Assessment of microbial contamination in instant prepared food within 30minutes and screening the post contamination up to 3 hours along with the final microbial status after Microwave oven heat treatment.

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Abstract:

In Bangladesh, the preparation and handling of street and home-cooked dishes frequently result in inadequate hygiene and the use of inferior ingredients. This can cause foodborne illnesses like diarrhea, dysentery, and botulism. This can cause foodborne illnesses like diarrhea, dysentery, and botulism. These foods are sometimes kept for a long time and are sometimes heated in a microwave before being served. Present study attempted to screen out the potential microbial contamination level at three points after preparing noodles, sandwiches and home-cooked fish and meat: after 30min of cooking, after being normally stored for 3 hours and after reheating through microwave. The microbial contamination level was observed through conventional cultural techniques and drug susceptible pattern of the isolated bacteria was determined by Kirby-bauer methods. The initial levels (within 30min of cooking) of bacteria and fungi were noticed around 3. 8 to 3. 9 log CFU/g. After 3hours, the growth of *Clostridium* spp. reached up to 5. 9 log CFU/g in noodles and fish while coliform bacteria appeared after storage e.g. 4. 3 log CFU/g in noodles. However, after heating with microwave the contamination level was found to be reduced but *Clostridium* spp., was in steady condition which is extremely alarming. Meanwhile, isolating bacteria like *Escherichia coli*, *Staphylococcus* spp., and *Clostridium* spp. exhibited their resistance against ampicillin and piperacillin. Staphylococcus spp. had some resistance to doxycycline and cefixime, but were more sensitive to ciprofloxacin. These findings indicate serious health risks of daily consumers who are having food after long storage or practicing improper heating before consumption. Current study found some spore forming bacteria and drug resistant bacteria those are the causative agent of food borne diseases and cane be the real threat for proper treatment strategies.

Keywords: Street and homemade foods, Foodborne pathogens, Microwave oven heat, Antimicrobial Resistance.

Introduction:

Efforts to enhance food safety and lessen the spread of foodborne illnesses are growing as a result of the socioeconomic impacts of the human population's health. According to the FAO, food consumption will reach roughly 60% by 2050, while animal protein production will rise by 1.7% annually, with meat accounting for roughly 70% of this growth, dairy products for 55%, and seafood for up to 90%. The availability of food that is safe for consumers and does not endanger their lives or health is significantly hampered by microbial contamination of food [1]. Food infections are one of the biggest problems facing the global healthcare community. These illnesses have slowed economic progress, especially in emerging nations like Bangladesh. The primary reason is that, in contrast to industrialized nations, traditional automated processes are used in developing nations to process and sell food. Ready-ready products such as noodles, sandwiches, burgers, vegetable breads, and more are sold mainly on the streets, especially in developing countries. Street products have prepared products on-site or at home before selling consumers who eat these products without treatment or heating [2]. Ultimately, these products can lead to environmental growth of bacteria that can transmit human diseases to humans and cause food poisoning [3]. In developed countries, there are complex criteria for food preparation, but in less developed countries, the main issue is the availability of proper, reliable water, which is generally an important object [4]. On the other hand, domestic products like precooked meat, fish, and other items can harbor microorganisms for a long period after they are prepared. Because germs multiply more readily as time passes [5]. The main causes of food contamination are the ability to convert food without using gloves, erase hands, erase hands with dangerous water, lack of storage of ingredients, inaccurate temperatures, and maintain cooked and raw meat near the fridge [6]. Microbiological evaluation of products is necessary from a public health perspective to ensure that domestic products are not only dangerous to consumers, but also in the market [7]. Therefore, methods to reduce microbial growth should be related to consumer safety.

The use of microwave heating is spreading in the food industry as well as in family practice for edible/thawing, holidays, drying, drying, whitening, cooking, cooking and heating [8]. Microwave applications provide the highest

quality (in terms of taste, color and nutrition) of the final product with rapid generation heat. Compared to traditional heating, microwave heating can have a positive impact on product quality due to shorter processing times, resulting in improved energy efficiency [9]. Microwave treatment can effectively reduce pathogenic microorganisms of food origin and ensure the microbiological safety of the product. In particular, microwave microwaves have great potential for pasteurization and sterilization of food [10]. Microwave sterilization does not provide significant changes in properties, such as antioxidant activity, enzyme activity and product color and biologically active ingredients due to disruption of short exposures [11]. However, irregular heating can lead to the survival of pathogenic bacteria in areas with low heat [12]. In addition to microbial safety issues, high microwave capacity and long-term treatment affects product quality, reducing expiration dates, causing tissue damage due to uneven heat and humidity loss, leading to poorly looking and poor-quality products. Based on all these effects, The goal of the current study was to screen for possible microbial contamination levels at three different stages following the preparation of noodles, sandwiches, and home-cooked beef and fish: 30 minutes after cooking, three hours after being regularly stored, and three hours after microwave warming.

Methods & Materials:

Sample Collection and Processing: Total 4 samples (2 were street food and 2 were homemade foods) of sandwich, noodles, cooked fish and meat were selected for this experiment and collected following the standard protocol [13]. The study was carried out within April to June 2025 in the department of Microbiology, Stamford University Bangladesh. All the samples were quickly transported into the laboratory within 15 minutes of preparation and executed the microbiological assay at three points: after 30min of cooking, after being normally stored for 3 hours and after reheating through microwave.

Microbiological Analysis of the samples:

Same samples were taken in three different time intervals like after 30min of cooking, after being normally stored for 3 hours and finally for reheating through microwave. The microbial contamination of the samples was determined within 30mins after cooking, after 3 hours and after heating with microwave. For that, 10g of each sample was homogenized with 90 ml of normal saline in 9:1 ratio and serially diluted up to 10^{-5}. From the dilution 10^{-4} each of the samples (freshly cooked, after 3 hours and after heating) of 0.1ml 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 Clostridium agar were used as selective media for the quantification of coliforms, *Staphylococcus* spp. *Clostridium* spp. consecutively [14,15]. 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 [14-16].

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 [17,18]. Lawns of bacterial suspensions including *Escherichia coli, Clostridium* spp., and *Staphylococcus* spp. (turbidity compared with the McFarland standard OD600-0.5) were prepared and introduced on to Muller Hinton agar. Some common antibiotics such as Ceftazidime 30 µg, Ciprofloxacin 5 µg, Doxycycline 30 µg, Ampicillin 10 µg, Cefixime 5 µg, Piperacillin 100 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) [19].

Result & Discussion:

Distribution of various pathogenic strains including coliforms, fecal coliforms and Clostridium spp. in food samples due to inadequate hygiene are the main pathogens of several diseases that are transferred by foods such as diarrhea, dysentery, and botulism. [20.21]. Lack of knowledge about maintaining good hygiene and maintenance of poor quality of raw materials can lead to serious illnesses in developing countries such as Bangladesh [22].

Microbial post contamination in commercial foods

In the figure 1, microbial load of frequently available commercial street foods like noodles and sandwiches are given where the count was measured within 30 minutes of preparation, after 3 hours of preparation and after heating. It was seen that, within 30 minutes of preparation stage, total viable bacterial count was 3.9 $\log_{10}$/g for noodles and 3.8 $\log_{10}$/g for sandwiches. Fungal growth of 3.7 $\log_{10}$/g for noodles and 4.3 $\log_{10}$/g for sandwiches was observed which might be due to the moisture content of the samples [23]. *Staphylococcus* spp. was in range of 3.8 $\log_{10}$/g for noodles and 4.2 $\log_{10}$/g for sandwiches were observed while *Clostridium* spp. was higher in sandwiches with the 4.3 $\log_{10}$ than noodles which had the 4.1 $\log_{10}$/g. As the time increases, the microbial growth can increase also due to the nutrients content, pH value, temperature ranges [24].

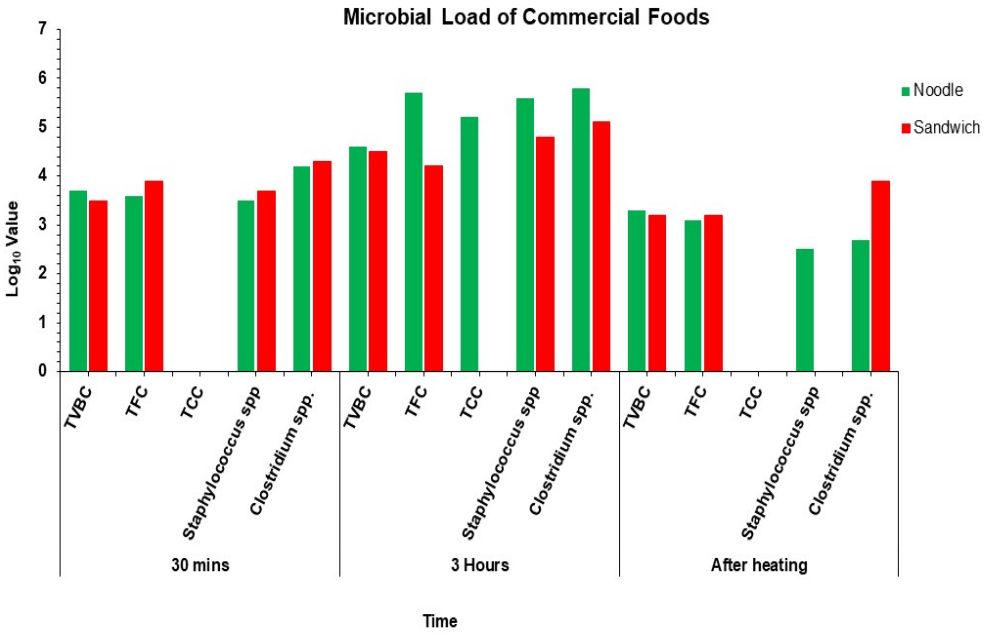

Fig-1: Microbial Load of commercially available street foods

To identify this, result was taken after 3 hours of preparation where it was noticeable that fungal growth was highest in noodles with the log $\log_{10}$/g of 5.8 while it was 3.8 for sandwiches. *Staphylococcus* spp. was also increased in amount in the foods as long time keeping of food can lead to the microbial growth and the range was 4.8 $\log_{10}$/g for noodles and 4.4 $\log_{10}$/g for sandwiches. This report also in affirmative form with another report which says that *Staphylococcus* spp. can spread easily during food preparation and processing by workers [25]. *Clostridium* spp. was present with the $\log_{10}$/g of 5.8 for noodles and 4.8 for sandwiches and it might happen because the resilient spores of *Clostridium* spp. can survive cooking temperatures and, if the food is then improperly stored, these spores can germinate and multiply into active, toxin-producing bacteria [26]. It was noticeable that, coliform bacteria were absent during the first 30 minutes of preparation in both samples but increased in noodles with the $\log_{10}$/g of 4.3 for noodles which is not acceptable. The reasons of coliform present might be because of the improper sanitation and environmental conditions [27]. As heating is a great source of sterilizing or freeing microorganisms so we heated the foods to check the quality where we found that heating actually minimizes the amount of microbes in the foods [28]. Total viable bacterial count was decreased to 2.8 $\log_{10}$/g for noodles while 2.9 $\log_{10}$/g for sandwiches. Fungal load was decreased simultaneously for noodles and sandwiches to 3.1 log value/g and 3.0 $\log_{10}$/g. It was noteworthy that coliform count was absent for both of the samples after heating. *Staphylococcus* spp. was found in average range of 2.6 $\log_{10}$/g for noodles while absent for sandwiches. It was noteworthy that, *clostridium* spp. was present in both samples after heating which is a risk to public health as it can cause botulism or release toxins which are dangerous for the consumers (figure 1).

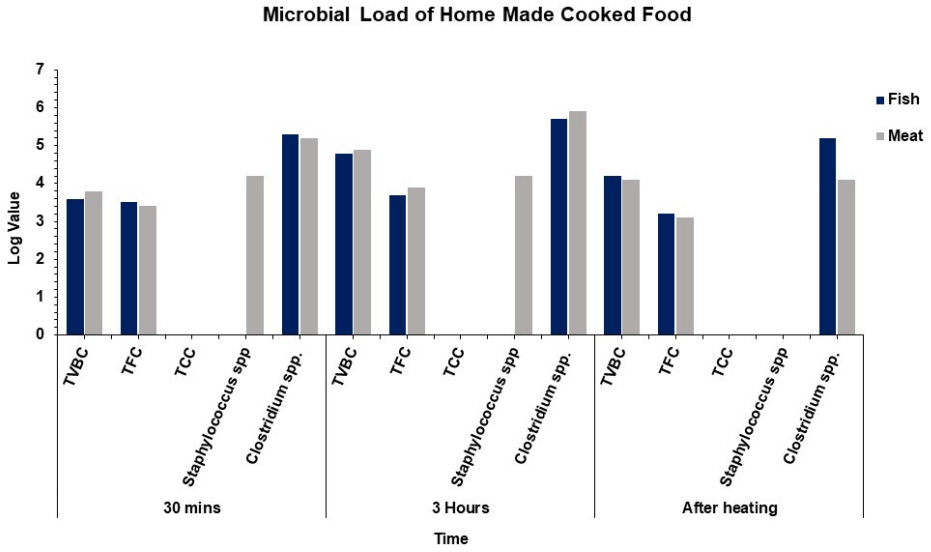

Fig 2: Microbial load of homemade cooked foods

Microbial post contamination in homemade foods

On the contrary, same time-consuming theme was observed for homemade foods also because it is very common in our nation to keep food for a long time and then consume it without any further processing which might lead to food poisoning as toxins releases from foods due to the microbial growth or chemical release. Here it was seen that freshly cooked foods contain total viable bacterial load of 3.8 $\log_{10}$/g for fish and 3.9 $\log_{10}$/g for meat. Fungal growth was also observed because of the moisture content ranging 3.7 $\log_{10}$/g for fish and 3.6 $\log_{10}$/g for meat. Total coliform count was absolute absent within 30 minutes of preparation which indicates that foods were coliform free and safe to consume. As staphylococcus spp. is a ubiquitous microorganism [25], it was present in meat with a $\log_{10}$/g of 4.8 but absent if fish samples. Although, *Staphylococcus* spp. was present in meat after 3 hours which shows that microbes use the nutrient to grow and comminute the food. But noteworthy was that *Clostridium* spp. was found in both fish and meat samples with a range of 5.2 $\log_{10}$/g and 4.9 $\log_{10}$/g and it was increased after 3 hours of keeping which was 5.9 $\log_{10}$/g and 4.1 $\log_{10}$/g which is not acceptable as the spores can produce toxins and can do diseases [26]. However, heating helped to decrease the number of microbes in food where we observed that the total bacterial count was decreased to 3.9 $\log_{10}$/g in fish and 3.8 $\log_{10}$/g in meat. But *Clostridium* spp. was in a range of 5.0log value/g for fish and 4.9 $\log_{10}$/g for meat which shows microwaving food to insufficient temperatures can fail to kill *Clostridium* spp. spores due to their remarkable heat resistance, which requires proper and thorough heating to reduce microbial growth and prevent foodborne illness [29].

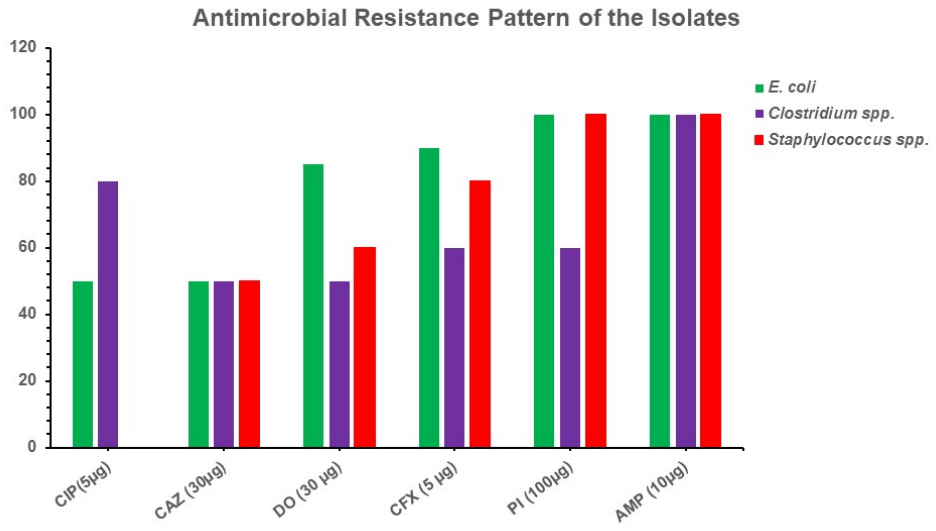

Fig-3: Antibiotic Resistance Pattern of the Isolates

As antimicrobial resistance is increasing day by day so the identified are resistant or sensitive to the commercially available antibiotics is of great concern. Commonly available some antibiotics were taken for the research where it was shown that *E. coli* was 100% resistant to ampicillin and piperacillin while moderately resistant to Doxycycline and Cefixime but 50% resistant to Ciprofloxacin and Caz which was also shown in another study [30]. Clostridium spp. was also 100% resistant to Ampicillin while 70% resistance to Ciprofloxacin and others were in 50% range of resistance. The resistance pattern of the clostridium spp. isolates was in a same state with the another study conducted in Saudi Arabia [31]. *Staphylococcus* spp. was resistant to ampicillin and piperacillin while moderate resistance was shown for Doxycycline and Caz, and it was noticeable that Ciprofloxacin was only sensitive to *Staphylococcus* spp. This result matches with the another study in our country [32]. Identified isolates are all multidrug resistant and it is of concern for the public health. Alternatives to these resistant insulators with multiple medical counters should now be concerned about future generations. Otherwise, simple food poisoning could pose a serious risk, even a death threat. The generalization and misuse of antibiotics in agriculture, aquaculture, and human medicine contributes to the homeostasis and spread of MDR pathogens throughout the food chain, particularly in RTE products. To combat this crisis, we need a reliable approach to health that integrates national antibiotics, observation, regulation, innovation and management of education. While outcomes in the field of molecular diagnostics, total genome-wide and profiling sequencing improve the ability to detect, control and predict stability diagrams, alternative interventions such as phagolis, bactericin, and probiotics provide intelligent tools to reduce antibiotic reliance.

Conclusion:

The current findings significantly highlighted that the microbial contamination occurred in the food samples within the time frame of after cooking to storage period and re-heating the storage food by microwave oven could not reduce the microbial load remarkably. Most importantly, the existent bacteria in food samples were showed resistance against the antibiotics. Therefore, implementing environmental surveillance, regulating antibiotic use in food systems, promoting behavioral changes through training and proper heating of storage food are necessary steps to reduce and dissemination of drug-resistant bacteria. As food systems continue to globalize, in the face of increased resistance, an international response coordinated to protect human health and food security is important. Furthermore, to address microbiological contaminants in prepared food samples, extensive measures are required, including enforcing better hygiene standards, protecting the food properly, accurate heat treatment and putting in place routine monitoring procedures. The integration of traditional and modern analytical methods ensures complete monitoring and prevention of food disease. Although the food safety authority should implement proper regulation and guidelines for the restaurant where the practices of proper heating the food before serve is not up to the mark. Our investigation would

aware the consumers who have huge fascination to have restaurant food as well as the people who apply microwave oven heat in house to make the storage food warm.

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