Assessment of Bacterial contaminants and nutritional profilesof Mung bean Sprouts (*Vigna radiate L*)

Abstract

Mung beans (*Vigna radiate L*) are well-known for their excellent nutritional value as well as their ease of digestion. They are rich in calories, total carbohydrate, dietary fiber, protein, macronutrients and vitamins. The nutritional composition of the sprouts was assessed using procedures suggested by the AOAC (Association of Official Analytical Chemists). The prevalence of bacterial isolates namely *Pseudomonas aeruginosa*, *Serratia marcescens*, *Enterobacter cloacae complex*, *Escherichia coli*, *Klebsiella pneumoniae* ,and *Acinetobacter baumannii* in sprouted beans produced in the research laboratory, which is worrying to the health-conscious people. Antimicrobial susceptibility testing of bacterial isolates from mung bean sprouts is performed in this study in order to decide which antimicrobial agent should be utilized against certain bacterial strains. Amikacin (30µg), Cefepime (30µg), Ceftriaxone (30µg), Cefuroxime (30µg), Ciprofloxacin (5µg), Colistin (10µg), Gentamicin (10µg), Meropenum (10µg), Nalidix acid (30µg), Nitrofurantoin (300µg), Tigecycline (15µg), Cefoperazone/Sulbactum (75/30µg), and Trimethoprim/Sulfamethoxazole (25µg) were used to test the antibiotic susceptibility of bacterial isolates from mung bean sprouts.

Keywords: Mung bean sprouts, Bacterial contaminants, Antibiotics susceptibility and nutritional profiles.

1. Introduction

Mung bean *(Vigna radiate L)* sprouts are a culinary vegetable made from mung beans that have been sprouted. They can be grown by planting sprouted beans in the shade and watering them until the hypocotyls appear. Mung bean sprouts are widely grown and consumed in East and Southeast Asia, and they are relatively simple to grow, requiring only a consistent supply of water [1].

Because of its adaptability and nutritious benefits, the mung bean plant, which belongs to the Fabaceae or legume family, has been grown since ancient times and is a mainstay of Asian cuisine. Mung bean sprouts are plump, silvery-white shoots with two little yellow leaves at one end that form after mung beans are germinated. Mung beans are one of the most common forms of sprouting beans for culinary purposes. They are crunchy and slightly nutty in flavor, with high water content, and can be used cooked or raw [2].

The germs that most commonly cause food poisoning from bean sprouts are *Salmonella* and *Escherichia coli (E. coli).* Other bacteria, including *Bacillus cereus, Staphylococcus aureus,* and *Listeria monocytogenes,* have been reported to cause disease when sprouts are consumed.

Microbial surveys show that there are high populations of aerobic bacteria in sprouts [3].There are different incidences of *Salmonella* or *E.coli* O157:H7 in sprouts. Mung bean sprouts (maskalai-Bengali name) is commonly consumed in Bangladesh. There is not much information on the quality of mung bean sprouts and other sprouts in this region. Therefore, the objective of the study was to

determine the risks associated with different types of sprout specially bean sprouts and to assess the nutritive values of domestic producing sprouts in Bangladesh.

2. Materials and Methods

2.1 Sprouts Sample Seed Collection

Sprouting mung bean seeds are extremely straightforward and raw seeds that have not been chemically treated. We purchased seeds avoiding toasted or roasted and avoiding milled or cracked sprouting seeds. We collected different types of sprouting seeds from different retail seed vendors like Hydrophonic Shad Krishi seed vandar, Seed Bazar BD and BD Garden Seed, Dhaka, Bangladesh.

Fig.1: Mung bean sprouts production

Fig.2: Harvested Mung bean sprouts

Fig.3: Dry sprouts for nutritional analysis

2.2 *Sampling:*

A total of 17 batches of bean sprouts samples were produced in the laboratory by maintaining aseptic condition at room temperature from July 2020 to April 2021. At each batch, 20 gram of sprouts was taken in sterile stomacher bag and then added 180 ml of sterile distilled water. After that blended with blender for 1 minutes until homogenized mixed well that is stock samples for further analysis.

2.3 Microbial analysis of bean Sprouts

Sprouts after growing of each batch of sprouting from 10.0 g of sprouts were suspended in 90 ml of sterile distilled water. The flasks were placed on a rotary shaker for 15 minutes and ten - fold serial dilutions were prepared. Aliquots of 0.1 ml of appropriate dilutions were spread on plate containing Plate count Agar (MacConkey Agar, Chromogenic Agar) and plates were incubated at 37^0C for 24-48 hours. The plates were observed after incubation for growth and colony characteristics.

Viable bacteria counting formula:

$$STANDARD\ FORMULA = \frac{Colony\ count\ on\ agar\ plate}{Total\ dilution\ of\ tube\ X\ Amount\ plated}$$

2.4 Cultivation of Bacterial Pathogens

Bacterial hazards were determined by spread plating of appropriate dilutions (10^-5 and 10^-6) of sprout homogenate (10 g sample in 90 ml of sterile distilled water) on Chromogenic agar (Biomaxima, Poland), MacConkey agar, Sorbitol (Biomaxima, Poland), SS agar (Biomaxima, Poland) and SDA agar (Biomaxima, Poland). The pathogens were counted after 24-48 hours of incubation at 35^0±2 C.

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2.5 Biochemical Parameters Analysis

For identification of the bacterial pathogens, standard biochemical parameters were performed in the laboratory and finally, antibiotics susceptibility and minimum inhibitory concentration (MIC) were confirmed with the VITEK®-2 Compact (BioMerieux, SA France).

2.6 Nutritional Value Assessment

Moreover, the nutritional composition evaluations of the sprouts were analyzed by using AOAC (Association of Official Analytical Chemists) recommended methods. Parameters of nutritional values were analyzed during the study period, such as protein, total fat, saturated fat, sodium, iron, manganese, magnesium, phosphorus, total carbohydrates and vitamins.

3. Statistical Data Analysis

Data obtained were analyzed by SPSS version 20 and Excel 2016. The percentage of frequencies was generated for categorical variables such as rate of isolation, type of bacteria, rate of antibiotic sensitivity, resistance, intermediate of the organisms.

4. Results and Discussion

In the present study microbial isolates, nutritional compositions and antibiotic susceptibility testing of bacterial isolates from mung bean sprouts was done among the 17 (seventeen) number of sprouts production batches with 31 positive bacterial contaminates were analyzed by gold standard culture methods following by Online Bacteriological Analytical Manual, USFDA for detection, enumeration and identification of individual organisms (USFDA, 2001). The observations of the experiments conducted were discussed as below:

Morphological characteristics of bacterial isolates

List of bacterial isolates, morphological characteristics on different dehydrated culture media from mung bean sprouts (**Table 1 and Table 2**).

Table 1: List of isolates bean sprouts on MacConkey Agar culture plates:

SL	Identified bacteria	Color	Bacterial colonies
1.	*Serratia marcescens*	Late lactose fermenter, Cream-white color	Flat, moist, non-mucoid colonies
2.	*Enterobacter cloacae complex*	Lactose fermenter, pink to red	Mucoid, moist, sticky and slimy but smaller than *Klebsiella* spp
3.	*Escherichia coli*	Lactose fermenter, red/pink	Flat, dry, pink, non-mucoid colonies
4.	*Klebsiella pneumoniae*	Lactose fermenter, pink	Mucoid, moist, sticky & slimy
5.	*Acinetobacter baumannii*	Non-Lactose Fermenter, Colorless	Transparent, round & dry
6.	*Pseudomonas aeruginosa*	Non-Lactose Fermenter, Colorless	Flat, smooth colonies, 2-3mm in diameter with greenish to brownish pigmentation.

In this study, 45% of bean sprout organisms, namely *Enterobacter cloacae complex* (26%), *Escherichia coli* (9%) and *Klebsiella pneumonie* (10%) that fermented lactose (LF) and thereby produced an acidic environment, appeared as pink colonies because of the neutral red turning red, and

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20% of non-fermenters (NLF), for example, *Acinetobacter baumannii* (10%) and *Pseudomonas aeruginosa* (10%), produced normally colorless colonies, but the rest of 35% were late lactose fomenters (LLF) *Serratia marcescens* produces a red pigment at 35°C (**Fig. 4, 5, and 6**). According to epidemiological investigations, Park CE and Sanders DW also isolated microbial hazards of *Klebsiella pneumoniae* and other bacterial isolates of LF, LLF, and NLF from mung bean sprouts that resembled our studies [4,5].

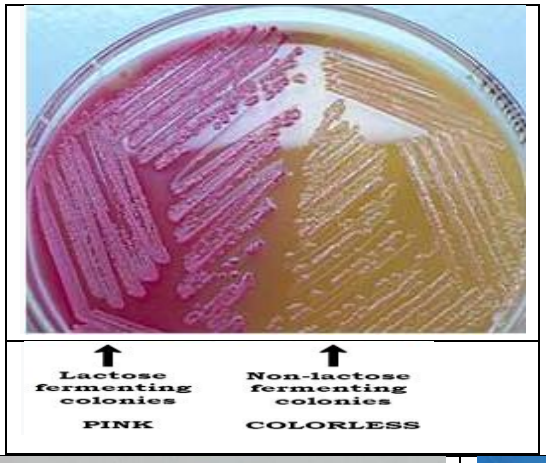

Fig.4: Lactose and non lactose fermenting bacterial colonies from sprouts samples on MacConkey agar plate.

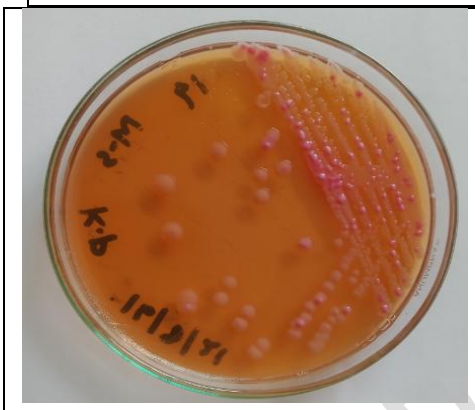

Fig.5: *Klebsiella pneumoniae* cultural growth on MacConkey agar plate

Fig.6: *Enterobacter cloacae complex* cultural growth on MacConkey agar plate

Figure7: *Klebsiella pneumoniae* cultural growth on Chromogenic agar plate

Table 2: List of isolates bean sprouts on Chromogenic Agar culture plates

Sl	Identified bacteria	Color	Bacterial colonies characteristics
1.	*Serratia marcescens.*	Light blue	Flat, moist, non-mucoid colonies
2.	*Enterobacter cloacae complex*	Blue	Mucoid, moist, sticky and slimy but smaller than *Klebsiella* spp
3.	*Escherichia coli*	Pink-purple	Flat, dry, pink, non-mucoid colonies
4.	*Klebsiella pneumoniae*	Deep blue to purple, mucoid	Mucoid, moist, sticky & slimy
5.	*Acinetobacter baumannii*	Colorless	Transparent, round & dry

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6.	*Pseudomonas aeruginosa*	Colorless (greenish pigment may be observed)	Flat, smooth colonies, 2-3mm in diameter with greenish to brownish pigmentation.

Sprouts contaminated with bacterial colonies produced multiple colors of colonies on the chromogenic agar plates, similar to *Serratia marcescens, Enterobacter cloacae complex, Escherichia coli,* and *Klebsiella pneumoniae,* which showed light blue, blue, pink-purple, and deep blue to purple colonies, respectively, but *Acinetobacter baumannii* and *Pseudomonas aeruginosa* produced colorless colonies.

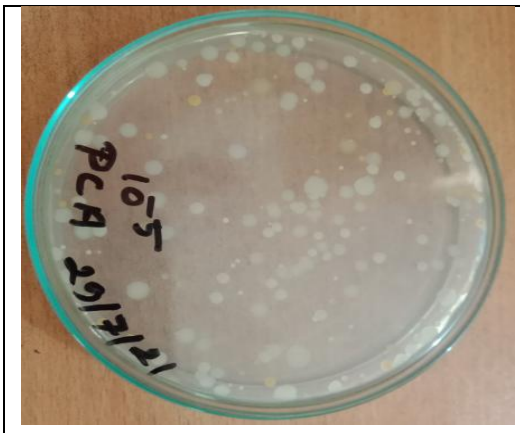

Fig.8: Total aerobic bacterial count on Plate count agar medium (Countable, 30-300CFU/g)

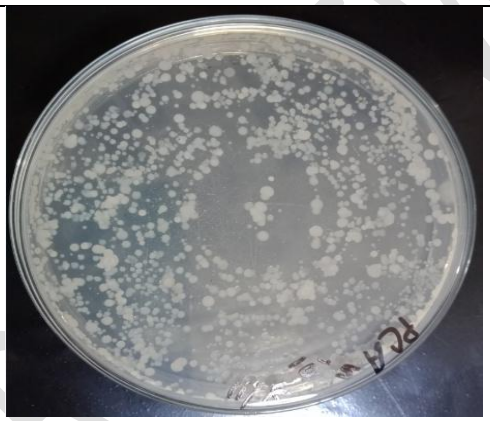

Fig.9: Total aerobic bacterial count on Plate count agar medium (Uncountable, >300CFU/g)

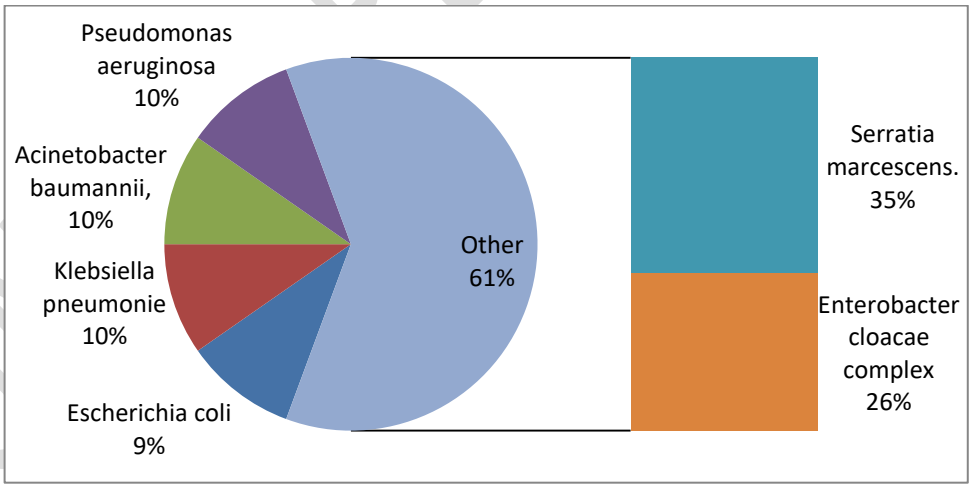

Fig.10: Occurrence of bacterial isolates from sprouts (Mung bean) sample.

Bacterial contaminants were isolated and identified through the standard biochemical parameters such as the catalase test, coagulase test, oxidase test, motility-indole urease test, citrate utilization test, and triple sugar iron test. Finally, all the suspected cultures were confirmed with the VITEK®-2 Compact automated ID/AST instrument (BioMerieux, France). The analyzed antibiotic susceptibility parameters are shown in **Table 3,** and the MIC of antibiotics is given in **Table 4.**

Table 3: Results of biochemical tests of the isolated bacterial species from sprouts samples.

Bacterial hazards	Gram Reaction	KIA				MIU			Oxidase	S.citrate	Catalase
		Slant	Butt	Gas	H_2S	Mot	Indole	Urease			
Serratia marcescens.	G-Ve	R/Y	Y	±	-	+	-	±	-	+	+
Enterobacter cloacae complex	G-Ve	Y	Y	+	-	+	-	-	-	+	+
Escherichia coli	G-Ve	Y[2]	Y	+2	-	+1	+3	-	-	-	+
Klebsiella pneumoniae	G-Ve	Y	Y	+	-	-	±	+	-	+	+
Acinetobacter baumannii	G-Ve	R	Y	+	_	+		-	-	±	+
Pseudomonas aeruginosa	G-Ve	R	R	-	-	+	-	±	+	+	+

Note: KIA = Kligler's Iron Agar *test*, MIU=Motility indole urease test, **(**+) =Positive; (-) =Negative reaction; (±)= Variable; R=Red (Alkaline reaction);Y=Yellow (Acid reaction); W=Weak positive, H2S=Hydrogen sulphide; 1-A few strains are non-motile; 2-A few strains produce red-pink stant; 3-Aminority strains give a negative result. Cat=Catalase test, Mot=Motility test.

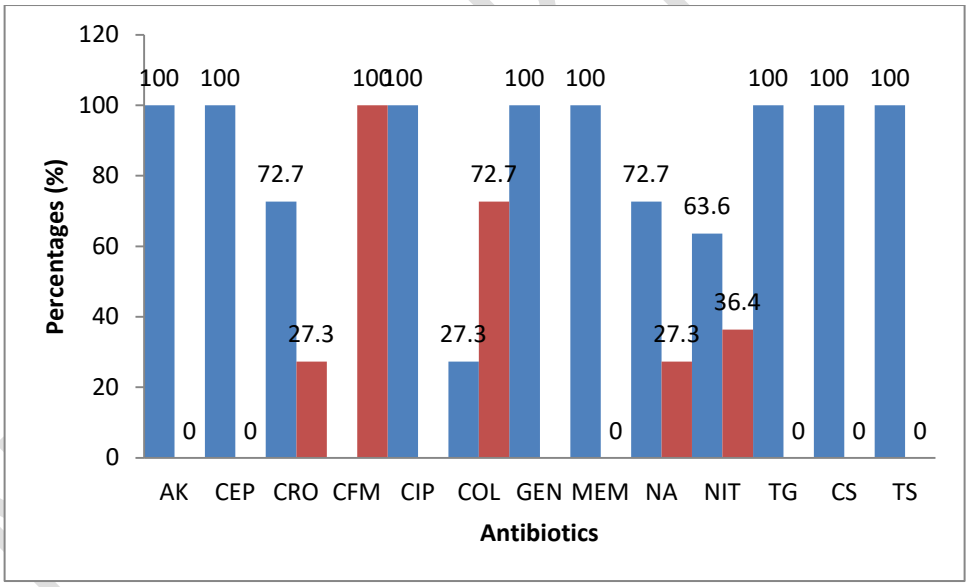

Fig.11: Anti-biogram sensitivity and resistant pattern of *Serratia marcescens* .

Note: Amikacin (AK), Cefepime (CEP), Ceftriaxone (CRO), Cefuroxime (CFM), Ciprofloxacin (CIP), Colistin (COL), Gentamicin (GEN), Meropenum (MEM), Nalidix acid (NA), Nitrofurantoin (NIT), Tigecycline (TG), Cefoperazone/Sulbactum (CS), Trimethoprim/Sulfamethoxazole (TS)

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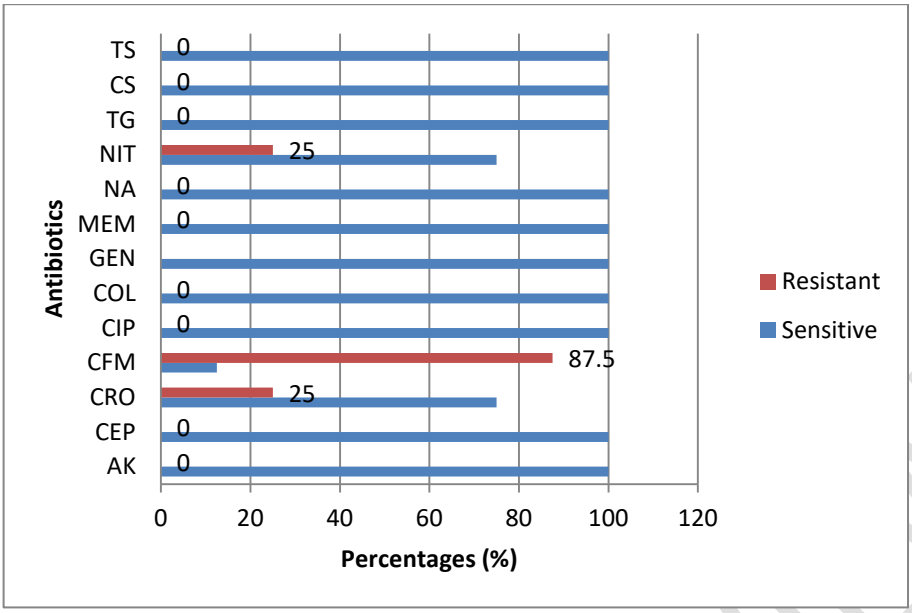

Fig.12: Anti-biogram sensitivity and resistant pattern of *Enterobacter cloacae complex*

Table 4: In-vitro antibiogram profile of Gram-negative bacterial isolates.

Bacterial isolates	Patterns	Amikacin	Cefepime	Ceftriaxone	Cefuroxime	Ciprofloxacin	Colistin	Gentamicin	Meropenum	Nalidix acid	Nitrofurantoin	Tigecycline	Cefoperazone/ Sulbactum	Trimethoprim/ Sulfamethoxazole
Escherichia coli (n=3)	S	100	100	100	66.67	100	100	100	100	100	100	100	100	100
	R	00	00	00	33.33	00	00	00	00	00	00	00	00	00
Klebsiella pneumonie (n=3)	S	100	100	100	66.67	100	100	100	100	100	66.67	100	100	100
	R	00	00	00	33.33	00	00	00	00	00	33.33	00	00	00
Acinetobacter baumannii (n=3)	S	100	100	100	33.33	100	100	100	100	100	66.67	100	100	100
	R	00	00	00	66.67	00	00	00	00	00	33.33	00	00	00
Pseudomonas aeruginosa (n=3)	S	100	100	66.66	33.33	100	100	100	100	100	100	100	100	100
	R	00	00	33.33	66.66	00	00	00	00	00	00	00	00	00

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Note: S=Sensitive, R=Resistant

In this experiment, *Serratia marcescens* and *Enterobacter cloacae complex* isolates were resistant to cefuroxime at 11/11 (100%) and 7/8 (87.5%), respectively, but both *Acinetobacter baumannii* and *Pseudomonas aeruginosa* were resistant at 2/3 (66.67%). *Klebsiella pneumoniae and Escherichia coli* were resistant to cefuroxime by 33.67%.

All the bacterial strains of sprouts showed 100% sensitive to amikacin, cefepime, ciprofloxacin, colistin, gentamicin, meropenum, tigecycline, cefoperazone/sulbactum, trimethoprim/sulfamethoxazo but few percentages of ceftriaxone, cefuroxime, ciprofloxacin, colistin, gentamicin, meropenum, nalidix acid, and nitrofurantoin were sensitive (**Fig.13 & 14).**

Table 5: In-vitro antibiotics MIC (minimum inhibitory concentration) of Gram-negative Bacterial isolates.

Antibiotics	***Bacterial hazards isolates***			
	Serratia marcescens (n=11)	***Enterobacter cloacae complex (n=8)***	***Klebsiella pneumoniae (n=3)***	***Acinetobacter baumannii (n=3)***
Amikacin (AK)	<=2 mcg/ml	<=2 mcg/ml	<=2 mcg/ml	<=2 mcg/ml
Cefepime (CEP)	<=1 mcg/ml	<=1 mcg/ml	<=1 mcg/ml	<=8 mcg/ml
Ceftriaxone (CRO)	<=1 mcg/ml	<=1 mcg/ml	<=1 mcg/ml	<=16 mcg/ml
Cefuroxime (CFM)	16 mcg/ml	4 mcg/ml	-	-
Ciprofloxacin (CIP)	<=0.25mcg/ml	<=0.25mcg/ml	<=0.25mcg/ml	<=0.25mcg/ml
Colistin (COL)	>=16 mcg/ml	<=0.5 mcg/ml	<=0.5 mcg/ml	<=0.5 mcg/ml
Gentamicin (GEN)	<=1 mcg/ml	<=1 mcg/ml	<=1 mcg/ml	<=1 mcg/ml
Meropenum(MEM)	<=0.25mcg/ml	<=0.25mcg/ml	<=0.25mcg/ml	<=0.25 mcg/ml
Nalidix acid (NA)	4 mcg/ml	<=2 mcg/ml	<=4 mcg/ml	-
Nitrofurantoin (NIT)	128 mcg/ml	<=32 mcg/ml	<=64 mcg/ml	-
Tigecycline (TG)	2 mcg/ml	<=1 mcg/ml	<=1 mcg/ml	<=0.5 mcg/ml
Cefoperazone/ Sulbactum (CS)	<=8 mcg/ml	<=8 mcg/ml	<=8 mcg/ml	<=8 mcg/ml
Trimethoprim/ Sulfamethoxazo(TS)	<=20 mcg/ml	<=20 mcg/ml	<= 20mcg/ml	<=20 mcg/ml

In this study, we discovered that different marketed antibiotics have varying minimum inhibitory concentrations (MIC). The highly MIC of meropenum (=0.25mcg/ml), ciprofloxacin (=0.25mcg/ml), gentamicin (=1 mcg/ml), and amikacin (=2 mcg/ml) were represented as highly MIC of Serratia marcescens (n=11*)*, *Enterobacter cloacae complex* (n=8), *Klebsiella pneumoniae* (n=3), nitrofurantoin had the lowest MIC, which was 128 mcg/ml.

Nutritional Value Assessment

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The nutritional composition evaluations of the sprouts were analyzed by using AOAC (Association of Official Analytical Chemists) recommended methods. The nutritional profiles of sprouts (mung bean) were examined in the present study as follows (Table 6).

Table 6: Nutritional parameters of sprouts by AOAC recommended methods.

SL	Parameters	Unit	Dry Sprouts	Raw sprouts
1.	Calories	kcal/100g	354.24	28.8
2.	Total Carbohydrate	%	54.84	2.73
3.	Dietary Fiber	%	14.5	4.1
4.	Sugar	%	13.6	7.5
5.	Protein	%	32.37	3.03
	Fat			
6.	Total Fat	%	0.6	0.64
7.	Saturated Fat	g/100g	< 0.005	< 0.005
8.	Trans Fat	g/100g	< 0.005	< 0.005
	Macronutrients:			
9.	Magnesium (Mg)	mg/kg	457.0	130.0
10.	Phosphorus (P)	%	0.22%	0.02
11.	Potassium (K)	mg/kg	6025.0	8218.0
	Micronutrients:			
12.	Copper (Cu)	mg/kg	< 0.2	< 0.2
13.	Manganese (Mn)	mg/kg	20.0	20.0
14.	Iron (Fe)	mg/kg	69.0	135.0
15.	Zine (Zn)	mg/kg	45.0	92.0
16.	Sodium (Na)	mg/kg	151.0	340.0
	Vitamins :			
17.	Vitamin-C (Ascorbic acid)	%		0.87
18.	Vit-B9 (Folic acid)	ppm	312	7.8

Mung bean sprouts are high in a variety of vitamins and minerals. While the exact nutrient ratio varies based on the type of sprout, they often contain significant levels of folate, magnesium, phosphorus, vitamin C (ascorbic acid), and vitamin B9 (riboflavin) (folic acid). They actually contain more of these nutrients than fully developed forms of the same plants [6].

Sprouts are a fantastic food with a high nutritional value. Grain or legume seeds are germinated to generate this superfood, which has more than 28.8 kcal/100g of calories in raw sprouts and 354.24 kcal/100g of calories in dry sprouts, respectively. This report has a higher score than the others [7]. Sprouts are high in protein and high in dietary fiber. In our study, raw and dried mung bean sprouts had 4.1 percent and 14.5 percent dietary fiber, respectively, whereas raw and dried sprouts included 3.03 percent and 32.37 percent protein. Surprisingly, sprouts contain a lot of fat as well as macro and micronutrients that are important for good health.

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Conclusion

The goal of this study was to look at bacterial contamination as well as nutritional characteristics. Six types of bacterial isolates were isolated from several batches of sprouts grown on various culture media and were found to be responsible for sprouts contaminated with organisms. If eaten raw or semi-cooked, these microscopic species can cause foodborne disease. This study also looked at the antibiotic susceptibility patterns of all the isolates. *Acinetobacter baumannii, Pseudomonas aeruginosa, Klebsiella pneumonie,* and *Escherichia coli* isolates were entirely resistant to cefuroxime, while isolates from *Acinetobacter baumannii, Pseudomonas aeruginosa, Klebsiella pneumoniae,* and *Escherichia coli* were only somewhat resistant. The nutritional content of dry sprouts is higher than raw sprouts. As a result, as a developing country, we can use it as an alternate source of nourishment to help us recover from malnutrition. As a result, raw sprouts should be ingested with caution, regardless of their source, i.e., whether they are prepared at home or bought at a store. Finally, we should attempt or make efforts to determine its eligibility for the development of various appealing food items from sprouts. Sprouts have a long list of health advantages. Because of the number of outbreaks linked to the presence of harmful organisms, proper safety requirements should be observed.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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