

Evaluation of Food borne Pathogens in Ready-to-Eat Noodles Collected from the Different Shops in the Dhaka City, Bangladesh

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Abstract:

Background: Food contamination is a serious public health problem around the world, causing foodborne illnesses to affect humans every year.

Cases: The present research was designed to evaluate the microbiological quality of cooked noodles samples collected from various places in Dhaka city, Bangladesh. Qualitative microbiological analyses of total 15 samples from 5 different shop (3 from each place) were performed through conventional culture methods and the drug susceptible array of the isolated bacteria were demonstrated through antibiotics disc diffusion methods.

Results: In case of microbial contamination, the total viable bacterial count, 10^5 cfu/g to 10^7 cfu/g was found in all the samples from different sources. The maximum contamination was recorded in the samples from food cart. Meanwhile, as the specific bacterial contamination, *E. coli*, *Klebsiella* spp and *Pseudomonas* spp were present in the entire sample. *E. coli* was estimated within the range of 10^2 cfu/g to 10^3 cfu/g while the *Klebsiella* spp. and *Pseudomonas* spp. were observed up to 10^3 cfu/g. However, *Staphylococcus* spp, *Salmonella* and *Shigella* spp were not found in any samples. Moreover, the drug resistance of *E. coli*, *Klebsiella* spp. and *Pseudomonas* spp. were performed against ten antibiotics; among 10, *Escherichia coli* was found to be resistant against three, *Klebsiella* spp. was resistant towards two and *Pseudomonas* spp. was resistant to one antibiotic.

Conclusion: However, the degree of initial contamination in samples may pose hazard to public health. This study emphasized that the hygienically maintained food retained the best quality attributes required for consumer's acceptability and safety.

Keywords: Cross contamination, Drug resistant, Food borne pathogens, Ready-to-eat food

1. Introduction

Food and waterborne infections have been documented predominantly among those who live in the busy or crowded city [1,2]. Due to the hectic lifestyle, ready-to-eat food, street food, vendor food items are getting huge attention from the resident's day by day [2,3,4,5]. As a result, numbers of mobile shops have been increased in the city area especially beside the school, collage, hospital, and stadium [6,7]. Foods that can be consumed immediately without extra processing are referred to as ready-to-eat food. [8,9]. From the microbiological point of view, such types of foods may harbor several food borne pathogens those are extremely hazardous for the health [10,11].

Since more than 200 different food borne diseases have been recognized by the researcher [12]. Generally, food borne illness happen due to the injection of pathogenic strains (bacteria, virus and parasites) into the human body with infected food [13,14,15,16]. Several issues are responsible for food contamination with the diseases causing bacteria such as unhygienic water, open atmosphere, polluted apparatuses, and personal unhygienic condition [1,15,16,17]. Meanwhile, the people of Dhaka city in Bangladesh are highly dependent on the supply water for their daily activities particularly the use of supply water is enormously noticeable in canteen and mobile food shop. According to the previously published data, the quality of supply water in Dhaka city is not microbiologically safe to consume due to the poor water distribution system and maintenance [18,19,20,20,21,22].

In the present study, we worked on the noodle samples, which is so much popular not only in Bangladeshi people but also in all over the world. To control the existence of microbial agent in noodles, drying and heating method have been recommended by the researcher [23,24]. Many studies have been focused previously in United states, Europe and Canada on the proliferation of pathogens in pasta products during the production from different [25,26,27,28]. Recently, a study on 629 samples was conducted in Canada, where the researchers discovered 94 percent aerobic plate count (APC) in 50,000 per g; 98 percent *S. aureus* in 25 per g; 98.1 and 99.7 of samples showed coliform and fecal coliform respectively in 1 per g; yeast and mold were found in 98 percent of the samples [28,29]. Because of the limited amount of data on the microbiological quality of chowmein and noodle products obtained at the retail level, that is why our current research was designed for microbial analysis of cooked noodles samples from various places of Dhaka city along with the drug resistant profile of the isolated bacterial strain from the samples.

2. Materials and Methods

2.1 Sampling and sample collection

To execute the microbiological analysis of noodles, the samples were collected from various street vendors and restaurants located in different places of Dhaka city. A total of 15 samples from five different food preparation zone (n=3) were composed. All the samples were collected within polyethylene bag or plastic cup from every place as the retailer distributed. The transportation period was variable; from ten min to two hours depending on the location of the individual street vendor or restaurant. Then the samples were transported into the laboratory straight away. All the samples were carefully handled during the transportation and maintain the thermostable condition to avoid the immediate contamination [1,16,30].

2.2 Processing and preparation of samples

For homogenization, 25g of each noodle's samples were weighted and blended with 75ml of normal saline with the help of blender. Before starting examination, all the samples were gently mixed. Then the samples were then revitalized for 1min. As a result, the solid elements of the samples were suspended at the bottom of the glass bottle and created some clear portion at the top. 1 mL of each sample was added into the test tube having 9 mL of sterile saline to make the ratio 9:1 then the serial dilutions were performed up 10^{-4} according to the standard method [1,30].

2.3 Detection of total bacterial count

Exactly, 100 μ l of each sample from the 10^{-4} dilution was spread out over the nutritional agar (NA) plate to determine total bacterial count. The pH of the medium was approximately 6.8. All the inoculated media were kept inside the incubator at 37°C temperature for 24 hours for their proliferation. On nutrient agar media, white, yellowish white, off-white, colored colonies were detected. The results of the total bacterial count were expressed as the number of organism or colony forming units per milliliter (CFU/mL) of all samples [1,19].

2.4 Detection of total coliform count

To observe the presence of total coliform, 100 μ l of each sample from the 10^{-2} dilution was transferred to MacConkey agar plate by following the spread plate technique. The has a pH of the media was approximately 7.1. After incubation period at 37°C temperature for 24 hours, all the plates were observed. Pink or red and colorless colonies were detected on MacConkey agar media [19].

2.5 Detection of total Staphylococcal count

100 μ l of sample from 10^{-3} dilution was transferred onto the Mannitol salt agar (MSA) plate and consistently spread onto the surface of the plate. All of the plates were incubated All the plates were incubated for 24 hours at 37°C temperature. Generally, white and yellowish colonies were observed on MSA media [19].

2.6 Detection of *Pseudomonas* spp.

For the detection 100 μ l of sample from 10^{-3} dilution was transferred onto the *Pseudomonas* agar (PA) agar plate by spread plate technique. The pH of the media was around 7.1. All the agar plates were incubated at 37°C temperature for 24h. Pyocyanin producing *Pseudomonas aeruginosa* showed blue-green water-soluble pigment or off white - greenish color into the media [1,19].

2.7 Detection of *Salmonella* and *Shigella* spp.

100µl of sample from the 10^{-3} dilution was inoculated onto the on the xylose lysine deoxycholate (XLD) media for the detection of *Salmonella* and *Shigella* spp. by spread plate technique. The agar plate was incubated at 37°C temperature for 24h [1,19].

2.8 Biochemical Identification of the isolates

All the isolated bacteria were further confirmed by observing their different attributes through several biochemical test such as Triple Sugar Iron test, Motility Indole Urease test, Citrate utilization test, Oxidase test, Catalase test and MR-VP test [31].

2.9 Antibiotic Susceptibility Test

Antibiotic susceptibility test was carried out with the *E.coli*, *Klebsiella* spp. and *Pseudomonas* spp. strains isolated from the different samples. The bacterial suspension was prepared (culture turbidity was adjusted to a 0.5 McFarland standard) and spread evenly over the entire surface of Muller-Hinton agar (Difco, Detroit, MI). Then the disc of ten standard antibiotics such as Azithromycin (30µg), Cefoxitin (30µg), Kanamycin (30µg), Tetramycin (30µg), Vancomycin (30µg), Rifampicin (5µg), Chloramphenicol (30µg), Ciprofloxacin (5µg), Cefixime (5µg) and Cefpodoxime (30µg) were placed on to the Muller-Hinton agar and the plates were incubated at 37°C for 4 hrs to observe the zone diameter [1,19,32,33].

3. Result

3.1 Existence of microbial load in noodles samples

In this study, five noodles samples were taken respectively from street food cart, fast food, canteen, Chinese restaurant and homemade. All the samples were found to be contaminated with total heterotrophic bacteria, 10^5 cfu/g was estimated in the samples from Chinese restaurant and homemade while the samples from fast food and canteen showed 10^6 cfu/g. The maximum contamination was recorded as 10^7 cfu/g in the sample from food cart (Table 1). In case of specific bacterial strain, the growth of *Pseudomonas* spp. was found in the samples from food cart, fast food, canteen, Chinese restaurant and homemade within the range of 10^2 to 10^3 cfu/g. All the samples exhibited the presence of coliform (*E. coli* and *Klebsiella* spp.). *E. coli* and *Klebsiella* spp. both were estimated within the range of as 10^2 cfu/g to 10^3 cfu/g (Table 1).

Table 1: Total viable count (TVC) and enumeration of coliforms, *Staphylococcus* spp, *Pseudomonas* spp, *Salmonella* and *Shigella* spp. from different noodles samples of Dhaka city.

Source of noodles sample	Total viable bacteria (cfu/g)	Coliform (cfu/g)		<i>Staphylococcus</i> spp (cfu/g)	<i>Pseudomonas</i> spp (cfu/g)	<i>Salmonella</i> & <i>Shigella</i> spp (cfu/g)
		<i>E. coli</i>	<i>Klebsiella</i> spp.			
Food cart (n=3)	9×10^7	1.9×10^2	2.24×10^3	0	3×10^3	0
Fast food (n=3)	2.12×10^6	0	0	0	1.3×10^3	0
Canteen (n=3)	2.11×10^6	0	0	0	2.66×10^3	0
Chinese restaurant (n=3)	1.5×10^5	0	0	0	1.62×10^3	0
Homemade (n=3)	1.01×10^5	0	0	0	1.24×10^2	0

Average count of all the samples were recorded in the table

Staphylococcus spp., *Salmonella* & *Shigella* spp. were absent in all the samples tested. All the isolates were identified according to their appearance on the agar plate as well as different biochemical attributes (Table 2 and Figure 1).

Table 2: Biochemical tests of different pathogens

Assumed Pathogenic microorganisms	TSI				Motility	Indole Production	MR	VP	Citrate utilization	Catalase	Oxidase
	Slant	Butt	Gas	H ₂ S							
<i>E. coli</i>	Y	Y	+	-	+	+	-	-	+	-	
<i>Klebsiella</i> spp	Y	Y	+	-	+	-	-	+	+	-	
<i>Pseudomonas</i> spp.	R	Y	-	-	+	-	+	-	+	+	

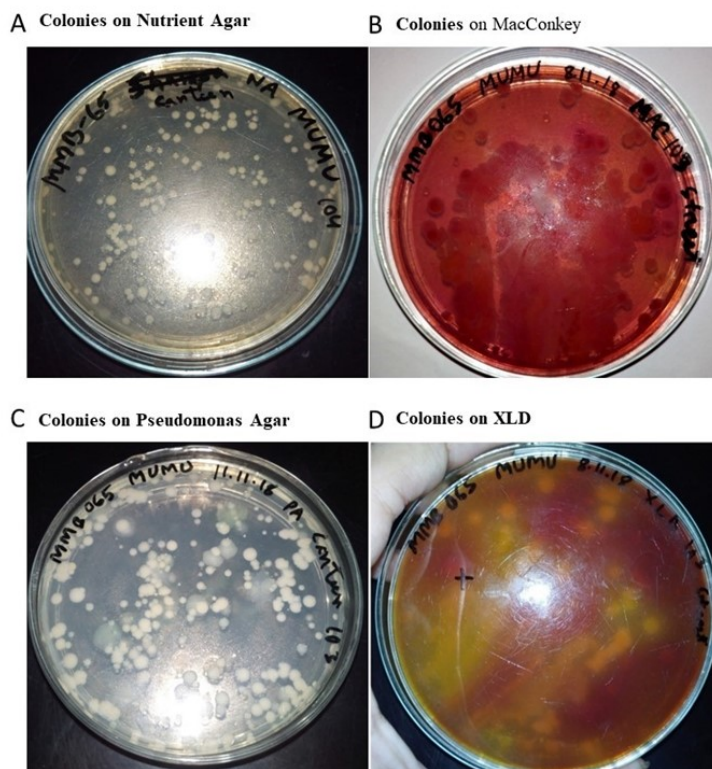


Figure 1: Morphological characteristics of the isolates on selective media: here panel A) indicates the colony characteristics of total viable bacteria on nutrient agar plate; the colonies were whitish, circular, smooth, convex and dry. Panel B) shows the growth of gram-negative bacteria (*E. coli* & *Klebsiella* spp.); two types of colonies were found, dry pink as *E. coli* and gummy pink as *Klebsiella* spp. Panel C) growth characteristics of *Pseudomonas* spp. on pseudomonas agar plate; most of the colonies were large, opaque, irregular colonies with fruity odor. Panel D) indicates the growth on Xylose Lysine Deoxycholate (XLD).

The overall microbial contamination (total viable bacteria) of the samples taken from the street food cart was in the highest position among all the samples. Noodles collected from fast food, canteen and chinese restaurant were containing medium amount of microorganisms. The sample collected from home was having the least amount of microorganisms (Figure 2).

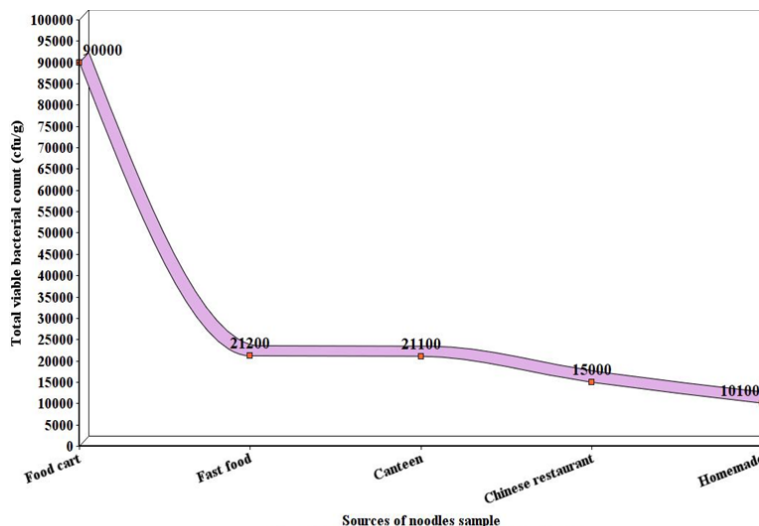


Figure: Microbial analysis of selected noodles samples

Figure 2: Microbial quality of the noodle’s samples collected from different places: based on the growth of total viable bacteria, the maximum count was recorded in food cart.

3.2 Drug susceptibility pattern of the isolates

According to the zone diameter produced by the synthetic drugs against the three isolates (*E. coli*, *Klebsiella* spp. and *Pseudomonas* spp.), the drug susceptibility feature of the isolates were determined (Table 3 and Figure 3).

E.coli was found to be resistant against Cefoxitin (30 µg), Vancomycin (30 µg) and Rifampicin (5 µg) while *Klebsiella* was resistant against Cefixime (5 µg) and Cefpodoxime (30 µg). On the other hand, *Pseudomonas* spp. was resistant to cefpodoxime and intermediate for cefixime.

Table 3. Antibiotic susceptibility test.

Name of the Antibiotics	Antibiotic Sensitivity level					
	Zone of inhibition (mm)	<i>E.coli</i>	Zone of inhibition (mm)	<i>Klebsiella</i> spp.	Zone of inhibition (mm)	<i>Pseudo-monas</i> spp.
Azithromycin (30 µg)	23	S	24	S	20	S
Cefoxitin (30 µg)	7	R	22	S	18	S
Kanamycin (30 µg)	26	S	25	S	23	S
Tetramycin (30 µg)	20	S	20	S	29	S
Vancomycin (30 µg)	7	R	26	S	30	S
Rifampicin (5 µg)	6	R	16	S	18	S
Choramphenicol (30 µg)	29	S	22	S	20	S
Ciprofloxacin (5 µg)	37	S	31	S	25	S
Cefixime (5 µg)	21	S	12	R	17	I
Cefpodoxime (30 µg)	24	S	22	R	20	R

*S = Sensitive, R = Resistant, I = Intermediate

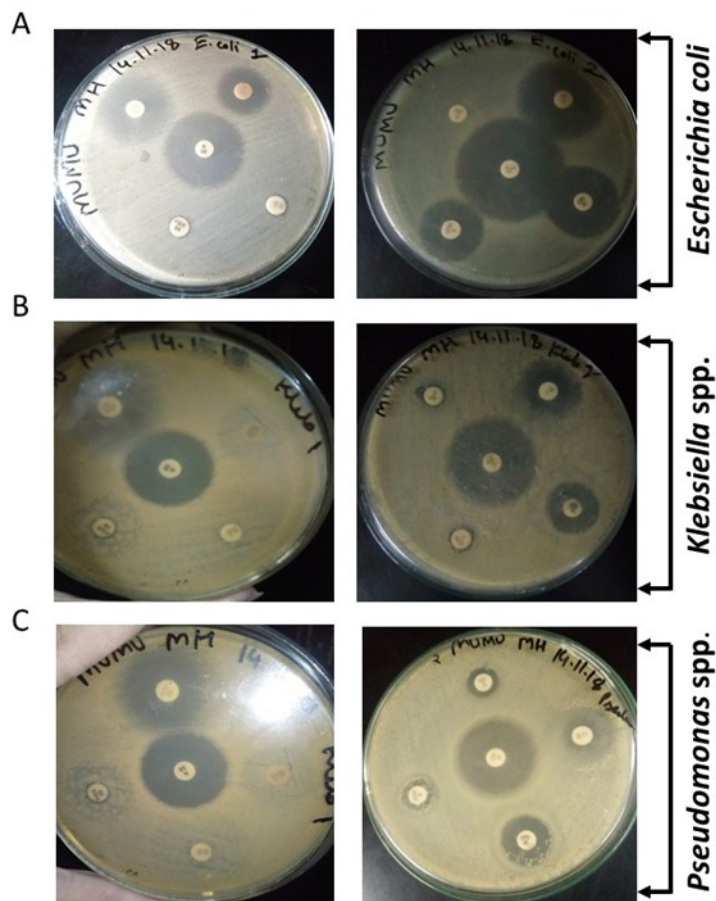


Figure 3: Antibiotic susceptibility pattern of the isolates against the commercially available drugs: here panel A) indicates the zone diameter produced by the antibiotics against *E. coli*. Panel B) shows the action of 10 antibiotics against *Klebsiella* spp. Panel C) represents the effects of antibiotics against *Pseudomonas* spp.

4. Discussion

Despite the expansion of technologies in food safety and security sector, food born outbreaks by the existence of food contaminants is still one of the most concerning issues especially for the highly populated countries like Bangladesh where the communities are not yet anxious regarding personal hygiene [2,4,5,34]. Beside the personal hygiene, cross-contamination during the food preparation is another serious threat for the consumers that mostly happened through polluted water and dirty utensils [13,14,16,35]. Based on the results of our study, noodles samples from different sources were not found to be complied with the requirements of good hygienic practices. This study has generally revealed that there is a great need for improving food safety in both inside and outside of our home. As the coliforms, *E. coli* and *Klebsiella* spp. counts in all the samples from different sources were higher than the food safety standard permissible limits. It is clear from these findings that the majority of individual respondents did not follow good food handling practices (e.g., smoking, chewing tobacco leaves while handling food, serving food without using hand gloves, hair restraints, or masks) and that there was a lack of frequent supervision by the relevant food safety authorities. The same level of coliform contamination (*E. coli* and *Klebsiella* spp.) was reported by the previous researchers in case of vendor and packet fruit juice [2]. Several bacterial contaminations may have existed in the food especially in ready-to-eat food even after processing which has been stated by a group of researchers [4,5]. One of the research published by [1] showed the occurrence of *Vibrio cholerae* in at least 40 different types of food items but our current study did not get any *Vibrio* species in the tested samples. Meanwhile, food technologists and food microbiologists have been implementing several methods to eliminate the proliferation of food borne diseases causing microorganisms from very earlier time [1,4,5,15,35]. Another important aspect of our study was to evaluate the drug susceptible parameter of the isolates from noodles samples. Here we observed that the *E. coli* and *Klebsiella* spp. showed their resistance against more than one antibiotic, which was fairly similar with the outcomes of earlier research [1,2,4,5,33]. Surprisingly, almost all the drugs were found to be effective against *Pseudomonas* spp. accept cefpodoxime.

The microbial contamination level was inconsistent in different sources of samples, which indicates that the contamination may occur due to the contaminated water, improper hygiene practice, or the uses of polythene bag for take away. Our study highly suggests that the proper education, awareness on food safety and better sanitary conditions are urgently needed for food handlers during the processing, preparation and manufacturing of noodles.

5. Conclusion

Unfortunately, no regulatory norm governing the sanitary manufacturing, processing, and distribution of street meals has yet to be enacted in Bangladesh. The microbial contamination in the food items may come from various sources including our environment. The current research focused on the post-harvest safety of cooked noodles and its impact on the potential health risks associated with their intake. The outcome aids in the formulation of recommendations for ensuring the delivery of safe food products in order to meet the demands of customers now and in the future. The following recommendations for ensuring hygienic food safety and obtaining hygienic quality noodles are likely to be reliable: acquisition of the highest possible microbiological quality raw materials, prevention of excessive food contamination prior to processing, proper food processing, quality packaging to keep instant noodles fresh, adequate storage, optimal transportation, and hygienic handling.

6. Conflicts of interest

All authors have equal contribution and have no conflicts of interest.

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