*Proceeding Paper*

# Isolation and Identification of Multidrug Resistant Bacteria from Street Food †

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**Abstract:** One of the unpleasant problems and one that could have significant effects in the near future is the sharp rise in zoonotic multi-drug resistant (MDR) bacteria (*Staphylococcus aureus*, *Streptococcus*, *Salmonella*). The enlarging and indiscriminate use of antimicrobials in the production of food, fish, and crops has led to a severe problem with multidrug resistance in human as well as animal health, particularly in school-age children. Therefore, it is crucial to learn whether Bangladeshi street food contains *Salmonella* and MDR *Staphylococcus*. *Staphylococcus*, *Streptococcus*, and *Salmonella* prevalence in street food variants and the pattern of antimicrobial resistance of isolated multidrug resistant bacteria were investigated in a cross-sectional study. The study was conducted at five street food carts near schools in Gopalganj Sadar, Bangladesh. MIU TEST, Oxidase, and Citrate tests) are carried out precisely in order to isolate and identify bacteria that are multidrug resistant. Each of the food samples examined for the research included MDR Salmonella (resistant to up to six of the eight tested antimicrobials). Erythromycin and Amoxicillin had the greatest resistance rates (100%) while Doxycycline had the lowest rates (approximately 15%) among identified Bacteria. Additionally, numerous street food products have different levels of *Salmonella* antibiotic resistance.

**Keywords:** multidrug resistance; prevalence; foodborne zoonoses; street foods

## 1. Introduction

Street foods are defined as a variety of ready-to eat foods and beverages prepared and sold by vendors in streets and other public places for immediate consumption [1]. The UN's FAO defined street food in 1989 as "ready- to-eat foods and beverages prepared and sold by vendors and hawkers, particularly in streets and other similar places [2]. Drinks, meals, and snacks sold by street food vendors are widely consumed by millions of people in developing countries, and a significant percentage of consumers suffer from diseases such as antimicrobial resistance, dysentery, diarrhea, enteric fever, and so on [3]. Microorganism contamination of street foods has emerged as a major public health concern around the world [1]. Foodborne diseases are among the most prevalent global public health issues in recent years, and their implications for health and the economy are becoming more widely recognized [4]. Among these pathogens, *Staphylococcus aureus*, *Bacillus cereus*, *Streptococcus salmonella* are most common foodborne pathogens worldwide and have long been recognized as an important zoonotic pathogen of economic importance in animals and humans, primarily in developing countries [5]. Food products of animal origin, which may be contaminated at the source or during handling, are an important route of transmission of *Staphylococcus*, *Bacillus*, and *Salmonella* organisms from animals to humans [6]. Street foods are appealing due to their convenience, low cost, and savory taste, making them a popular daily food option [2]. School-aged children, who frequently consume these street-vendor foods, are particularly vulnerable to most

foodborne diseases. A large number of street food vendors sell various appealing and colorful food items to school-aged children in and around several school-based locations in Dhaka and its outskirts [7]. Antimicrobial resistance (AMR) is one of the century's major global public health concerns, making effective treatment of an ever-increasing array of infectious diseases extremely difficult [8]. Antimicrobial resistance, including multidrug resistance (MDR), is becoming a global issue. MDR bacteria are frequently found in humans and animals from a variety of countries. According to a WHO report from 2014, this is becoming an increasingly serious threat to human health [9].

## 2. Materials and Methods

### 2.1. Study Design and Sampling Area

Between January and April of 2023, a cross-sectional study was conducted in five schools (SM Model, Binapani Govt. Girls, Sarnakoli High School, Wahad School, and Notun School) on the side street food card shop of Gopalganj Sadar in Bangladesh. These are the hotspots of street food trading where school-aged children can easily collect these foods.

### 2.2. Sampling Collection and Preparation

There are different types of street food that are consume by the school kids. Depending on the high rate of consumption, I have collected three types of street food that includes first one is Vorta that is prepared from potatoes with addition of spices. Second one is Panipuri is a liquid street food which made from water and combination of different types of spices. Last one is Ice Ball which core composition is ball shaped ice that is covered with various types of colored juice.

### 2.3. Bacteria Culture and Isolation

For the isolation of bacteria 1 mL of sample is pour into mannitol salt agar (MSA) and EMB medium respectively and raping the petri plate with parafilm and place in the incubator for overnight at 37 degree that gives the grow of bacterial colony on the medium according to the diluted sample that are loaded in the petri plate. After incubation the bacterial culture in the MSA and EMB medium, a loopful culture from EMB medium is streaked over the fresh EMB medium and another loopful culture of bacterial colony from MSA medium is streaked over the fresh MSA medium and wrapped with parafilm and placed into the incubator for growth of the streaked colony.

### 2.4. Characterization and Identification

After the streaking process the single colony which is arisen from the streaking is taken into the glass slide with the help of sterile loop and smearing the bacterial sample on the glass slide and then fix the smearing sample by heat and allow them to air dry. Then the gram staining procedure is performed by adding 4 reagent (Crystal violet, Iodine, Ethanol, Safranine) respectively. Between addition of two reagent, the glass slide should be washed with the distilled water. When the proper staining done it is time to visualize the bacteria under the microscope. After morphological studies by microscopic observation, different types of biochemical test is performed for identification of bacteria. Biochemical test includes MR test, VP test, Catalase test, Oxidase test, Citrate test, MIU test.

## 3. Results

### 3.1. Staining Result

After Gram staining procedure, these types of bacterial colony observed under microscope and both Gram positive and negative bacteria is identified. Visualized bacteria are morphologically rod in shape and coccus in shape.
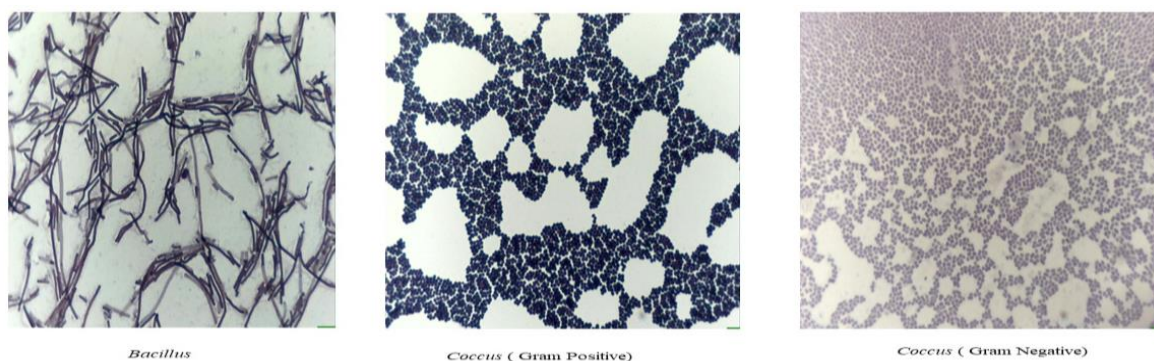
**Figure 1.** Morphological studies of isolated bacteria.

**Table 1.** Isolated bacteria from food sample.

| Vorta | Panipuri | Iceball |
|---|---|---|
| Gram staining (+) | Gram staining (+) | Gram staining (−) |
| *Bacillus* | *Coccus* | *Coccus* |

*3.2. Antibiotic Susceptibility Result*

The disk diffusion method, as described by the Clinical and Laboratory Standards Institute, was used to test antimicrobial susceptibility. For the antibiotic susceptibility testing nutrient medium is prepared by exact composition of peptone, agar, NaCl, yeast extract. All the component are mixed in the distilled water and then autoclave the media at 121 degree centigrade for 15 min and then pore into the petri plate and allow them to dry. After then swab the bacterial culture by cotton swab method, the antibiotic disk are placed on the medium and placed into the incubator under appropriate condition and grow overnight at 37 degree. After incubation the zone of inhibition is measured and measured the diameter for antibiotic susceptibility and resistant.
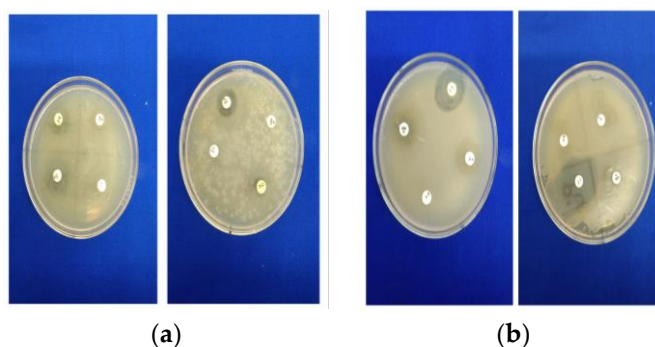
Antibiotic test (Figure 2 and Table 2).



(**a**)        (**b**)

**Figure 2.** Antibiotic susceptibility test using antibiotic disk method (**a**) Among 8 widely used antibiotic, bacteria showed resistant 5 of them; (**b**) Here also used 8 widely used antibiotic, bacteria showed resistant nature to 6 antibiotic among 8.

**Table 2.** Antibiotic resistant result.

| Antibiotics | Resistant | Intermediate | Susceptible |
|---|---|---|---|
| Cefotaxime | 100% | 0% | 0% |
| Erythromycin | 100% | 0% | 0% |
| Penicillin | 100% | 0% | 0% |
| Azithromycin | 100% | 0% | 0% |

* Antibiotic susceptibility test result.

## 4. Discussions

The purpose of this study was to determine the prevalence of *Staphylococcus*, *Streptococcus*, *Salmonella* in street foods, as well as the prevalence and severity of infection. Antimicrobial resistance pattern of isolated bacteria against commonly used antimicrobials in Gopalganj Bangladesh. The current study found a significant prevalence of isolated bacteria in selected street foods, and a similar finding was reported in Vietnam [1]. In this antibiotic susceptibility testing, vorta exhibit high rate of antibiotic resistance against Erythromycin, Penicillin, Azithromycin, Cefotaxime, Tetracycline that show 100% resistance. In case of Ice Ball Erythromycin, Penicillin shows 100% resistant. However, a lack of proper training, awareness, and poor personal hygiene contribute to the contamination of street foods continue, and the public health risk will rise [10]. The presence of pathogens with a high microbial load in foods is a good indicator of food quality and the potential health risk they pose to consumers. Amoxicillin and Tetracycline are the most commonly used antibiotics in Bangladesh, which may be the cause of the higher resistance found in this study and the findings agreed with those of previous Bangladeshi and Indian researchers [11]. High resistance to Amoxicillin, Erythomycin by *Staphylococcus*, *Bacillus*, *Salmonella* isolates was observed in this study, and this finding is significant. Many previous studies on street foods in developing countries, including Bangladesh, have found similar results [12]. This study also confirmed the prevalence of different drug resistance patterns in *Staphylococcus*, *Streptococcus*, and *Salmonella* isolates. This could be due to the presence of multiple bacterial serovars in the various food items. Southern Brazil has a higher proportion of Salmonella enteritidis with antibiotic resistance. Antibiotic resistance can limit physicians' therapeutic options for clinical cases that require antibiotic treatment [13]. Furthermore, multi-drug resistance of foodborne microorganisms made the food safety situation more vulnerable in public health [14]. Millions of people are fed every day by street food, which offers a diverse range of inexpensive and easily accessible foods [15]. Vendors are frequently uneducated, unlicensed, and untrained in food hygiene, and they work in filthy conditions with little or no knowledge of the causes of food-borne disease [10]. The goal of this study was to isolate and identify the bacteria found in various street foods, as well as to investigate their antimicrobial susceptibility patterns.

## 5. Conclusions

The presence of MDR *Streptococcus*, *Staphylococcus*, *Bacillus*, and *Salmonella* at a high rate in street foods indicates poor hygiene in street food production and poses a significant risk for the spread of foodborne zoonoses especially in the school going kids who are very much found of these food item. The existence of MDR *Streptococcus*, *Staphylococcus*, *Bacillus*, *Salmonella* notably a high rate in the street foods cues poor hygiene in street food production and it is a major threat for the advent of foodborne zoonoses. The whole world is moving towards to the excessive use of antimicrobial product in the different areas of our daily life. The current study found a higher prevalence of *Staphylococcus*, *Bacillus*, and *Streptococcus* among street foods. Furthermore, most of the bacteria isolates from food samples were multidrug resistant. The current study's findings indicate that food-borne drug resistant bacteria are one of the most concerning issues in Gopalganj, Bangladesh.

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