# Safety Assessment of Spices and Herbs Consumed In Saudi Arabia: Microbiological Quality and Toxin Production

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**Abstract:** This study was carried to estimate the bacterial load and the presence of five enterotoxigenic bacterial pathogens included *Salmonella* spp., *Listeria monocytogens*, *Bacillus cereus*, *Escherichia coli* O157:H7 and *Clostridium perfringens* using multiplex polymerase chain reaction (M-PCR) on dried spices and herbs retailed in Saudi Arabia. The study further aimed to analyze antibiotic resistance rates against commonly used antibiotics among bacterial population of dry spices and herbs. Results revealed that none of them contained *Salmonella* spp. *Listeria monocytogenes* and *Escherichia coli* O157:H7. However, *Clostridium perfringens* and *Bacillus cereus* were detected in one and six samples, respectively. The bacterial loads ranged from 2.8 to 8.4 $\log_{10}$ CFU $g^{-1}$ for aerobic bacteria, 1 to 3.8 $\log_{10}$ CFU $g^{-1}$ for aerobic sporeformes bacteria and thermophiles and 1 to 3.3 $\log_{10}$ CFU $g^{-1}$ for coliforms. The isolates exhibited resistance in decreasing order for ampicillin (74.5%), cephalothin (69.5%), sulfonamides (36.2%), aminoglycosides (25.2%), phenicoles (19.1%), tetracycline (8.5%), fluoroquinolones (10.6%), and amoxycillin-clavulanic acid (5.0%). Maximum resistance to extended-spectrum beta-lactam antibiotics occurred in 11.3% of isolates and the production of extended spectrum beta-lactamase was achieved by 3.5% of isolates. Multiple resistances to three or more antimicrobial agents were documented. Theses investigation indicate the occurrence of resistant bacteria contaminating some spice and herb samples. Therefore quality of the products may be regularly checked to ensure safety and make them fit for human consumption.
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**Key wards**: Dried food, Bacterial load, Enterotoxigenic bacterial pathogens, Antibiotic resistance, Saudi Arabia.

## 1. Introduction

Spice and herb preparations are widely used in food formulation. Some of these preparations harbor a great number of microorganisms that may contribute to the deterioration of food products or representing a direct health hazard to consumers. Assessment of microbial load of these preparations to assure safety and quality is therefore worth investigation. The Kingdom Saudi Arabia imports spices from several countries and a lot of these preparations are commonly used in formulation of chicken parts, minced meat, and dressing of fishes as well as added to many ready to eat sandwiches. As with many other agricultural products, spices and herbs became contaminated at any point from production to consumption (de Boer *et al*., 1985; McKee, 1995; Chan, 2003). In addition, because of the overuse of antibiotics in plant agriculture and use of contaminated fertilizer or irrigated water to croplands, antibiotic resistant bacteria could colonize these herbal products (Falkiner, 1998; Witte, 1998; McManus *et al*., 2002).

Contamination of spices and herbs with food-borne pathogens and antibiotic resistant bacteria is of special concern, since these products are likely added raw or even minimally processed to foods and do represent some risk to public health (Sospedra *et al*., 2010). Untreated spices can be a source of microbial contamination (McKee, 1995) and vehicle for potential hazards (D Aoust, 1994). The outbreaks of illness associated with consumption of contaminated spices are few compared to fresh foods; however outbreaks of illness associated with the consumption of dried spices or foods containing these ingredients overseas have been reported (Gustavsen and Breen, 1984; Lehmacher *et al*., 1995).

Several microorganisms detected in spices and herbs have the potential to cause human illness including *Bacillus cereus*, *Clostridium perfringens*, *Escherichia coli*, *Salmonella* spp., *Listeria monocytogens* and *Staphylococcus aureus* (Cho *et al*., 2008; Casano *et al*., 2009). In Saudi Arabia, the microbiological quality of spices was mainly evaluated to their contents of fungi and aflatoxins (Al-Jassir *et al*., 1991; Bokhari, 2007; Hashem and Alamri, 2010; Al-Juraifani, 2011). However, limited data were obtained in relation to their bacterial content (El-Nawawy and Al-Jassir, 1991). In addition, limited information worldwide exists regarding development of antibiotic resistance due to use of these nutraceutical products (Brown and Jiang, 2008).

Therefore the study was carried out to characterize the microbiota and its attributes to the microbiological quality and safety of food related spices and herbs. The study further aimed to detect enterotoxigenic bacterial pathogens and antibiotic resistance rates among bacterial population of dry spices and herbs.

## 2. Materials and Methods

### Sample collection and preparation

A total of fifty-nine single sample of dry spices and herbs, representing different types, were purchased from retail premises (ethenic food shops, food markets, herbal shops) at Taif City, Western Region of Saudi Arabia (Table 1). Each sample weighted not less than 100 g. All samples were stored and transported under dry and ambient conditions.

### Bacteriological analysis

The initial microbial populations in the spices and herbs was determined by homogenizing 25 g of each sample with 225 ml of buffered peptone water (Oxoid) to give 0.1 dilution ($1:10^1$). Serial dilutions up to $1:10^6$ were prepared and 1 ml aliquots were pour plated in duplicates for the isolation and enumeration of total aerobic plate count (TABC) and coliforms (TC) using plate count agar (Scharlau) and violet red bile agar (Oxoid), respectively. In order to isolate and count the mesophilic spore former bacteria (MSFB) and thermophilic spore former bacteria (TSFB), a heat treatment was applied for the different dilutions at 95°C for 15 min in water bath and immediately cooled at 45°C. Standard plate counts agar for MSFB were incubated at 37°C and for TSFB were incubated at 55°C for 72 h. All microbial counts were calculated as CFU $g^{-1}$ and then transferred to $\log_{10}$ CFU $g^{-1}$.

Presence of thermotolerant *Escherichia coli* (*E. coli*) was determined using eosin methylene blue agar (Scharlau) after incubation at 44.5 °C for 24h. Concerning the identification of *Staphylococcus. aureus* (*S. aureus*), typical and presumptive *Staphylococci* spp. colonies on Baird-Parker agar supplemented with egg-yolk tellurite emulsion (Oxoid) were examined by Gram stain, coagulase, catalase and latex agglutination (Oxoid) tests. For *Pseudomonas aeruginosa*, Cetramide agar (BioLife) was used. The methods used were of the Association of Official Analytical Chemists (AOAC, 1990) and Compendium of Methods for the Microbiological Examination of Foods (Downs and Ito, 2001). The microbiological quality of dried spices and herbs samples was assessed according to the using criteria outlined in the international commission of microbiological specifications for foods (ICMSF, 2005) and European spice Association (ESA) specifications (Muggeridge and Clay, 2001).

### Multiplex PCR of pathogenic bacteria

The genomic DNA of five enterotoxigenic food-related *E. coli* O157:H7, *Bacillus cereus* (*B. cereus*), *Salmonella* spp., *Listeria monocytogenes* (*L. monocytogens*), and *Clostridium perfringens*(*Cl. perfringens*) were extracted as per the methodology of Makino *et al*., (1995). The obtained culture (approximately 1 ml) was centrifuged in a microcentrifuge (Sigma, USA) at 6000 rpm for 10 min. The recovered pellet was resuspended in 100 µl of sterilized DNAse and RNAse-free milliQ water (Millipore, USA), heated in a boiling water bath for 10 min. and then snap chilled in crushed ice. The obtained lysate (5µl) was used as a DNA template in PCR reaction mixture.

The PCR conditions were performed as previously described (Lei et al., 2008). The PCR was conducted in a Thermal Cycler *PXE*-0.5 (THERMO; Electron Corporation) and the amplified products were sized by electrophoresis on 1.5% agarose gels. Sequences targeting the *hly* gene in *L. monocytogenes* (Furrer *et al*., 1991), enterotoxin *cpe* gene *Cl. perfringens* (Fach and Popoff, 1997). Enterolysin *hly*A gene (O157-3 and O157-4) in *E. coli* O157:H7 and *hly* gene in *B. cereus* (Wang *et al*., 1997), meanwhile invasion *invA* gene (Sal-3 and Sal-4) in *Salmonella* spp. (Lei *et al*., 2008) virulence determinant were selected from the previously reported studies. All primers were synthesized by Invitrogen Life Technologies (Austria).

### Antimicrobials susceptibility testing

The antibiotic susceptibility behavior of the bacterial populations from dry spice and herb preparations were determined on cation-adjusted Mueller-Hinton agar (Hi-Media) using Kirby-Bauer disk diffusion method according to the standards and interpretive criteria described by NCCLS (2006). The used antibiotics (µg/g) were ampicillin (AMP 25), amoxycillin-clavulanic acid (AMC 20/10), kanamycin (KAN 30), nalidixic acid (NAL 30), streptomycin (STR 10), ciprofloxacin (CIP 10), chloramphenicol (CHL 30), cefoxitin (FOX 30), cefotaxime (CTX 30), ceftazidime (CAZ 30), cephalothin (CEF 30), gentamicin (GEN 10), tetracycline (TET 30), and trimethoprim-sulfamethoxazole (SXT 1.25/23.75). All disks were purchased from Hi-Media and Mast-Diagnostics (Mast Group Ltd.) except for tetracycline and trimethoprimsulfamethoxazole, which were purchased from Bioanalyse Ltd., and the results were recorded on the bases of the zone size interpretative chart supplied by the manufacturers.

The quality control was performed to check the quality of medium and potency of antibiotic disks before use against some sensitive ATCC reference strains, including *S. aureus* ATCC 29213, *E. coli* ATCC 25922 (beta-lactamase negative), *E. coli* ATCC

35218 (beta-lactamase positive), and *P. aeruginosa* ATCC 27853 (MicroTrol Discs; BD Diagnostics).

**Phenotypic detection of ESBL**

The first step of ESBL detection criteria was resistance or reduced susceptibility to cefotaxime and/or ceftazidime. Phenotypic confirmation of ESBL production was performed by double disk diffusion synergy test ( Jarlier *et al*., 1988).

**3. Results**

**Bacterial quality**

The bacteriological quality of dry spice and herb samples with regard to the population size of total aerobic mesophilic bacteria (TAMBC), mesophilic aerobic sporeforming bacteria (MASBC), thermophilic sporeforming bacteria (TSFB), total coliform (TC) and *E. coli* is presented in Table 1. No bacterial contamination was detected in aniseed, cinnamon, thyme and turmeric. The prevalence of aerobic mesophilic bacteria was 85.3% of samples ranged from 2.8 to 8.4 $\log_{10}$ CFU $g^{-1}$. According to the ICMSF and ESA specification, 24.2% of spices and herbs samples were unacceptable or in unsatisfactory in terms of TMAB counts (> 6 $\log_{10}$ CFU $g^{-1}$) included: cumin, basil, caraway, and with the maximum load of coriander 8.4 $\log_{10}$ CFU $g^{-1}$.

Regarding spore forming bacteria were detected in 58.9% of samples in a range of 1.5 – 3.8 $\log_{10}$ CFU $g^{-1}$ with highest level in garlic powder. The growth of thermophilic aerobic bacteria was achieved by 32.6% of samples in a range of 1.2 – 3.2 $\log_{10}$ CFU $g^{-1}$. Coliforms were detected in 53.7% of samples with the range of 1.01 – 3.3 $\log_{10}$ CFU $g^{-1}$, the highest counts was in coriander.

A total of 141 bacterial isolates was recovered based on selective isolation (Table 2). Bacterial identification of randomly selected colonies revealed the presence of ten genera and species, namely *Bacillus* spp. (20.6 %), *Acintobacter* spp. (16.3 %) *Pseudomonas aeruginosa* (12.8 %), *Enterobacter* spp. ( 12.1 %), %) *Erwina* spp. (9.2 %), *Klebsiella* spp. (7.8 %), *Hafni alvei* (6.4 %), *E. coli* (5.7 %), *Aeromonas salmoniside (5.0%), Shigella* spp. (2.8 %), and *S. aureus* (2.1 %).

The PCR results for spice and herb preenrichment cultures revealed that none of them positively reacted with the primers coding specific regions of *Salmonella* spp., *L. monocytogens* and *E. coli* O157:H7, however detect *Cl. perfringens* and *B. cereus* in one and six samples, respectively.

**Table 1: Average mean counts ($Log_{10}$ CFU $G^1$) of microbial items analyzed in various spice and herb preparations sold and consumed on Taif (Western Region of Saudi Arabia)**

| *Spice and herbs* | *Total (n=95)* | *TAMB* | *MASB* | TASB | *Coliform bacteria* | *E. coli* |
|---|---|---|---|---|---|---|
| Red pepper (spice) | 5 | 5.4 | 0.0 | 0.0 | 0.0 | 0.0 |
| Cumin (spice) | 6 | 6.9 | 2.7 | 0.0 | 2.02 | 1.2 |
| Coriander (spice) | 8 | 8.4 | 2.1 | 1.2 | 3.31 | 2 |
| Curry ( | 4 | 3.1 | 0.0 | 0.0 | 0.0 | 0.0 |
| Parsley (spice) | 8 | 5.7 | 2.8 | 0.0 | 3.04 | 1.8 |
| Dill (herb) | 6 | 4.5 | 2.1 | 2.7 | 2.67 | 1.2 |
| Ginger (spice) | 6 | 3.3 | 0.0 | 0.0 | 0.0 | 0.0 |
| Cinnamon ( | 3 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| Clove (spice) | 4 | 3.8 | 3.1 | 0.0 | 0.0 | 0.0 |
| Turmeric (spice) | 5 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| Thyme (herb) | 4 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| Celery (herb) | 5 | 5.2 | 3.3 | 2.6 | 1.02 | 0.0 |
| Saffron (spice) | 4 | 4.8 | 1.5 | 0.0 | 0.0 | 0.0 |
| Fennel ( | 3 | 3.2 | 2.1 | 0.0 | 1.01 | 0.0 |
| Rosemary(herb) | 5 | 5.3 | 0.0 | 0.0 | 0.0 | 0.0 |
| Garlic (spice) | 3 | 4.9 | 3.8 | 3.2 | 1.2 | 1 |
| Basil (herb) | 3 | 7.0 | 3.1 | 2.4 | 2.2 | 3 |
| Caraway (spice) | 6 | 6.2 | 2.2 | 1.8 | 1.3 | 3 |
| Sesame ( | 2 | 2.8 | 0.0 | 0.0 | 0.0 | 0.0 |
| Aniseed ( | 2 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| Spice mixtures | 3 | 3.5 | 0.0 | 0.0 | 2.7 | 1 |

TAMP; total aerobic mesophilic bacteria
MASB; mesophilic aerobic sporforming bacteria
TASB; thermophilic aerobic sporeformer bacteria

**Table 2: Recovery of bacterial species from spice and herb samples retailed in Taif City, Western Saudi Arabia**

| Spices and herbs *(n= 95)* | *E. coli* | *Enterobacter* spp. | *Havni alvei* | *Klebsiella* spp. | *Acintobacter* spp. | *A. salmonside* | *Shigella* spp. | *Erwina* spp. | *Bacillus* spp. | *S. aureus* | *P. aeruginosa* |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Red pepper | 0 | 1 | 0 | 0 | 2 | 0 | 0 | 1 | 3 | 0 | 0 |
| Cumin | 1 | 2 | 1 | 1 | 2 | 0 | 1 | 0 | 3 | 0 | 1 |
| Coriander | 1 | 2 | 1 | 1 | 3 | 1 | 1 | 1 | 1 | 1 | 2 |
| Curry | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 2 | 0 | 0 | 0 |
| Parsley | 1 | 2 | 1 | 1 | 2 | 1 | 0 | 0 | 2 | 0 | 1 |
| Dill | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 1 | 0 | 1 |
| Ginger | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 1 |
| Cinnamon | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Clove | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 2 | 2 | 0 | 2 |
| Turmeric | 0 | 0 | 1 | 0 | 2 | 1 | 0 | 2 | 2 | 1 | 1 |
| Thyme | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| Celery | 0 | 0 | 1 | 1 | 1 | 0 | 0 | 0 | 1 | 0 | 2 |
| Saffron | 0 | 1 | 1 | 1 | 2 | 1 | 0 | 1 | 3 | 0 | 0 |
| Fennel | 0 | 0 | 1 | 1 | 1 | 0 | 0 | 0 | 2 | 0 | 2 |
| Rosemary | 0 | 1 | 0 | 1 | 1 | 1 | 0 | 1 | 0 | 0 | 0 |
| Garlic | 1 | 1 | 0 | 0 | 2 | 0 | 0 | 2 | 3 | 0 | 0 |
| Basil | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 2 | 1 | 2 |
| Caraway | 1 | 0 | 0 | 1 | 2 | 0 | 1 | 1 | 4 | 0 | 2 |
| Sesame | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Aniseed | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Spice mixtures | 1 | 2 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 |
| Total, *n*= 141 (%) | 8 (5.7) | 17 (12.1 ) | 9 (6.4) | 10 (7.8) | 23 (16.3) | 7 (5.0) | 4 (2.8 ) | 13 ( 9.2) | 29 (20.6) | 3 (2.1) | 18 (12.8) |

**Table 3: Antibiotic resistance phenotype among bacterial population isolated from spices and herbs.**

| Bacterial species | *Beta-lactams* | | | | | | *Aminoglycosides* | | | *Fluoroquinolones* | | *Sulfonamides* | *Phenicoles* | *Tetracycline* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | AMP | AMC | FOX | CAZ | CTX | CEF | KAN | GEN | STR | NAL | CIP | SXT | CHL | TET |
| *Escherichia coli* | 8 | 0 | 0 | 0 | 1 | 6 | 1 | 0 | 2 | 1 | 1 | 3 | 2 | 0 |
| *Enterobacter* spp. | 14 | 0 | 0 | 0 | 1 | 11 | 0 | 1 | 3 | 2 | 0 | 2 | 4 | 3 |
| *Havni alvei* | 6 | 0 | 0 | 0 | 0 | 7 | 0 | 1 | 1 | 2 | 0 | 1 | 0 | 1 |
| *Klebsiella* spp. | 8 | 0 | 0 | 0 | 0 | 8 | 0 | 1 | 2 | 2 | 1 | 6 | 2 | 1 |
| *Actinobacter* spp. | 16 | 0 | 0 | 0 | 1 | 11 | 2 | 0 | 6 | 3 | 0 | 5 | 6 | 2 |
| *A. salmonside* | 5 | 0 | 0 | 0 | 0 | 3 | 0 | 0 | 1 | 0 | 0 | 2 | 0 | 2 |
| *Shigella* spp. | 2 | 0 | 0 | 0 | 0 | 3 | 1 | 0 | 2 | 0 | 0 | 1 | 0 | 2 |
| *Erwinia* spp. | 9 | 0 | 0 | 0 | 1 | 7 | 0 | 0 | 4 | 2 | 2 | 6 | 1 | 1 |
| *Bacillus* spp. | 26 | 4 | 2 | 0 | 8 | 21 | 4 | 1 | 9 | 0 | 0 | 13 | 7 | 0 |
| *P. aeruginosa* | 18 | 3 | 2 | 3 | 2 | 18 | 3 | 1 | 5 | 3 | 3 | 11 | 5 | 0 |
| *S. aureus* | 3 | 0 | 0 | 0 | 2 | 3 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 0 |
| Total n=141(%) | 105 (74.5) | 7 (5.0) | 4 (2.8) | 3 (2.1) | 16 (11.3) | 98(69.5) | 11 (7.8) | 5 (3.5) | 36 (25.5) | 15 (10.6) | 7 (5.0) | 51 (36.2) | 27 (19.1) | 12(8.5) |

AMP, ampicillin; AMC, amoxyicillin-clavulanic acid; KAN, kanamycin; NAL, nalidixic acid; CHL, chloramphenicol; CIP, ciprofloxacin; FOX, cefoxitin; CTX, cefotaxime; CAZ ceftazidime; GEN, gentamicin; TET, tetracycline; SXT, trimethoprim-sulphamethoxyzol; STR, streptomycin; CEF, cephalothin.

## Antibiotic resistance

The relative frequencies of antibiotic resistance in bacterial isolates evaluated (n = 141) was ampicilin (74.5%), cephalothin (69.5%), sulfamethxazole-trimethoprim (36.2%), streptomycin (25.5%), chloramphenicol (19.1%), cefotaxime (11.3%) and nalidexic acid (10.6%) (Table 3). Most isolates were sensitive or exhibiting low resistance to ceftazidime (2.1%), cefoxtin (2.8%), gentamycin (3.5%), ciprofloxacin (5.0%), kanamycine (7.8%) and tetracycline (8.5%).

Of the 141 isolates tested for resistance, 21.3% were resistant to one antibiotic and 48.9% to two antibiotics, meanwhile multiresistance to three or more was exhibited by 18.4% of isolates (data not shown). Sensitivity to all antibiotic tested was presented by 11.4 % of isolates. Maximum resistance to extended-spectrum beta-lactam antibiotics occurred in 11.3% of isolates and by ESBL selective screening the production of extended spectrum beta-lactamase was achieved by 3.5% of isolates.

## 4. Discussion

### Bacteriological quality

Dried spices and herbs are commonly used in a variety of ways and food preparations. Recently, the microbiological concerns of these products are recognized by center for Food Safety and Applied Nutrition (CFSAN) of the US Food and Drug Administration (FDA) (Vibha *et al*., 2006). The results of hygienic quality parameter analysis demonstrated that the population size of aerobic bacteria had a varied count depending on sample type. Similar reports of varied aerobic mesophilic bacteria in food related spices and herbs have been documented (Hampikyan *et al*., 2009; Sago *et al*., 2009; Sospedra *et al*., 2010; Vitullo *et al*., 2011; Witkowska *et al*., 2011; Dababneh, 2013). Generally, plate count of aerobic bacteria in herbs is considered as a sign of general sanitation and quality parameter (Kneifel *et al*., 2002). In the current study, 24.2% (23 of 95) of retail spices and herbs samples did not comply with the ICMSF standards for total aerobic mesophilic bacterial counts (TAMB >6 $\log_{10}$ CFU $g^{-1}$). These findings are similar to data reported by several researchers (Moreira *et al*., 2009; Sago *et al*., 2009; Vitullo *et al*., 2011; Witkowsk *et al*., 2011).

The preparations found to be within an unacceptable range (TAMB >6 $\log_{10}$ CFU $g^{-1}$) included: cumin, coriander, basil and caraway. These preparations were also reported to contain high incidences of contamination in a number of studies carried out by other researchers (Garcia *et al*., 2001; Banerjee and Sarkar, 2003; Abou Donia, 2008).

Growth was absent in cinnamon, turmeric, thyme, rosemary and aniseed preparations. Certain herbs and spices are known to contain active antimicrobial compounds, which may contribute to lower contamination levels, when compared to other spices (Burt, 2004; Souza *et al*., 2006; Turgis *et al*., 2009; Sospedra *et al*., 2010; Witkowsk *et al*., 2011). This may provide a possible explanation of the presence of relatively low TAMB levels (<4 log $\log_{10}$ CFU $g^{-1}$). A higher microbial load of aerobic bacteria (9 $\log_{10}$ CFU $g^{-1}$) was reported earlier by several researchers (Czech *et al*., 2001; Mousumi and Prabir, 2003; Phianphak *et al*., 2007; Abou-Donia, 2008; Gil *et al*. 2009; Khanzadi *et al*., 2012). While in contrast to the finding of present study, Idu *et al*. (2008) reported comparatively low bacteria counts (4 $\log_{10}$ CFU $g^{-1}$).

Contamination with coliforms denotes unhygienic conditions. The bacterial populations of coliforms were detected at a low limit among samples (< 4 $\log_{10}$ CFU $g^{-1}$). Similar population sizes have been reported in other studies (Abou-Donia, 2008; Vitullo *et al*., 2011; Khanzadi *et al*., 2012). However, higher coliform counts ( > 4 $\log_{10}$ CFU $g^{-1}$) have been reported by other workers (Czech *et al*., 2001; Phianphak *et al*., 2007; Idu *et al*., 2008). The occurrence of coliforms show the possibility of fecal contamination and inadequate sanitation conditions while the plants were being grown.

Spices and herbs are a principal source of spore forming aerobic bacteria (*Bacillus* spp.) and anaerobic bacteria (*Clostridium* spp.). Bacterial spores are potential risk because they may survive cooking temperatures and multiply leading to food spoilage (Pafumi, 1986; Little *et al*., 2003). Our results indicate that 58.9% and 32.6% of spices were contaminated with spore forming bacteria and thermophiles, respectively. Data in the present study would agree with the other researchers that certain spices may act as potential sources of contamination by harboring highly heat-resistant spores of bacteria (Baxter and Holzapfel, 1982; Kovacs-Domjam, 1988; Kneifel and Berger, 1994; Banerjee and Sarkar, 2003; Witkowsk *et al*., 2011).

Although herbs and spices are not major contributors to foodborne disease they occasionally contain pathogenic microorganisms which may pose a risk to public health, especially when added to meals without further treatments (Banerjee and Sarkar, 2003; ICMSF, 2005). Of particular significance are *Salmonella* spp., *E. coli* O157:H7, *S. aureus* , *L. monocytogenes* , *B. cereus* and *Cl. perfringens*. The results of this study indicated that no sample was found to be contaminated by *Salmonella* spp., *L. monocytogenes* or *E. coli* O157:H7. However, Low levels of contamination by *S. aureus*, *B. cereus* and *Cl. perfringens* were detected and this may be occur because of the natural occurrence in the soil environment of these bacteria. This finding is constituents with other published studies in which these foodborne pathogens were uncommon in spices and herbs (Abou Donia, 2008; Garcia *et al*., 2001;Vitullo *et al*., 2011 ) or could be at low prevalence (Kneifel and Berger, 1994; Little *et al*., 2003; ICMSF, 2005; Hampikyan *et al*., 2009; Sago *et al*., 2009; Sospedra *et al*., 2010). Escherichia coli was found in eight samples and may indicate faecal contamination by humans, livestock and wildlife or the poor hygiene of the workers (Leifert *et al*. 2008; Elviss *et al*. 2009). Varied prevalence of *E. coli* was

achieved by previous studies (Hampikyan *et al*., 2009; Sago *et al*., 2009; Sospedra *et al*., 2010; Vitullo *et al*., 2011). Other microorganisms like *Shigella* spp., *Enterobacter* spp., *Acinetobacter* spp., *Pseudomonas aeruginosa*, *Hafni alvei* were also isolated. Similar to our results, previous researches recovered a variety of bacteria from dry spices and herbs (Sospedra *et al*., 2010; Dababneh, 2013).

**Antimicrobial resistance**

The broad use of antimicrobials in agriculture selects for resistant bacteria, which may enter the food chain and potentially to humans (Khachatourians, 1998). Among the most common antimicrobial agents are drugs that are either identical or related to those administered to humans. In this study, 88.6% of bacterial population from spice and herbs at retail level showed resistance phenotypes to one or more antibiotic. This is in agreement with previously data that found high resistance rates in bacterial populations from dry spices and herbs (Brown and Jiang, 2008). Various levels of antibiotic resistant bacteria were detected in several products in this study. Currently, limited information exists regarding development of antibiotic resistance due to use of

Of dry spices and herbs. In one previous study, bacterial population from dry spice and herbs reported varied resistance to nine antibiotics tested (Brown and Jiang, 2008). The resistance to extended-spectrum beta-lactam and beta-lactamase inhibitors is of great clinical significance. Resistance to beta-lactam antibiotics is primarily mediated by beta-lactamases production. Many different beta-lactamases had been described (Livermore and Woodford, 2006). In this study, maximum resistance rate to the extended-spectrum beta-lactam antibiotics was expressed by 11.3 % of isolates. A lower detection to ceftriaxon was reported in bacterial population from spices and herbs (Brown and Jiang, 2008).

A CTX resistance most found in Enterobacteriaceae and recently in *Pseudomonas aeruginosa*. The association of CTX b-lactamase–encoding genes with mobile elements (Eckert *et al*., 2006) may facilitate the spread of $bla_{\text{CTX-M}}$ genes among bacteria. The CTX-resistant *P. aeruginosa* in this study may be due to horizontal resistance transfer or loss of membrane permeability. Other possible mechanisms, such as a non–beta-lactamase mediated resistance to beta-lactam drugs, could be also encountered. Non–beta-lactamase mechanisms included loss or deficiency of specific porins (Benz, 1994), PBPs alteration (Wilke *et al*., 2005), and increased efflux pumps (Poole, 2004; Fisher *et al*., 2005).

Despite the resistance to narrow and extended-spectrum beta-lactam drugs, we detected resistance to fluoroquinolones including ciprofloxacin (5.0% of isolates) and nalidixic acid (10.6% of isolates). Resistance to quinolones is chiefly mediated through chromosomal mutation in DNA gyrase, topoisomerase, and plasmid-mediated quinolone. Plasmid-mediated quinolone resistance is of great concern, because these resistance determinants are potentially spread among bacteria due to plasmid mobility (Robicsek *et al*., 2006a). More recently, another new mechanism of plasmid-associated quinolone resistance that involves ciprofloxacin-modifying aminoglycoside acetyltransferase gene has been discovered (Robicsek *et al*., 2006b). Further, resistance to aminoglycosides was observed most commonly against streptomycin (25.5%) and less against gentamicin (3.5%), and kanamycin (7.8%). The nalidixic acid and gentamicin resistance in bacterial population isolated from spices and herbs has been previously reported (Brown and Jiang, 2008). Appearance of fluoroquinolone, gentamicin, and cephalosporins resistance in bacteria identified in our study suggested animal or human sources, because these classes are not used in plant agriculture, or plant-associated bacteria through horizontal transfer could be also supposed.

A comparative data on the resistance patterns of the bacterial species described in our study with those of bacterial species originating from clinical settings in Saudi Arabia and Arabic Gulf region revealed a correlation. In Saudi Arabia, *E. coli* isolated from chicken intestine was found to be resistant to many antibiotics (Al-Ghamdi *et al*., 1999). In line with this result, Altalhi *et al*. (2010) observed high rates of resistance among *E. coli* isolates from chicken meat against sulfonamides, nalidixic acid, gentamicin, chloramphenicol, and streptomycin. Further, a gradual increase in resistance rates against fluoroquinolones, aminoglycosides, and trimethoprime-sulfamethoxazole and multidrug resistance among bacteria had been reported in many retrospective analysis (Kader and Kumar, 2005; Al-Tawfiq, 2006, 2007; Al-Harbi and Al-Fifi, 2008).

In recent years, data from Arabian Gulf region show high occurrence of ESBL-producing isolates, with rates as high as 31.7% in Kuwait, 41% in United Arab Emirates, and 55% in Saudi Arabia (Al-Zarouni *et al*., 2008; Mokaddas *et al*., 2008; Al-Agamy *et al*., 2009).

**Conclusion**

In summary, the present study indicates bacterial contamination and occurrence of antimicrobial resistance in bacterial populations in spices and herbs at retails can affect the products quality and shelf life as well as may constitute threats to consumers, possibly via resistance transfer. Further, the potential public health risk of using spices and

herbs as ready-to-eat foods that potentially undergo no further processing is highlighted in this study. Prevention of microbial contamination in dried herbs and spices lies in the application of good hygiene practices and HACCP at all stages of food chain production. At the time of this study, it is obvious that there is a lack of microbiological specification and standard of spices and herbs at the national and international level. It requires a great effort to be completed and uniformed. The data reported in this investigation represents a primary evaluation for the microbiological states of spices and herbs that could promote detailed studies leading to set up microbiological standards and a complete safety program in Saudi Arabia.

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