasons like the selective pressure of antibiotics as well as other preservative chemicals which are used in food. In the presence of these components, the microorganisms make a way to resist their mechanism of destroying themselves. On the other hand, genetically modified plants having antibiotic resistance genes can produce crops with these genes and upon consumption such genes can be transferred to other microbes like the probiotics. Misuse of antibiotics is another big reason for drug resistance and in Bangladesh 70% infection causing bacteria have become resistant toward several antibiotic drugs (Tenover, 2006; Canton, 2009; Bennett, 2008; Noor *et al.*, 2013a, 2013b; Jilani *et al.*, 2008; Hung and Kaufman, 2010; Tasnia *et al.*, 2013; Lucky *et al.*, 2016; Malek *et al.*, 2015; Piangka *et al.*, 2016). The current study was designed to determine the presence of pathogenic bacteria found in locally produced sauces as well as the drug resistance pattern of the isolates found these sauces sold in some popular fast food shops in Dhaka city to identify the risk of public health.

## II. Materials and Method

### 2.1 Study area and sample processing

Ketchups are very popular item consumed with the fast foods and are available in all kinds of food shops where people can eat as well as for takeaway service. As people are obsessed with the varieties of sauces, there prevails a competition between branded fast food shops for making sauces with better tastes with more variations. Four different categories of sauces including tomato sauce, tamarind sauce, mayonnaise and mustard sauce which are very popular these days were collected from five different popular fast food shops in Dhaka city for microbiological analysis. The study included total twenty samples (five samples from each categories) prepared locally by themselves. These samples were collected during the time span of September, 2018 to November, 2018. Samples were collected aseptically and transferred to the microbiological laboratory for microbial assay. The samples were homogenized in normal saline and serially diluted up to  $10^{-3}$  dilution (Cappuccino & Sherman, 1996).

### 2.2 Total bacterial and total fungal count

0.1 ml diluted sample from  $10^{-1}$  to  $10^{-4}$  dilution of each dairy samples was spread over nutrient agar and Sabouraud Dextrose Agar (SDA) medium and incubated overnight at  $37^{\circ}\text{C}$  and  $25^{\circ}\text{C}$  respectively and the average CFU was counted.

### 2.3 Determination of *Pseudomonas* spp., *Staphylococcus* spp., *Escherichia coli*., *Klebsiella* spp. and *Proteus* spp.

0.1 ml sample was spread on *Pseudomonas* agar for the determination of *Pseudomonas* spp., MSA (Mannitol Salt Agar) for determination of *Staphylococcus* spp., MaC Conkey agar for identification of *Escherichia coli*., *Klebsiella* spp., and *Proteus* spp. respectively and incubated overnight at  $37^{\circ}\text{C}$ . All the suspected isolates were then confirmed by biochemical tests (Cappuccino and Sherman, 1996).

### 2.4 Detection of *Vibrio* spp., *Salmonella* spp. and *Shigella* spp.

As *Vibrio*, *Salmonella* and *Shigella* remain in the environment as VBNC state, they don't appear readily during microbiological test procedures. Before conducting serial dilutions, we enriched all the dairy samples with alkaline peptone water for *Vibrio* spp. and selenite cystein broth for *Salmonella* and *Shigella* spp. We enriched the samples for six hours at  $37^{\circ}\text{C}$  and then serially diluted up to  $10^{-3}$  with normal saline for microbiological analysis. The samples were spread on the SS agar and TCBS agar for detecting *Salmonella* spp (with black precipitates), *Shigella* (redish or pink color) and *Vibrio* (yellow colored colony) (Cappuccino and Sherman, 1996).

### 2.5 Biochemical identification of the isolates

All of the isolates found in different specific media were subjected to biochemical identification for confirmation of the identification. TSI, oxidase, MR, VP, motility, indole and citrate utilization tests were done and then compared with standard charts to identify the bacteria (Cappuccino and Sherman, 1996).

## 2.6 Antibiotic sensitivity of the isolated pathogens

The pathogenic bacterial isolates found in the sauce samples were subjected to the determination of their sensitivity patterns towards some antibiotic drugs which are commonly used. About 10 antibiotics such as Amikacin (AK 10), Aztreonam (ATM 30), Ceftriaxone (CTR 30), Cefpodoxime (CPD 30), Azithromycin (AZM 30), Cefotaxime (CTX 30), Ciprofloxacin (CIP 30), Cefepime (FEP 30), Meropenem (MEM 10) and Ceftazidime (CAC 30/10) were selected for the antibiotic sensitivity test following the Kirby-Bauer method. At first, Mueller Hinton agar plates were used to make a lawn of bacteria using the suspension prepared from the isolated pathogenic bacteria and then selected antibiotic discs were placed over the lawn culture. Finally, the plates were incubated at 37°C for 24 hours. After incubation, the plates were observed for the presence of the zone of inhibition around the antibiotic discs and the diameters were measured to determine whether it was in sensitive or intermediate category.

## III. Results and Discussion

In Bangladesh, the popularity of fast foods has become tremendously increased especially among the young people. The consumption of fast foods is almost impossible without the mouth watering ketchups which are really very much popular among the young generations. Consumers take a handsome amount of ketchups taking fast foods not only occasionally but for several times in each day. So the quality of the ketchup is very important as its consumption has been drastically increased. Very young children find these ketchups very tasty and often eat without any additional food items. So this age group is in more risk of health associated problems if the quality is not good enough. As the ketchups are made of several fruits and vegetables, the quality of them also determines the quality of the final ketchup. In addition, the preparation process, additives, flavors, preservatives, processing, packaging materials, storage conditions etc also determine the product quality. Food items available from the vendors have been showed that they often contain a good account of pathogenic microbes which can cause public health problems (Yasmin et al., 2015; Ahmed et al., 2014; Marjan et al., 2014; Sultana et al., 2014; Fatema et al., 2013; Noor et al., 2013a, 2013b; Sarker et al., 2013; Uddin et al., 2011). Presence of pathogenic bacteria in the vegetables used for making ketchup can remain in not processed properly and can cause gastrointestinal diseases. Local homemade ketchups which are sold in one time packaging have more chance of getting contamination during the ketchup making procedures. The personnel who are engaged in such local productions don't have the proper knowledge about the sanitation, transmission of microorganisms from their simple actions (Barro et al., 2006; WHO, 2002; Bhaskar et al., 2014). Additionally the equipments used during ketchup preparation are also important factor. Contamination can occur during any stages of these procedures (Afroz et al., 2013;). Current study headed to determine the pathogenic load of microorganisms present in locally prepared ketchups available in five different popular fast food shops.

Five samples of tomato sauces were collected from five different fast food shops showed nearly similar results for total viable bacterial count (Table 1) ranging from  $2.7 \times 10^6$  cfu/gm to  $3.6 \times 10^5$  cfu/gm. Total fungal count was observed only in sample 02 ( $4.3 \times 10^5$  cfu/gm) and sample 03 ( $2.0 \times 10^4$  cfu/gm). *Escherichia coli* and *Salmonella* spp. were totally absent from all of the five samples. *Vibrio* spp. and *Klebsiella* spp. were found to be present only in one samples whereas *Pseudomonas* spp. (sample 01, 02), *Proteus* spp. (Sample 01, 03) and *Shigella* spp. (Sample 01, 05) were present in two samples respectively. The most significant bacteria found in tomato sauces was *Staphylococcus* spp. ranging from  $2.0 \times 10^4$  cfu/gm to  $1.1 \times 10^6$  cfu/gm respectively. Sample 04 was the only sample that was free from the contamination.

Tomato sauces varied in their quality and all the samples contained different ranges of bacteria. Sample 4 was the only sample having so pathogenic bacteria proving the good quality of raw materials, proper handling, storage condition and maintenance condition. The second product according to the good quality was sample 05 which showed the presence of *Staphylococcus* spp. and *Shigella* spp. *Staphylococcus* spp. can easily gain access into the sauce during manual preparation from the hands. But the presence of *Shigella* spp. confirms the unhygienic condition during processing which should be reduced. Most contaminating tomato sauce was sample 01 which showed the growth of several kinds of pathogens including *Vibrio* spp. and is not safe for consumption at all. According to the recommendation by Standardization organization for G.C.C. (GSO). GSO/FDS 1016/2014, all samples are free from *Salmonella* spp. and *Escherichia coli* but the limit exceeds for TVBC, TFC and *Staphylococcus* spp.

During the study, five tamarind ketchups were subjected for microbiological analysis (Table 2) and it has been found out that the quality of these sauces were not much good and not safe for human consumption. All samples were highly contaminated and the total viable bacteria were almost same for all of them. Highest fungal count was present in sample 03 ( $1.0 \times 10^6$  cfu/gm) and the lowest fungal count was present in sample 02 (no growth). Presence of *Escherichia coli* and *Klebsiella* spp. in high numbers indicates the unhygienic condition during making ketchups and the contamination from fecal oral route. *Salmonella* spp. and *Proteus* spp. were present in sample 03 ( $1.7 \times 10^5$  cfu/gm) and 02 ( $3.6 \times 10^5$  cfu/gm) respectively. *Pseudomonas* spp. was

the most predominant contaminant in all of the five samples. Other pathogenic bacteria present in the sauces include *Staphylococcus* spp., *Vibrio* spp. and *Shigella* spp.

It was quite shocking to reveal that tamarind sauces are not safe to eat with fast foods. As it needs to be cleaned thoroughly and deseeding manually hands unlike other sauces, the contamination sources are many as well as easy to get contaminated. Proper hygiene should be maintained and only purified water must be used during washing and preparing the thick tamarind puree.

Mustard sauces collected from different local shops were free from any fungal contamination (Table 3) as well as contamination by *Salmonella* spp., *Staphylococcus* spp. and *Escherichia coli*. *Shigella* spp. was present only in one sample (sample 03:  $1.2 \times 10^6$  cfu/gm), whereas *Klebsiella* spp. (sample 01, 05) and *Proteus* spp. (sample 02, 03) were present in two samples. *Pseudomonas* spp. was the most predominating pathogen like other sauces too. Surprisingly, it was observed that *Vibrio* spp. was present in three samples ranging from  $4.0 \times 10^4$  cfu/gm in sample 02 to  $2.0 \times 10^6$  cfu/gm in sample 05.

Mustard sauces were the least contaminated sauces than the other sauces studied in this research. Dried mustards are used as raw materials lot of oil, seasonings and small amount of water. Dry ingredients using might the major reason for lesser growth of microbes compared to other sauces where fresh fruits were used to make puree. In comparison with other mustard sauces, sample 01 was the least contaminating sauce and other samples for this sauce can be consumed by putting a little more concentration on hygiene during preparation.

Mayonnaise is also another popular spread or paste which makes the taste of any kind of fast food better. This can also be used in combination with other sauces like mustard or tomato sauces as well. In current study, we observed that the overall quality of mayonnaise is not satisfactory at all (Table 4). There prevailed huge contamination by *Escherichia coli* (in two samples ranging from  $1.4 \times 10^5$  cfu/gm to  $1.8 \times 10^6$  cfu/gm), *Pseudomonas* spp. (only in sample no. 01), *Vibrio* spp. (in sample 01), *Klebsiella* spp. (in 3 samples with maximum count in sample 04:  $5.0 \times 10^5$  cfu/gm and minimum count in sample 01:  $3.0 \times 10^4$  cfu/gm). *Proteus* spp. and total fungal growth was found to be present in all of the five samples. *Staphylococcus* spp and *Shigella* spp. were second most predominant contaminant after *Proteus* spp. and fungal contamination ((Lateef et al, 2006). All of the isolates were confirmed after biochemical identification by conducting several biochemical tests like TSI, MR, VP, citrate test, motility test, oxidase and indole test (Table 5).

Finally mayonnaise samples were subjected to microbial analysis and huge load of microbes were found to be present. As mayonnaise is produced using raw eggs, pathogens can easily get access in the final product if proper hygiene is not strictly maintained. The bacteria from the infected chicken, eggs from unhygienic storage condition and also the unhygienic handling of the raw materials are the main reason for such bad quality of the mayonnaise.

The isolates found in different sauce samples were subjected to antibiotic susceptibility test by Kirby-Bauer method. All *Vibrio* spp. and *Pseudomonas* spp. were found to be susceptible for all of the antibiotics used in this study (Table 6). Five isolates of *Escherichia coli* and nine isolates of *Shigella* spp. were susceptible for all antibiotics except ceftazidime and amikacin respectively. Surprisingly *Klebsiella* spp. showed to be 70% and 80% resistant for Aztreonam and Ceftazidime respectively. The VBNC bacteria *Salmonella* spp. was resistant against meropenem (100%) and amikacin (30%). *Staphylococcus* spp. was 100% resistant to both aztreonam and ceftazidime. Ceftazidime antibiotic was not active for three pathogenic isolates among eight isolates. Meropenem and aztreonam was inactive only against *Salmonella* spp. and *Klebsiella* spp. accordingly.

As the result shows (Table 6) for commonly used antibiotics, most of the isolates are completely susceptible towards most of the drugs prescribed in our country Bangladesh. Meropenem and aztreonam showed inactivity for only a few cases. Ceftazidime was the drug against which several isolates showed resistance. But the overall result indicates the susceptibility of the isolates found during the study from the sauce samples. It is satisfactory that there prevail several drugs of choice to defeat these bacteria if people get infected or suffer from disease after consuming these bacteria with sauces. But still there are few resistant bacteria which pose a threat to transfer the drug resistance genes to the other susceptible bacteria encouraging the risk for more resistance in the future. So strict maintenance and hygiene should be followed during these non-commercial ready to eat sauces.

As ketchups are very popular, adequate knowledge should be provided to the common people raising the awareness about the health hazards of the chemicals present in the ketchup and the food laws should be enforced strictly on the preparation procedures. This must be helpful in decreasing food borne illness among the general people.

IV. Tables

Table 1: Microbiological analysis of tomato ketchup (cfu/gm).

Sample no.	Total Viable Bacterial Count (TVBC)	Total Fungal Count (TFC)	<i>Escherichia coli</i>	<i>Klebsiella</i> spp.	<i>Pseudomonas</i> spp.	<i>Proteus</i> spp.	<i>Salmonella</i> spp.	<i>Shigella</i> spp.	<i>Staphylococcus</i> spp.	<i>Vibrio</i> spp.
01	2.7×10 <sup>6</sup>	0	0	0	3.7×10 <sup>5</sup>	2.4×10 <sup>5</sup>	0	2.4×10 <sup>6</sup>	0	5.5×10 <sup>5</sup>
02	2.1×10 <sup>6</sup>	4.3×10 <sup>5</sup>	0	2.3×10 <sup>6</sup>	3.0×10 <sup>4</sup>	0	0	0	2.0×10 <sup>4</sup>	0
03	2.0×10 <sup>6</sup>	2.0×10 <sup>4</sup>	0	0	0	7.8×10 <sup>5</sup>	0	0	1.0×10 <sup>5</sup>	0
04	3.6×10 <sup>5</sup>	0	0	0	0	0	0	0	0	0
05	7.0×10 <sup>5</sup>	0	0	0	0	0	0	1.0×10 <sup>4</sup>	1.1×10 <sup>6</sup>	0

\*\*Standard limit for sauces: Total viable bacteria= 10<sup>4</sup>, Yeast and mold= 10<sup>2</sup>, *E. coli*= 10, *Salmonella* spp.= nil, *Staphylococcus aureus*= 10<sup>2</sup>[Standardization organization for G.C.C. (GSO). GSO/FDS 1016/2014]

Table 2: Microbiological analysis of tamarind ketchup (cfu/gm).

Sample no.	Total Viable Bacterial Count (TVBC)	Total Fungal Count (TFC)	<i>Escherichia coli</i>	<i>Klebsiella</i> spp.	<i>Pseudomonas</i> spp.	<i>Proteus</i> spp.	<i>Salmonella</i> spp.	<i>Shigella</i> spp.	<i>Staphylococcus</i> spp.	<i>Vibrio</i> spp.
01	2.9×10 <sup>6</sup>	5.0×10 <sup>5</sup>	0	2.0×10 <sup>6</sup>	1.4×10 <sup>6</sup>	0	0	0	1.7×10 <sup>6</sup>	0
02	1.6×10 <sup>6</sup>	0	1.0×10 <sup>6</sup>	6.0×10 <sup>5</sup>	8.0×10 <sup>5</sup>	3.6×10 <sup>5</sup>	0	0	0	0
03	2.5×10 <sup>6</sup>	1.0×10 <sup>6</sup>	0	1.6×10 <sup>6</sup>	1.0×10 <sup>6</sup>	0	1.7×10 <sup>5</sup>	0	1.4×10 <sup>6</sup>	1.1×10 <sup>6</sup>
04	2.4×10 <sup>6</sup>	9.0×10 <sup>5</sup>	1.7×10 <sup>6</sup>	0	7.5×10 <sup>5</sup>	0	0	1.0×10 <sup>5</sup>	0	0
05	2.9×10 <sup>6</sup>	7.0×10 <sup>5</sup>	1.8×10 <sup>6</sup>	0	1.7×10 <sup>6</sup>	0	0	1.5×10 <sup>5</sup>	0	6.5×10 <sup>5</sup>

\*\*Standard limit for sauces: Total viable bacteria= 10<sup>4</sup>, Yeast and mold= 10<sup>2</sup>, *E. coli*= 10, *Salmonella* spp.= nil, *Staphylococcus aureus*= 10<sup>2</sup> [Standardization organization for G.C.C. (GSO). GSO/FDS 1016/2014]

**Table 3:** Microbiological analysis of mustard sauce (cfu/gm).

Sample no.	Total Viable Bacterial Count (TVBC)	Total Fungal Count (TFC)	<i>Escherichia coli</i>	<i>Klebsiella</i> spp.	<i>Pseudomonas</i> spp.	<i>Proteus</i> spp.	<i>Salmonella</i> spp.	<i>Shigella</i> spp.	<i>Staphylococcus</i> spp.	<i>Vibrio</i> spp.
01	2.9×10 <sup>6</sup>	0	0	1.7×10 <sup>6</sup>	0	0	0	0	0	0
02	2.8×10 <sup>6</sup>	0	0	0	1.7×10 <sup>5</sup>	7.6×10 <sup>5</sup>	0	0	0	4.0×10 <sup>4</sup>
03	2.7×10 <sup>6</sup>	0	0	0	1.2×10 <sup>6</sup>	1.5×10 <sup>6</sup>	0	1.2×10 <sup>6</sup>	0	0
04	2.4×10 <sup>6</sup>	0	0	0	2.2×10 <sup>6</sup>	0	0	0	0	8.0×10 <sup>4</sup>
05	2.3×10 <sup>6</sup>	0	0	7.8×10 <sup>5</sup>	2.1×10 <sup>6</sup>	0	0	0	0	2.0×10 <sup>6</sup>

\*\*Standard limit for sauces: Total viable bacteria= 10<sup>4</sup>, Yeast and mold= 10<sup>2</sup>, *E. coli*= 10, *Salmonella* spp.= nil, *Staphylococcus aureus*= 10<sup>2</sup> [Standardization organization for G.C.C. (GSO). GSO/FDS 1016/2014]

**Table 4:** Microbiological analysis of mayonnaise (cfu/gm).

Sample no.	Total Viable Bacterial Count (TVBC)	Total Fungal Count (TFC)	<i>Escherichia coli</i>	<i>Klebsiella</i> spp.	<i>Pseudomonas</i> spp.	<i>Proteus</i> spp.	<i>Salmonella</i> spp.	<i>Shigella</i> spp.	<i>Staphylococcus</i> spp.	<i>Vibrio</i> spp.
01	5.2×10 <sup>5</sup>	1.0×10 <sup>6</sup>	0	3.0×10 <sup>4</sup>	6.5×10 <sup>5</sup>	8.2×10 <sup>5</sup>	0	1.2×10 <sup>6</sup>	3.0×10 <sup>4</sup>	7.0×10 <sup>4</sup>
02	2.9×10 <sup>6</sup>	2.1×10 <sup>6</sup>	1.4×10 <sup>5</sup>	0	0	1.2×10 <sup>6</sup>	0	1.0×10 <sup>5</sup>	1.7×10 <sup>5</sup>	0
03	2.4×10 <sup>6</sup>	3.0×10 <sup>4</sup>	1.8×10 <sup>6</sup>	0	0	1.4×10 <sup>6</sup>	0	0	1.0×10 <sup>4</sup>	0
04	2.3×10 <sup>6</sup>	1.4×10 <sup>6</sup>	0	5.0×10 <sup>5</sup>	0	1.0×10 <sup>6</sup>	0	5.0×10 <sup>5</sup>	1.1×10 <sup>6</sup>	0
05	2.9×10 <sup>6</sup>	2.0×10 <sup>4</sup>	0	5.0×10 <sup>4</sup>	0	2.0×10 <sup>5</sup>	0	2.0×10 <sup>5</sup>	0	0

\*\*Standard limit for sauces: Total viable bacteria= 10<sup>4</sup>, Yeast and mold= 10<sup>2</sup>, *E. coli*= 10, *Salmonella* spp.= nil, *Staphylococcus aureus*= 10<sup>2</sup> [Standardization organization for G.C.C. (GSO). GSO/FDS 1016/2014]

**Tables 5:** Confirmative biochemical tests for the isolates.

Assumed Organism	TSI									
	slant	Butt	Gas	H <sub>2</sub> S reaction	Indole test	MR test	VP test	Citrate test	Motility	Oxidase Test
<i>Escherichia coli</i>	Y	Y	+	-	+	+	-	-	+	-
<i>Staphylococcus</i> spp.	Y	R	+	+	-	+	-	+	+	-
<i>Pseudomonas</i> spp.	R	R	-	-	-	-	-	+	+	+
<i>Klebsiella</i> spp.	Y	Y	+	-	-	-	+	+	-	-
<i>Shigella</i> spp.	R	Y	+	+	+/-	+	-	-	-	-
<i>Salmonella</i> spp.	R	Y	-	+	-	+	-	-	+	-
<i>Proteus</i> spp.	Y	Y	+	+	-	-	+	+	+	-
<i>Vibrio</i> spp.	Y	Y	-	-	+	+	-	+	+	+

TSI Triple Sugar Iron Test  
 Y Yellow (Acid)  
 R Red (Alkaline)  
 MR Methyl red  
 VP Voges-Proskauer

**Tables 6:** Antibiotic susceptibility test of the isolates.

Organisms	Antibiotics	Amikacin	Aztreonam	Ceftriaxone	Cefpodoxime	Azithromycin	Ceftazidime	Cefotaxime	Ciprofloxacin	Cefepime	Meropenem
<i>Escherichia coli</i> (n=5)	R	0%	0%	0%	0%	0%	100%	0%	0%	0%	0%
	S	100%	100%	100%	100%	100%	0%	100%	100%	100%	100%
<i>Klebsiella</i> spp. (n=9)	R	0%	30%	0%	0%	0%	80%	0%	0%	0%	0%
	S	100%	70%	100%	100%	100%	20%	100%	100%	100%	100%
<i>Vibrio</i> spp. (n=7)	R	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%
	S	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%
<i>Pseudomonas</i> spp. (n=12)	R	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%
	S	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%
<i>Salmonella</i> spp. (n=1)	R	30%	0%	0%	0%	0%	0%	0%	0%	0%	100%
	S	70%	100%	100%	100%	100%	100%	100%	100%	100%	0%
<i>Proteus</i> spp. (n=10)	R	0%	0%	0%	100%	0%	0%	0%	0%	0%	100%
	S	100%	100%	100%	0%	100%	100%	100%	100%	100%	0%
<i>Shigella</i> spp. (n=9)	R	30%	0%	0%	0%	0%	0%	0%	0%	0%	0%
	S	70%	100%	100%	100%	100%	100%	100%	100%	100%	100%
<i>Staphylococcus</i> spp. (n=9)	R	0%	100%	0%	0%	0%	100%	0%	0%	0%	0%
	S	100%	0%	100%	100%	100%	0%	100%	100%	100%	100%

## V. Conclusion

Ketchups in their different flavors are very popular among young generation. But this nutritious substance can also be harmful for the health if harbors adequate amount of pathogenic bacteria. Pathogenic bacteria can be introduced from the raw fruits, ingredients, equipments and multiply rapidly using the nutrition of the ketchup which can be corrected by making the pH very low. Overdose of acids and preservatives can cause health problems also. Food law enforcement should be strict enough to control the condition specially in case of street vending.

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