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Research Article

Population-Based Study of Environmental Food Pathogens and their Antibiotic Resistance Pattern

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Abstract: Foodborne diseases are the current public health issue. These diseases occur due to the microbial contamination of water, mishandling, and improper cooking of foods. Nowadays, eating junk foods, street, smoked, contaminated homemade foods, and the foods from average class or below average class hotel are the significant sources of various stomach disorders. This study was aimed to determine the consequences of unhygienic, overeating of different foods by different communities of Hyderabad. A detailed survey, interviews, and microbial analysis by culturing, microscopy, and biochemical identification and antibiotic sensitivity were performed. The findings revealed the population of 4-22 (41%) 23-35 (26%), 36-50 years (20%) of both genders are habitual of taking junk foods, street foods, smoked foods whereas the age group of 51-60 (13%) eat homemade foods compared to other foods. The health issues analysis showed that 17%, 4%; 6%, 4%; 8%, 18%; 8%, 5.5% of males and females of 4-22; 23-35; 36-50; 51-60 years had suffered from various stomach problems and few were undergone surgical operation. The hygienic status revealed that food handlers and cook wash hands before handling and cooking (27%), wash food items before cooking (15%), wash utensils with detergent and hot water (11%), use oven-dried crockery after washing to serve customers (11%) compared to others with less percentage of hygienic measures. Different food samples showed *Staphylococcus aureus* 30, 35, 20% in burgers/bun kabab, pizza, roasted meat, and commercial milk, *Salmonella enterica* 35% in ojhri / tripe, *Campylobacter jejuni* 20% in burgers/bun kabab, ojhri, *Escherichia coli* 35, 30 and 25% in ojhri / tripe, hotel foods, daleem, Streptococcus lactis 45% and *Pseudomonas aeruginosa* 15%, 5% in golgapa, samosa, kachori, ojhri / tripe Bacillus cereus 20% in hotel foods, *L. monocytogenes* 20% in commercial milk and Proteus mirabilis 10% in golgapa, samosa, kachori respectively. The antibiotic sensitivity pattern revealed the greater resistance 38%, 22%, 28.5%, 35%, 25%, 37.5%, 36%, and 25% of *Staphlococcus aureus*, *S. lactis*, *L. monocytogenes*, *B. cereus*, *C. jejuni,* S. *enterica*, *E.coli*, *P. aeruginosa* whereas *Shigella dysenteriae* and *P. mirabilis* showed no resistance against test antibiotics.

Keywords: Population-based study, Foodborne bacteria, Consumers' food, Antibiotic resistance.

1. INTRODUCTION

Food is known to be a consumable item in any form either liquid, solid, or partially solid that regulates the body functions and metabolism for growth provide energy for survival, and protects the body from any kind of physical disability. Mainly, human edibles are not complete and do not contain the suggested content of vitamins, iron, minerals [1-2]. All humans including males and females of all age groups have different types of eating foods such as junk foods, smoked foods, oily foods, homemade and hotel-made normal foods with or without knowing the quantity, quality, caloric values, and freshness of foods.

The eating practice among the children and young persons is mainly depending upon the environment and the use of fast food is a newly emerged craze among youths [3-4]. The less expensive, quick serving foods with variable taste, attractive brands, and different selling approaches are publically famous, particularly in children and adolescents. The increased intake of unhealthy foods by adolescence may create physical, mental, and communicative changes [3, 5-6]. Globally, food

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safety measures are considered at a peak level. WHO reported 1.5 billion cases of diarrhea in children per annum that resulted in 30,00,000 deaths in under developing countries and the developed nations. The Centre for Disease Control and Prevention reported more than 70 different human complications that occurred by intake of food resulted in 3.25 million population is hospitalized. Mainly the prevalence of food-related infections is provoked by unhygienic food management practices [7-10].

Nowadays, a trend of using foods during short visits anywhere and picnics. The children and youths usually avoid homemade foods. The poor nutrition of any age factor has great effects on human health including weakened learning ability, unstable concentration, obesity, depression, difficulty in breathing, insulin resistance, reduction in conversion of glucose into energy, constipation, cancer, and early death. A good nutritious diet is a basic need of every child for their growth and development and the same also supports microbial growth. Consumable foods may be contaminated by the environmental microorganisms that include the Gram-positive and Gram-negative bacterial flora of the family of Bac*illaciae, Enterobacteriaceae, Pseudomonadaceae, Micrococaceae, Staphylococaceae Campylobacteraceae, and Listeriaceaes* [11-17].

A lot of work has been done on human health issues. This demographic study of different consumers was undertaken that include the comparative study of the frequency of the bacterial contamination of foods, which are frequently consumed by the local population of different age groups in Hyderabad city. The variety of foods under this study were junk, smoked, street, hotel, and homemade foods that include chips, burger, pizza, chats, samosa, kachori, bread roll, French fries, bun kabab and daleem, ojhari/tripe, fried meat (Karahi), roasted meat, curry and other meat items, milk products, and vegetables respectively. The essence of this study is to expose the age-wise, gender-wise eating habits, commercial hygienic status, most frequent bacteria in food samples their percentage, and the antibiotic resistance.

2. MATERIALS AND METHODS

2.1 General survey of food consumers and their eating habits

A general survey of 550 males and females of 4-60 years was undertaken. Food consumers of all age groups were examined for their eating habits once a week, twice a week, once a month, and twice a month of junk, smoked, and street food.

2.2 Collection of data on health issues from different consumers and hospitals

Detailed data were collected from all age groups of male and females consumers of different foods.

2.3 Collection of food samples

A random sampling of 20 samples (Total 200 samples) of Burgers/ Bun Kabab, Daleem, Roasted meat, Chana-chat, Golgapa, Samosa, Kachori, Chips / French fries, Pizza, Ojhri / tripe, fried meat (Karahi), and commercial milk was collected in sterile containers and transported to the laboratory for microbial investigations.

2.4 Data collected hospital ward of various hospitals of Hyderabad

Data of different pathogens from the male and female patients 4-60 years of food poisoning and other stomach disorders were collected from the medico-legal and general wards of various hospitals with the consent of hospital administration.

2.5 Determination of bacterial isolates from food samples

Quantitative analysis was performed by immersing aseptically a small quantity of each food sample in 100 mL of flasks containing sterile nutrient broth and allowed for incubation at 37°C for 4 hours (h) for activation of microbial growth. A little volume (0.5-1 mL) of each food sample was transferred on nutrient agar, MacConkey's agar plates (Merck), and Pylori agar (Sigma) and spread by sterile glass spreader near the Bunsen burner. Incubation of test sample plates was done at 37°C overnight. The next day the colonies were observed and pure culture study was undertaken by streaking each diverse colony over the surface of nutrient agar and MacConkey's agar plates for differentiation of G^{+ve} and G^{-ve} bacteria and finally identified by cultural and morphological characters by standard methods and biochemical characterization by API strips (API CAMPY, API Listeria, API 50 CHB, API 20 E and API Staph).

2.6 Determination of drug resistance of the clinical isolates

All Gram-positive and Gram-negative isolates were examined by Kirby-Bauer disc diffusion method for the antibiotic resistance on different commercial antibiotic discs with varying disc contents (µg) (Oxoid-UK) such as Ampicillin (10), Amoxicillin (25), Azithromycin (15), Clindamycin (2), Chloramphenicol (30), Ciprofloxacin (5), Erythromycin (15), Imipenem (10), Linezolid (30), Levofloxacin (5), Methicillin (5), Oxacillin (5), Ofloxacin (5), Tetracycline (30), Vancomycin (30) and Penicillin (10 U).

3. RESULTS

The population of various areas of Hyderabad city; 550 males and females of poor, average, and above-average class of different age groups were interviewed for their choice of food and eating habits. The findings revealed that apart from the homemade foods, 225 (41%) local population of 4-22 years age of Hyderabad used to eat junk foods including chips, grain (Chana) chat, French fries, burgers mainly zinger burgers, golgappa, samosa along with soft drinks; 142 (26%) of 23-35 years were found habitual of eating mainly burgers, roasted meat, pizza with soft drinks, 110 (20%) of 36-50 years were found habitual of mainly homemade foods compared to junk and hotel foods with or without soft drinks whereas 73 persons (13%) persons of 51-60 years of poor, labors, shopkeepers were found to have their meals from hotels, street vendors, home foods and beggars of both genders were also having beggared foods (Remained foods) from hotels and homes respectively.

Food consumers of all tested age groups were examined for their eating habits once a week, twice a week, once a month, and twice a month of junk, smoked, and street food. The findings 225 persons of 4-22, 142 of 23-35, 110 of 37-50 and 73 of 51-60 years revealed 66 (29%), 93 (41%), 24 (11%) and 42 (19%) of 4-22 years (225 ; 28 (20%), 64 (45%), 20 (14%) and 30 (21%) of 25-36 years; 14 (13%), 23 (23%), 28 (25%) and 42 (38%) of 37-50 years whereas 11 (15%), 24 (33%), 13 (18%) and 25 (34%) of 51-60 years of age of both genders respectively (Table 1).

The survey of health issues revealed that out of 550 persons of both genders, 342 (62%) persons of elite, average, below average and poor class communities of both genders had suffered from several complications that include abdominal cramps, stomach pain with different intervals, vomiting, diarrhea, lose motions with long term weakness, stomach ulcers and were hospitalized for 2-5 days for their diagnosis, treatment and surgical operation The data revealed that 17 (5%) males, 13 (4%) females of 4-22 years had suffered from stomach pain, abdominal cramps, constipation and appendicitis, 21 (6%) males 17 (4%) females of 23-35 years suffered from stomach pain, vomiting diarrhea, constipation and ulcer, 27 (8%) males 18 (5%) of females of 36-50 years showed symptoms like abdominal cramps, constipation, few showed vomiting, diarrhea and ulcer whereas 28 (8%) males, 19 (5.5%) females of 51-60 years showed stomach pain with intervals, vomiting, diarrhea, loose motions and stomach ulcers (Fig.1). The data was collected from 422 food vendors for the maintenance of hygienic conditions including washing hands, utensils, food items, crockery, wearing gloves, head covering, apron, etc. (Table 2).

The food microflora was isolated and identified by API strips that revealed *C. jejuni*, *S. entartica*; *S. aureus, Shigella dysenteriae, E.coli, Bacillus cereus*, *Listeria monocytogenes, Proteus mirabilis, Pseudomonas aeruginosa* as major Gram-positive and Gram-negative foodborne pathogens all test foods including in Burgers / Bun Kabab *C. jejuni* (20%), *S. aureus* (30%), *S. entartica* (10%), *S. dysentriae* (5%), *E.coli* (20%), *B. cereus* (10%); Pizza, Roasted meat *C. jejuni* (15%), *S. aureus* (35%), *S. entartica* (15%), *L.* monocytogenes (10%), *E.coli* (10%); Ojhri / Tripe *C. jejuni* (20%), *S. entartica* (35%), *L. monocytogenes* (20%), *E.coli* (35%), *P. aeruginosa* (5%); Channa, Frech fries, Chips *S. aureus* (20%), *S. entartica* (25%), *L. monocytogenes* (10%), *E.coli* (20%), *B. cereus* (10%); Golgapa, Samosa, Kachori *Streptococcus lactis* (45%), *E.coli* (10%), *Proteus mirabilis* (10%), *P. aeruginosa* (15%); Daleem *B. cereus* (15%), *S. dysentriae* (5%), *S. entartica* (15%), *L. monocytogenes* (10%), *E.coli* (25%); Hotel foods *B. cereus* (20%), *S. dysentriae* (5%), *S. entartica* (15%), *Listeria monocytogenes* (5%), *E.coli* (30%); Commercial milk *C. jejuni* (5%), *S. aureus* (20%), *S. entartica* (15%), *L. monocytogenes*

Table 1. Determination of eating habits of different food consumers of different age groups

Age factor	Total numbers of persons interviewed	Eating Habits							
		Once a week		Twice a week		Once in a month		Twice a month	
		No. of persons	%	No. of persons	%	No. of persons	%	No. of persons	%
4-22	225	66	29	93	41	24	11	42	19
23-35	142	28	20	64	45	20	14	30	21
36-50	110	14	13	25	23	28	25	42	38
51-60	73	11	15	24	33	13	18	25	34

(20%), *E.coli* (15%) (Table 3, Fig. 2).

Various numbers of strains of foodborne isolates showed resistance zone (mm) against Azithromycin 12, Clindamycin 12.5, Ciprofloxacin 15 Erythromycin 14, Methicillin 09 (*Staphylococcus aureus*); Chloramphenicol 13.4, Ofloxacin 10, Oxacillin 13 (*Streptococcus lactis*); Ampicillin 11, Chloramphenicol 13, Ofloxacin 11, Oxacillin 10.5, Tetracycline 14 (*Bacillus cereus*); Ampicillin 12.5, Chloramphenicol 12, Cephalosporin 16, Oxacillin 11 (*Listeria monocytogenes*); Ampicillin 14, Clindamycin, 11.4 Erythromycin 10.5, Imipenim 11, Oxacillin 11.5, Tetracycline 10 (*Campylobacter jejuni*); Chloramphenicol 16, Cephalosporin 13, Imepenim, 13.5, Levofloxacin, 15, Ofloxacin 14 (*Salmonella enterica*); Chloramphenicol 12, Cephalosporin 15, Imipenim 13, Linezolid 10.5, Oxacillin 13 (*E.coli*) and Ampicillin 11.5, Amoxicillin 13, Imipenim 13, Levofloxacin 12, Ofloxacin 11.5 mm zone of growth inhibition of *Pseudomonas aeruginosa*. The observation further revealed the percentage of antibiotic-resistant strains of *S. aureus* (38%), *Streptococcus lactis* (22%),

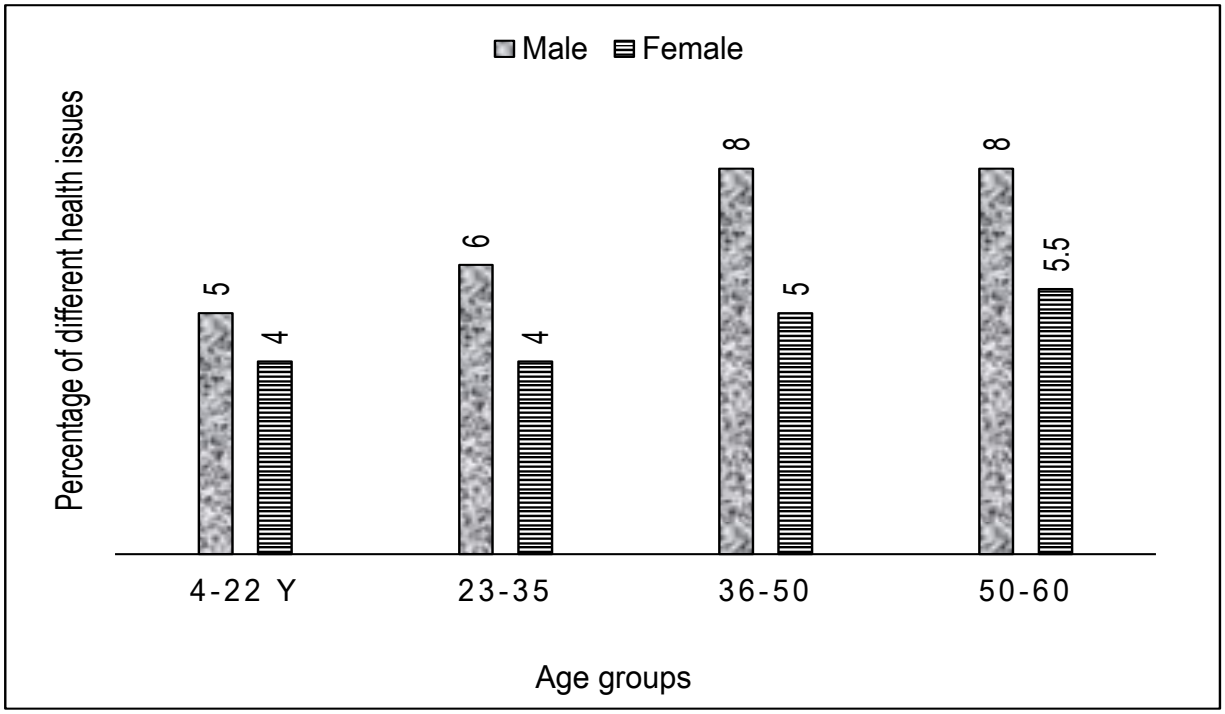

Fig 1. Data of vomit, stool, urine, and food materials tested from local hospitals and different consumers

Table 2. Data of 422 food vendor of standard hygiene

S. No	Hygienic standards	No. of the food vendors interviewed	Percentage (%)
1	Wash hands before handling foods and cooking.	114	27
2	Wash utensils with detergent and hot water	45	11
3	Wash utensils with tap water only	22	5
4	Washing food items (meat and vegetables) before cooking	63	15
5	Using new/ fresh tissue papers for drying hands	21	5
6	Using and re-using normal cloth for drying hands before cooking.	42	10
7	Using hot air oven for drying of crockery after washing to serv customers.	48	11
8	Using head covering only during cooking	47	11
9	Using Gloves, head covering, and apron during cooking	20	5

Table 3. Determination of total numbers and percentage of bacterial isolates from food samples

Food samples	Bacterial isolates	No. of isolates	Percentage of isolates
Burgers / Bun Kabab (I)	*Campylobacter jejuni*	4	20
	Staphylococcus aureus	8	30
	Salmonella enterica	2	10
	Shigella dysenteriae	1	05
	E.coli	4	20
	Bacillus cereus	2	10
Pizza, Roasted meat (II)	*Campylobacter jejuni*	3	15
	Staphylococcus aureus	7	35
	Salmonella enterica	3	15
	Listeria monocytogenes	2	10
	E.coli	2	10
Ojhri / tripe (III)	*Campylobacter jejuni*	4	20
	Salmonella enterica	5	35
	Listeria monocytogenes	4	20
	E.coli	7	35
	Pseudomonas aeruginosa	1	5
Chana / grains, French fries, Chips (IV)	*Staphylococcus aureus*	4	20
	Salmonella enterica	5	25
	Listeria monocytogenes	2	10
	E.coli	4	20
	Bacillus cereus	2	10
Golgapa, Samosa and Kachori (V)	*Streptococcus lactis*	9	45
	E. coli	2	10
	Proteus mirabilis	2	10
	Pseudomonas aeruginosa	3	15
	B. cereus	3	15
Daleem (VI)	*Shigella dysenteriae*	1	5
	Salmonella enterica	3	15
	Listeria monocytogenes	2	10
	E.coli	5	25
	B. cereus	4	20
Hotel foods (VII)	*Shigella dysenteriae*	1	5
	Salmonella enterica	3	15
	Listeria monocytogenes	2	5
	E.coli	4	30
Commercial milk (VIII)	*Campylobacter jejuni*	1	5
	Staphylococcus aureus	2	20
	Salmonella enterica	3	15
	Listeria monocytogenes	4	20
	E.coli	3	15

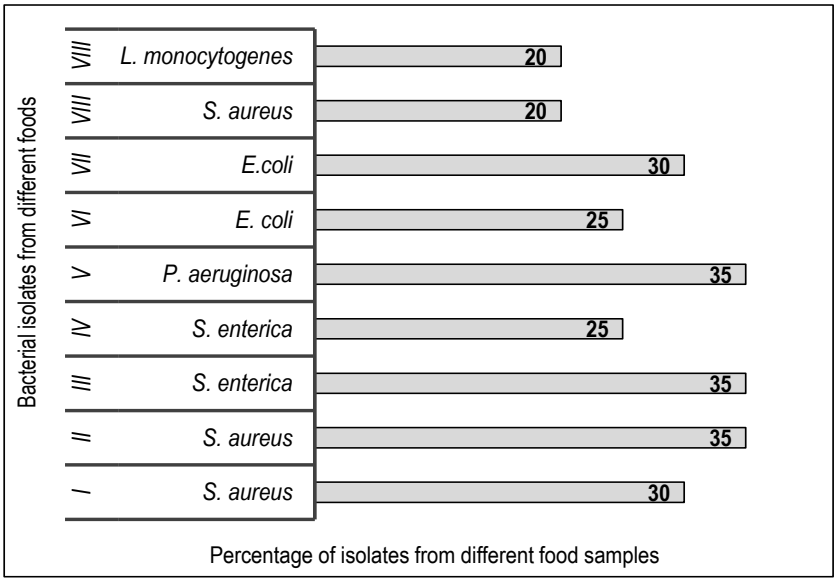

Fig 2. Determination of food-borne pathogens with the greater percentage in different food samples

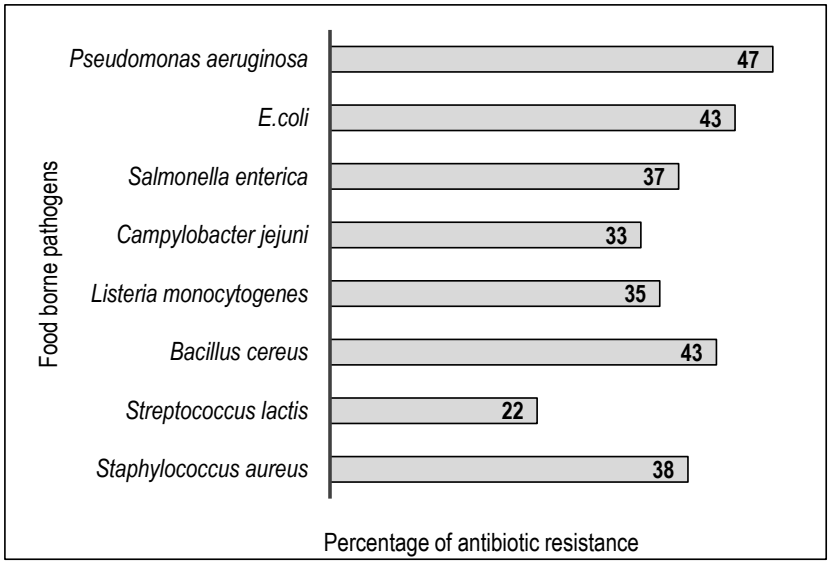

Fig 3. Determination of the percentage of antibiotic resistance of foodborne pathogens

Table 4. Determination of total numbers and general percentage of Gram-positive and Gram-negative bacterial pathogens from food samples

Gram reaction	Bacterial pathogens	Number of isolates	Percentage (%) of isolates
Gram-positive	*Staphylococcus aureus*	21	10.5
	Streptococcus lactis	9	4.5
	Bacillus cereus	7	5.5
	Listeria monocytogenes	17	8.5
	Campylobacter jejuni	12	6
	Salmonella enterica	24	12
	Shigella dysenteriae	3	1.5
Gram-negative	*Escherichia coli*	33	16.5
	Pseudomonas aeruginosa	4	1.5
	Proteis mirabilis	2	1

Bacillus cereus (41%), *Listeria monocytogenes* (35%), *Campylobacter jejuni* (33%), *Salmonella enterica* (37%), *E.coli* (43%) and *Pseudomonas aeruginosa* 47% antibiotic resistance (Fig. 3).

4. DISCUSSIONS

Human food is all carbohydrates, proteins, lipids, vitamins that in specific caloric value maintain the physiological functions of the body. Overeating at irregular timings develops taste and charm but the consequences are alarming for health. All age groups of both genders more likely to eat junk foods of different types besides their homemade foods at irregular timings. In this 20 months study, it is observed that the children from 4 to elderly age 22 years (41%) prefer to eat junk foods twice a week and the adults of 23-35 years (45%), 36-50 years (38%) and 51-60 years (38%) prefer to eat twice a month. The increasing ratio of eating junk foods is due to their time factor [18], the taste [19-20], advertisement of the food products, shelf life, easy availability, simplicity of manufacturing process, and cost-effectiveness [21- 22] sight attraction, pleasant smell. Various foods contain different substances that are not suitable for human health. The nutrient composition such as the increased quantity of sugar, white flour, trans-fat, polyunsaturated fat, salt, and different additives [21].

The consensus of the population of 4-22, 23-35 and 36-50 years revealed that from 41, 45 and 38% of junk food eater, 11, 13, and 16% of the population had suffered from obesity beyond 3-6% in their normal body weight within 30 days. This issue particularly 4-22 years of the population may be due to the greater extent of affections of the parents, irregular eating habits in different intervals, publicity, and promotional campaign of the food vendors through television, newspaper, and social media. A little percentage of consumers of 36-60 years avoid such foods due to unhealthy consequences such as increased fat contents, cholesterol level, sugar, a high salt concentration that lead to overweight, high blood pressure, paralytic stroke, cardiovascular and renal disorders, generalized weakness, sleeplessness, less attention, and partial memory loss. The facts behind these consequences may be due to lack of energy, low valued cooking practices, use of low standard cooking oils, irregular timings of eating, unavailability of natural phytochemicals that saturates the free radicals, use of high additives that may motivate brain receptors to enhance dopamine level. These findings are in accordance of [23, 21].

The percentage of below average, average were observed to have fewer health issues compared to poor and elite class due to the less consumption of junk foods, proper selection of time, proper selection of unpolluted areas and proper selection of food vendors where the hygienic status is better than the other food vendors. The findings also revealed that the poor population including beggars, sweepers, and laborers working at the welding shop, chemical distribution offices, hospital wards, congested vegetable market and slaughters houses were more sensitive to health issues. The fact behind this may be the type and nature of food taken, unhygienic storage of food before and after cooking, and the hygienic status of food vendors such as washing utensils with tap water only (5%), use of normal cloth for drying hands before cooking and the same cloth may be reused for cleaning the crockery and some mobile food vendors may also serve the same cloth to customers (10%) and using head covering only during cooking (11%) which could be the vehicles of transmission of diverse groups of pathogens. Our findings are per [24].

The potent food-borne multidrug-resistant strains of different bacterial isolates were observed from the test food items in *Pseudomonas aeruginosa*, *E.coli*, *Bacillus cereus, S. aureus, Salmonella enterica, Listeria monocytogenes Campylobacter jejuni, Streptococcus lactis* to respective antibiotics. The emergence of pathogenic bacteria may be due to improper storage of raw and cooked food for a longer duration, storage of food at stove charcoal at unacceptable temperature for food preservation that could support the microbial multiplication particularly S. aureus in uncovered foods and also overheating beyond need, holding foods at ground level, exposure to dust contamination and attraction of house flies could lead to the foodborne diseases [25-32].

5. CONCLUSIONS

It is concluded that the kids and the elderly age children eat junk foods as scavengers whereas the

36-60 years persons of both genders are less affected compared to 4-35 years of age. The cleanliness and the general hygiene concluded that few food vendors and cooks at home wash utensils with tap water, use normal cloth for drying hands before cooking and reuse them for cleaning the crockery for serving foods especially the street food vendors e.g. golgappa, French fries, chana, samosa, kachori, and ojhri/tripe sellers and use head covering only during cooking. It is concluded that a greater extent of both Gram-positive and Gram-negative foodborne pathogens are actively found in all test food items. The sensitivity to various antibiotics showed that *S. aureus*, *S. enterica*, *E.coli*, and *L. monocytogenes* have greater resistance to the test antibiotics.

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