

## BACTERIOLOGICAL STUDY OF SOME COMMON DRIED SPICES AND NUTS OF BANGLADESH

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### Abstract

The cultivation of spices and nuts in different parts of the world characterized by high humidity and warm climate provides optimum conditions for the development of microorganisms, including the undesirable ones. To identify the microbial risk factor associated with dried spices and nuts samples available in local markets of Bangladesh, samples were collected and analyzed through standard microbiological procedure. In all the spices and nuts samples, the total aerobic heterotrophic bacterial (TAHB) level was below the standard unacceptable range ( $>10^6$  CFU/g). Both Gram positive and Gram negative pathogenic bacteria viz., *Staphylococcus aureus*, *S. epidermidis*, *Bacillus* sp. and *Enterobacter* sp. were isolated from samples through screening staining properties, genus and group specific characters on selective and differential media along with morphological and biochemical tests. It could be assumed that dried spices and nuts might be the carrier of these types of pathogenic bacteria in foods when cooked using these ingredients and causes food borne illness.

### Introduction

Food poisoning is a common and sometimes life-threatening problem for millions of people all over the world. Foodborne illness resulting from eating food contaminated with bacteria or their toxins are most common and usually caused by bacteria such as *Campylobacter*, *Salmonella*, *Shigella*, *E. coli*, *Listeria* and *Clostridium botulinum* (Ahmed *et al.* 2014).

All over the world spices are virtually indispensable in the culinary art and used for flavor, color, aroma, taste and preservation of food and beverages. The spices are produced from roots, barks, leaves, bulbs, stems, flowers and seeds of certain plants and mostly grown and harvested in warm, humid areas of the world (Aguilera *et al.* 2005). On the other hand, worldwide, nuts are esteemed and highly priced food delicacy because of their pleasant taste and flavor in addition to their content of proteins and antioxidants (Alhussaini 2012). Different kinds of edible nuts including almonds, peanuts, hazelnuts, pistachios, walnuts and cashew nuts in addition to many dried fruits are frequently imported in Bangladesh for consumption. Although nuts and spices have continued to be popular food commodities, possible contamination with certain pathogenic microorganisms is a major safety and quality concern for both the industry and consumers. The spices may get contaminated by unwanted microorganisms during their growth and development or when these are passed through collection, processing, storage and marketing (Khan 2012). The traditional methods of harvesting and preparations of these products result in contamination. Finally, at consumers' level these are obviously found with multitude of organisms (Das 2002).

Many workers reported alarming microbiological quality of spices from different parts of the world (Antai 1988, Satchell *et al.* 1989, Koch *et al.* 2005, Moreira *et al.* 2009) and instances of recalls of nut and spice products in USA and Canada (CDC 2009). The high microbial loads in spices and nuts prompted an upsurge in monitoring and research related to these food commodities

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to maintain the quality of nuts and spices all over the world. However, Indian subcontinent, especially in Bangladesh very little attention has been given on this matter. Therefore, the present study was conducted to investigate the microbial loads and identify the pathogenic bacteria associated with microbial contamination of spices and nuts available in the commercial market of Bangladesh.

### Materials and Methods

Six different spices and three nuts were collected from different local markets of Dhaka city. Samples were collected in sterilized plastic bags, closed the opening, brought into the laboratory immediately and preserved for detailed study. The entire experiment was carried out in the laboratory of Microbiology of Botany department, Jahangirnagar University.

Bacterial enumeration and isolation were carried out by spread plate method in nutrient agar medium (NA) (Eklund and Lankford 1967) at pH 6.0. One gram of sample was suspended in 100 ml of sterile water in a conical flask and vortexed vigorously for five min. The plates were inoculated with ten-folds diluted suspension and incubated at 37°C for 24 hrs in an incubator (Memmert GmbH + Co Kg 8540 Schwabach) at the inverted condition. After incubation, plates having well discrete colonies were selected for counting. Discrete bacterial colonies were isolated immediately after counting. Based on distinct colony morphology, further selection was made and isolates were purified by repeated streaking and stored in NA slants at 4°C for further analysis.

The selected bacterial colonies were observed to study various characters *viz.*, color, form, elevation, margin, surface, optical characters (Eklund and Lankford 1967). Bacterial colonies were isolated and cultured on different selective and differential media such as: MSA, SSA, EMB, MacConkey, Bouillon agar, King's B, XLD, Simmon Citrate etc. Presence of *Staphylococcus aureus* and *S. epidermidis* was confirmed by the appearance of yellow and pink colonies on MSA media respectively. Presence of *Bacillus* sp. was confirmed by growth of white colonies on Bouillon agar media while the presence of *Enterobacter* was confirmed by the appearance of pink colonies on EMB medium. Different major biochemical tests *viz.*, casein, fermentation, indole, starch hydrolysis, catalase were also performed. Results of the physiological and biochemical tests of selected isolates were analyzed following Bergey's Manual of Systematic Bacteriology (Sneath *et al.* 1986). Coagulase test and endospore staining were used as a confirmation test of bacterial genus or species of *Staphylococcus aureus* and *Bacillus* sp., respectively.

### Results and Discussion

Six different spices and three nuts were collected from different local markets of Dhaka city (Table 1). Total aerobic heterotrophic bacterial (TAHB) load of the spice and nut samples were found to range from  $4 \times 10^2$  to  $2.1 \times 10^5$  CFU/g on nutrient agar media (Table 1). In case of spices, maximum heterotrophic bacterial counts were observed in the fennel sample. While, the lowest bacterial counts were found in cinnamon sample. According to guidelines elaborated by the International Commission on Microbiological Specifications for Foods (ICMSF 2005), the total bacterial count in spices below  $10^4$  CFU/g is indicative of their acceptable quality, the count of  $10^4$  -  $10^6$  CFU/g indicates their permissible quality, whereas bacterial count exceeding  $10^6$  CFU/g is unacceptable. In view of these guidelines, CFU results showed relatively good quality of the analyzed spices (Table 1). In case of spice cinnamon, TAHB did not exceed  $10^4$  CFU/g, which is consistent with the available data proving the antimicrobial activity of this spice (Nabavi *et al.* 2015). However, research around the world presented different scenario where in some cases spices and nut samples were contaminated with different bacterial species which are higher than acceptable limit *viz.*, black cumin seeds in Saudia Arabia (Al-Jassir 1992) (with  $7 \times 10^7$  CFU/g)

while in India were in the range from  $1 \times 10^4$  CFU/g to  $1 \times 10^8$  CFU/g (Bhar *et al.* 1987), all these values were higher than the present observed value for the cumin. Similarly, in black pepper TAHB was also found to be higher than acceptable range in different regions (Banerjee and Sarkar 2003). In addition, in ground cinnamon of Turkey CFU/g was  $5.2 \times 10^3$  to  $1.2 \times 10^5$  (Karapinar and Aktug 1988), clove oil in Australia (Pafumi 1986) and India (Sharma *et al.* 1984) recorded  $1 \times 10^2$  and  $8.7 \times 10^2$  CFU/g respectively, which was lower than the present findings. Furthermore, Stankovic *et al.* (2006) also demonstrated that about 50% of their analyzed spice samples were contaminated with bacteria higher than the acceptable limit. These varying degrees of microbial contamination might be due to different factors, viz., natural antibacterial effects of spice (Burt 2004) and handling and processing involved in production (Witkowska *et al.* 2011).

**Table 1. Collected common dried spices and nuts samples and their bacterial load (CFU/g).**

Sample no.	Bengali name	English name	Scientific name	Used part	CFU/g
1.	Ellachi	Lesser cardamom	<i>Elettaria cardamomum</i>	Seedpod	$2.61 \times 10^4$
2.	Daruchini	Cinnamon	<i>Cinnamomum zeylanicum</i>	Bark	$6.6 \times 10^3$
3.	Golmorich	Black pepper	<i>Piper nigrum</i>	Drupe	$1.5 \times 10^4$
4.	Kalozira	Black cumin	<i>Nigella sativa</i>	Seed	$6.1 \times 10^4$
5.	Mouri	Fennel	<i>Foeniculum vulgare</i>	Seed	$2.1 \times 10^5$
6.	Long	Clove	<i>Syzygium aromaticum</i>	Flower bud	$1.41 \times 10^4$
7.	Kath badam	Almond	<i>Prunus dulcis</i>	Seed	$2.37 \times 10^3$
8.	China badam	Peanut	<i>Arachis hypogaea</i>	Seed	$4 \times 10^2$
9.	Kaju badam	Cashew nut	<i>Anacardium occidentale</i>	Seed	$1.47 \times 10^3$

Identified bacteria of isolated bacterial colonies from spices and nuts samples are shown in Table 2. In the present study the identified bacteria were *Staphylococcus aureus* (Fig.1B), *S. epidermidis* (Fig.1B), and *Enterobacter* sp. through their respective color in specific culture medium. In addition, results of biochemical tests of different isolates revealed that out of 17 isolates 16 were Gram positive and 1 was Gram negative (Fig.1C) and rod shaped. Among the Gram positive isolates 11 were cocci (Fig.1D) and 5 were rod shaped. All the isolates were catalase positive, casein negative, lactose fermentation negative and indole negative (Table 3). Out of 17 isolates only 5 were positive in starch hydrolysis test (Fig.1E) and endospore staining showed the presence of *Bacillus* sp. Among 11 cocci shaped bacteria 10 were positive for coagulase test (Fig. 1F) which confirmed the presence of *Staphylococcus aureus*.

Instead of low CFU count in the present examined samples, presence of pathogenic bacteria, *Staphylococcus aureus*, *S. epidermidis*, *Bacillus* sp. and *Enterobacter* sp. suggested health risk to the consumers (Table 2). The presence of different pathogenic bacteria has been reported by different authors in spice samples also. For example, *Escherichia coli*, *Serratia* sp., *Klebsiella* sp., *Bacillus* sp., *Staphylococcus* sp. and *Streptococcus* sp. were identified from the composite sample of black and red pepper in the United States (Christensen *et al.* 1967). Salmeron *et al.* (1989) found four to six times as much pathogenic *Staphylococcus* in black pepper than white pepper samples in Spain. All of these findings are in agreement to the present study for the presence of *Staphylococcus aureus* and other *Staphylococcus* sp. in observed spice samples. Karapinar and

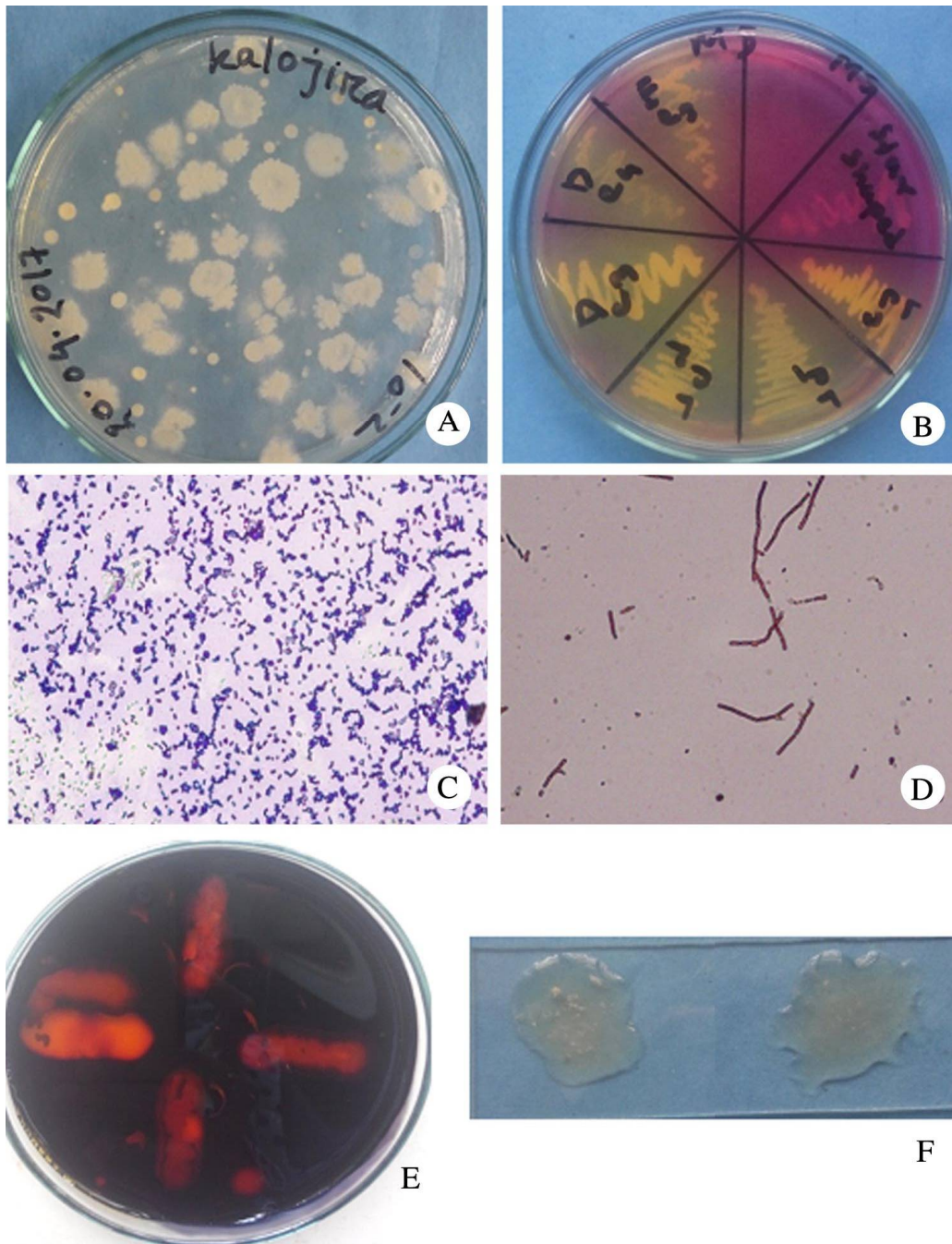


Fig 1. Bacterial isolates on nutrient agar (A), *Staphylococcus aureus* (yellow colonies) and *S. epidermidis* (pink colonies) on MSA media (B), Gram staining represents Gram positive (cocci shaped) *S. aureus* (C) and rod shaped Gram negative *Enterobacter* (D), starch hydrolysis test (E), coagulase test (+ (left) - (right) (F)).

**Table 2. Results of some major biochemical tests of isolated bacterial colonies from spices and nuts samples.**

Sample	Isolate No.	Gram reaction	Catalase	Casein	Fermentation	Starch hydrolysis	Indole	Coagulase	Endospore	Identified Bacteria
Clove, Cinnamon, Lesser Cardamom	LDEC1	+	+	-	-	+	-	-	+	<i>Bacillus</i> sp.
Clove	LC2	+	+	-	-	-	-	+	-	<i>Staphylococcus aureus</i>
	LC3	+	+	-	-	-	-	+	-	<i>S. aureus</i>
Cinnamon	DC4	+	+	-	-	-	-	+	-	<i>S. aureus</i>
	DC5	+	+	-	-	-	-	+	-	<i>S. aureus</i>
Lesser Cardamom	EC6	+	+	-	-	-	-	+	-	<i>S. aureus</i>
Fennel	KC1	-	+	-	-	-	-	-	-	<i>Enterobacter</i> sp.
	KC2	+	+	-	-	-	-	+	-	<i>S. aureus</i>
Black cumin	KC3	+	+	-	-	-	-	+	-	<i>S. aureus</i>
	KC4	+	+	-	-	-	-	-	-	<i>S. epidermidis</i>
Black pepper	KC5	+	+	-	-	-	-	+	-	<i>S. aureus</i>
	KC6	+	+	-	-	-	-	+	-	<i>S. aureus</i>
	KC7	+	+	-	-	-	-	+	-	<i>S. aureus</i>
Cashewnut	NC1	+	+	-	-	-	-	-	+	<i>Bacillus</i> sp.
	NC2	+	+	-	-	-	-	-	+	<i>Bacillus</i> sp.
Almond	NC3	+	+	-	-	-	-	-	+	<i>Bacillus</i> sp.
Peanut	NC4	+	+	-	-	-	-	-	+	<i>Bacillus</i> sp.

Aktug (1988) studied the microbiological quality of ground cinnamon with emphasis on occurrence of *Bacillus cereus* in Turkey. Microbial content of eight Nigerian spices was determined by Ofuya and Uduma (1988) who found *Bacillus cereus* only in 15% of the samples tested. Seenappa and Kempton (1981) found *Bacillus* sp. as predominant microflora in dry, unprocessed Indian spices in a study conducted in Canada. *Bacillus* sp. was also present in the present spice samples. Therefore, it can be assumed that *Staphylococcus aureus*, *S. epidermidis*, *Bacillus* sp. are the most common bacteria that contaminated the spices and nuts all over the world especially in tropical countries. The presence of *Bacillus*, *Enterobacter* and *Staphylococcus* sp. like pathogenic bacteria made the dried foods health hazard. *Staphylococcus* sp. is one of the most common bacteria that could cause food poisoning. It is a pathogenic bacterium which can produce enterotoxin in foods. *Bacillus cereus* is also associated with food poisoning. It is able to produce endospores which persist in foods for long time and cause foodborne illness. All of these bacteria could cause serious health problems to the consumers when consumed with contaminated spices or nuts. Therefore, microbiological control is very important in food industry to prevent food poisoning and other foodborne illness during handling and processing of these spices and nuts.

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