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Evaluation of Antibiotic Resistant Bacteria from Pre and Post Microwave Oven Treated Burger Collected form Different Retailer in Dhaka City.

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Abstract: *Present study attempted to isolate the food born micro-florain some pre and post microwave oven treated burger those were collected from popular shop in Dhaka City, Bangladesh. Aadditionally, drug resistant profile of the isolates wasdetermined against some common synthetic drug through Kirby Bauer method. Total 10 samples (5belonged to pre heated and 5 belonged to post heated) exhibited huge microbial load up to 10^7 cfu/g including total viable bacteria and fungi. Both of the samples(pre and post heated) harbored Staphylococcus, E. coli and Pseudomonas as specific pathogens. In case of pre heated burger, total viable count (TVC), total coliform count (E. coli) and fungal load were recorded up to 7.22 log cfu/g, 4.3 log cfu/g & 5.2 log cfu/g consecutively whereas the specific pathogens Staphylococcus* spp. *and Pseudomonas*spp. *were found up to 6.3 log cfu/g & 4.23 log cfu/g respectively. Likewise, post microwave oven treated samples showed total viable count (TVC), total coliform count (E. coli) and fungal load up to 3.22 logcfu/g, 2.3 logcfu/g & 2.2 log cfu/g consecutively while the pathogenic bacteria Staphylococcus* spp. *and Pseudomonas*spp.*were up to 2.3 log cfu/g & 2.23 log cfu/g respectively. Only 60-second microwave heat treatment remarkably eliminated the growth s TVC, fungal, E. coli, Staphylococcal and Pseudomonas count up to 4 log in burger. The Staphylococcus* spp. *and Pseudomonas*spp. *were found to be 100% resistant to tetracycline and kanamycin while sensitive against Azithromycin, Gentamycin and Streptomycin. On the other hand, E. coli showed 100% resistance against Azithromycin and Streptomycin whereas sensitive to Gentamycin, Kanamycine and Tetracycline. This data suggested that the 60seceond microwave heat was effective to reduce the microbial growth but resistant strains from burger may pretense serious public health threat.*

Keywords*: Food born microbes, Antibiotic resistance; Microwave oven heat*

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I. Introduction

Burger is one of the most popular fast food item, which is found in almost all fast food shops. It is a food that made by bread, cutlet, salads, sausages, sauces and others [1]. It can be eaten anytime for example during lunch, or even eaten as a snack during birthday parties or marriage ceremony and it is very much famous to children as a result they take burger for tiffin. Burger is a ready to eat meat products that has high demand throughout the world due to its high biological value, reasonable price, desired taste, and easy to serve [2].

However, there are several cases of food poisoning caused by burger consumption. Burger may harbour a number of pathogenic organisms such as, *Campylobacter* spp.,*E.coli, Listeria monocytogenes*, *Salmonella* spp., *Staphylococcus* spp., etc[3,4]. These pathogens usually spread by eating burgers that not only affects large groups of people but also can lead to death sometimes. Transmission of human pathogens through food is a serious global problem mainly in developing countries where gastrointestinal diseases are one of the most common causes of morbidity and mortality**[1]**. However, eating habits can increase the risk of hazards, which can be minimized by proper monitoring of microbiological quality of the ingredients used to make a burger and by creating awareness among people about food hygiene and sanitation [1].

Major sources of contamination of burgers are, handling of food without using any gloves, washing hands and other utensils with unsafe water, improper storage of burger buns, inaccurate temperature, keeping cooked and uncooked meat side by side in the refrigerator are some possible sources[5]. Microbiological assessment of burgers is required to ensure that the burgers sold in the market safe for the consumers, in a point of view to public health[1].

The control of food contamination by microorganisms or in other words, the practice of food protection largely depends on the knowledge on types and modes of food hazards, the pathogenic trait as well as of the virulence factors of the food contaminating bacteria or fungi, knowledge on toxins associated with food deterioration, and finally the urge of practical implementation of food protection means both by the

governmental or non-governmental organizations (NGOs) along with adequate research facilities on food microbiology[6,7].Considering all these facts, the present study attempted to (1) evaluate the microbiological profiling of some pre and post microwave heat treatment burger and (2) drug resistant pattern of the isolates.

II. Material And Methods

Study Area, Sampling, Sample Processing and Microbiological Analysis

Total 10 samples(5 were pre heat and 5 were post heat) of burger were randomly collected following standard protocol[8]. The study was carried out within October to December 2019 in the department of Microbiology, Stamford University Bangladesh. All the samples were quickly transported into the laboratory for microbiological assay. 10g of each pre-heated sample was homogenized with 90 ml of buffer peptone water (pH 7.2 ± 0.2) in 9:1 ratio and serially diluted up to 10^{-3}. Rest of the 5 samples was heated by microwave for 60 second afterward the samples was homogenized and diluted through the above mentioned procedure.

From the dilution 10^{-2} each of the samples (pre and post microwave heated) of 0.1 ml was introduced on to the nutrient agar and Sabouraud dextrose agar for the isolation of total viable bacteria and fungi, respectively. Subsequently, MacConkey agar, Manitol Salt agar and Cetrimide agar were used as selective media for the quantification of coliforms, *Staphylococcus* spp. *Pseudomonas* spp. consecutively[9,10]. All the inoculated plates were incubated at 37 °C for 24 hours except SDA plates, which were incubated at 25 °C for 48 hours.

Biochemical identification of the isolates

The biochemical properties of identified isolates were confirmed through standard biochemical methods [9,11].

Antibiotic susceptibility test of the identified bacteria.

The pathogenic isolates were examined for the detection of antibiotic susceptibility traits (either drug resistant or sensitive) by disc diffusion assay on Mueller-Hinton agar (Difco, Detroit, MI) against commonly used antibiotics following the standard protocol[12,13]. Lawns of bacterial suspensions including *Escherichia coli*, *Pseudomonas* spp., and *Staphylococcus* spp. (turbidity compared with the McFarland standard OD_{600}-0.5) were prepared and introduced on to Muller Hinton agar. Some common antibiotics such as Kanamycin (30 µg), Streptomycin (10 µg), Gentamycin (10 µg), Azithromycin (15 µg), Tetracycline (30 µg)were introduced against the target bacteria. All the plates were incubated at 37 °C for 12-18 hours and examined the zone of inhibitions (mm).

III. Result

Propagation of different pathogenic strain including coliform and fecal coliform in food samples due to the poor sanitation and hygienic condition are the main causative agent of several food borne diseases like diarrhea and dysentery[14,15,16]. Such diseases are very common in developing countries like Bangladesh due to the lack of proper knowledge on personalhygiene as well as poor maintenance quality of raw materials[7,17,18].

Existence of pathogenic bacteria in pre heated samples.

All the samples were found to be contaminated with total viable bacteria, Fungus, *E. coli*, *Staphylococcus* and *Pseudomonas* (Figure 1A,B,C,D&E). In case of American burger total viable count and fungus was found 7.22 log cfu/g and 5.2 log cfu/g respectively. While 4.3 log cfu/g, 6.3 log cfu/g and 4.23 log cfu/g forspecific pathogens *E. coli*,*Staphylococcus*spp. and *Pseudomonas* spp. were present in the samples consecutively. For the swiss burger, total viable bacterial and fungal growth were recorded 6.5 & 4.3 log cfu/g whereas the *E. coli*, *Staphylococcus*and *Pseudomonas* showed 4.2, 5.2 & 3.2 log cfu/g consecutively. On the other hand, Total viable bacterial and fungal contamination were observed 5.5 & 3.3 log cfu/g respectively though the *E. coli*, *Staphylococcus*spp. and *Pseudomonas*spp. were 3.2, 4.3 & 3.2 log cfu/g accordingly in alif burger. The sample from popayes the total viable bacteria and fungus was 6.2 and 3.3 log cfu/g respectively conversely 4.2, 4.3, & 3.2 log cfu/g were recorded for *E. coli*, *Staphylococcus* spp. and *Pseudomonas*spp. consecutively. For the burger queen the total viable bacteria and fungus load was noticed 6.3 log cfu/g and 4.2 log cfu/g respectively while the *E. coli*, *Staphylococcus*spp. and *Pseudomonas*spp. were 3.2, 3.2 & 4.2 log cfu/g consecutively (Figure 1 A,B,C,D&E).

Biochemistry of the isolates.

All the isolates showed their physiological and metabolic activitythrough several biochemical tests (Table 1).

Table 1 Biochemical identification of the pathogens isolated from burger samples.

Isolated Strain	TSI			H_2S reaction	Indole Test	MR test	VP test	Citrate Test	Motility test	Oxidase test
	Slant	Butt	Gas							
E. coli	Y	Y	+	-	-	+	-	-	+	-
Staphylococcus spp.	Y	R	+	+	-	+	-	+	+	-
Pseudomonas spp.	R	R	-	-	-	-	-	+	+	+

The experiments were conducted three times independently, and the results were found to be reproducible.

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

Detection of microbial load after 60-second of microwave oven heat treatment.

In all the samples the total viable bacteria, Fungus, *E. coli*, *Staphylococcus* and *Pseudomonas* (Figure 1A,B,C,D&E). After 60 second of microwave oven heat, the microbial load was eradicated up to 4 log from the previous count. In case of American burger total viable count and fungus was reduced 4 & 3 log after heat respectively while the specific pathogens *E. coli*, *Staphylococcus*spp. and *Pseudomonas* spp. were found to be reduced 2log, 4log & 2log consecutively (Figure 1a). Likewise, the swiss burger showed nearly 3log reduced growth for total viable bacteria and fungus from the pre heated samples whereas the *E. coli*, *Staphylococcus*spp. and *Pseudomonas*were eliminated 2, 3 & 1log consecutively (Figure 1B).

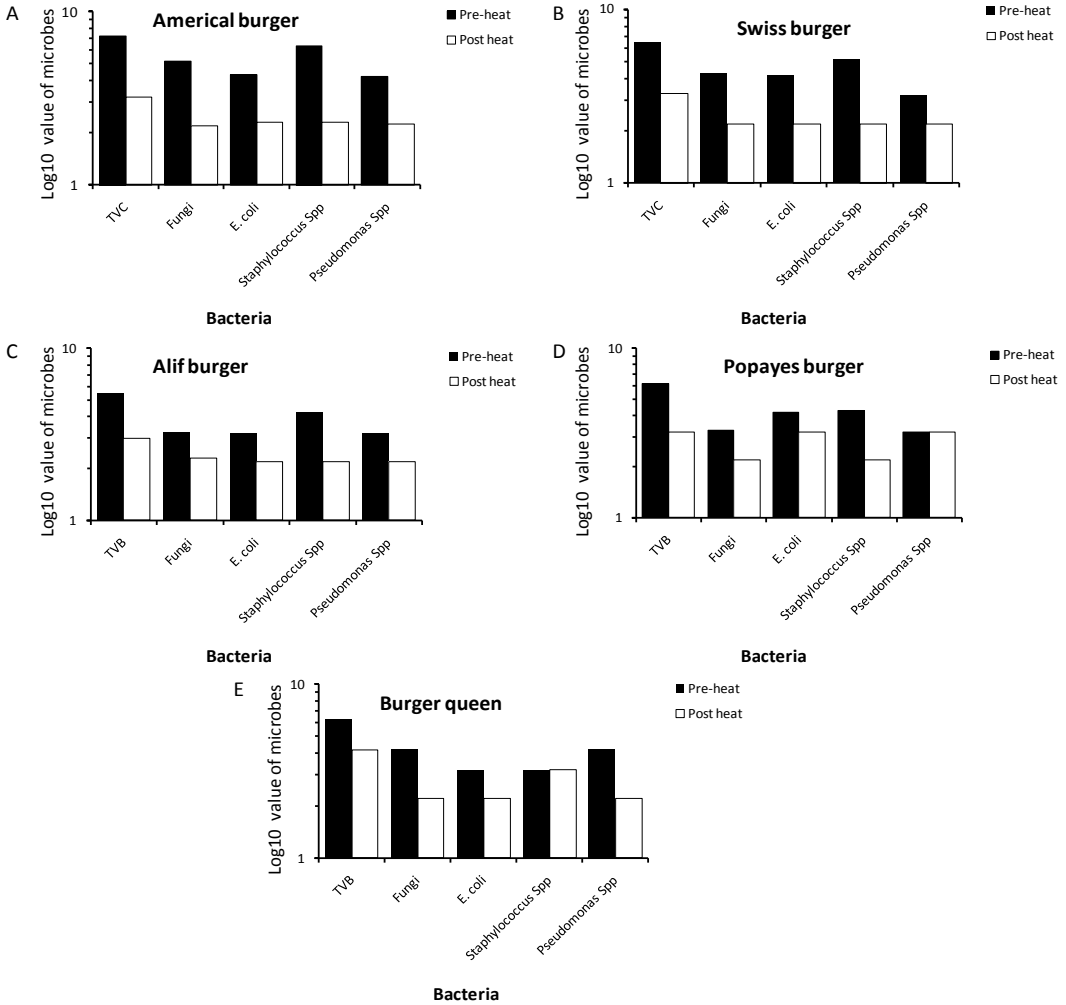

Figure 1: Effects of 60 second heat on the growth of microbes.

Panel A,B,C& D indicating the microbiological status of five burger samples. The black bar indicating the existence of bacteria before microwave oven heat and the white bar indicating the bacterial load after microwave oven heat.

In the same way, for alif burger total viable bacterial and fungal contamination were found to be reduced 2log and 1log from the previous count respectively though the *E. coli*, *Staphylococcus*spp. and *Pseudomonas*spp. were also reduced nearly 2log (Figure 1C). The sample from popayes the reduction rate was 3log for total viable bacteria and 1log for fungus conversely the growth was reduced 1log for*E. coli* and2log for *Staphylococcus*spp.However, no reduction was found for *Pseudomonas*spp. after heat (Figure 1D). For the burger queen the reduction of total viable bacteria and fungus load was noticed up to 2log while the*E. coli*and *Pseudomonas*spp. were found to be eliminated 1log & 2log respectively. Nevertheless, the growth of *Staphylococcus*spp. was unchanged (Figure 1E).

Couple of previous study investigated that the existence of contaminating microflora in food samples may occur during the processing of raw material, mixing of ingredients and packaging of the end products[19.-25]. According to the International Commission on Microbiological Specifications for Foods (ICMSF) 2011, the specific pathogen in food should not be exceeded 10^3cfu/g. This study exhibited that the heat of microwave oven up to 60 seconds has huge impact on the reduction of microbial growth. One of the research group also focused on the effect of heat on the burger sample previously and they said that the 60 seconds heat before eating anything is very much effective which may eliminate substantial amount of bacterial load[1].

Detection of drug resistant bacteria

The isolates *Staphylococcus* and *Pseudomonas*were found to be 100% resistant to tetracycline and kanamycin though sensitive against azithromycin, gentamycin and streptomycin. On the other hand,*E. coli* showed 100% resistance against azithromycin and tetracycline while sensitive to gentamycin, kanamycin andstreptomycin. This data suggested that the 60-seceond microwave heat was effective toreduce the microbial growth but resistant strains from burger may pretense serious public health threat (Table 2).

Table 2: Antibiogram of the isolates

Antibiotic	Disc content	Isolates					
		E. coli *n*=3		*Staphylococcus* *n*=5		*Pseudomonas* spp. n=1	
		R (%)	S (%)	R (%)	S (%)	R (%)	S (%)
Kanamycine	30 µg	0	100	100	0	100	0
Streptomycine	10 µg	0	100	0	100	0	100
Gentamycine	10 µg	0	100	0	100	0	100
Azithromycine	15 µg	100	0	0	100	0	100
Tetracycline	30µg	100	0	100	0	100	0

R Resistant
S Sensitive

IV. Conclusion

Overall, the present investigation discussed thecompact microbiological profile of some burger from popular shop consumed by Bangladeshi people. This study also exhibited the importance of heat before eating any food. Only 60-sceond microwave oven heat can successfully reduce the microbial growth. This study identified the presence of fungi and specific pathogens, which may possibly get entry from the storage condition. Coordinated efforts from government sector and private industry together with federal agencies are urgently needed in this context. There is a need for routine surveillance systems to investigate the quality of the different food to avoid any future outbreaks.

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