PAPER

PREVALENCE AND ANTIBIOTIC RESISTANCE OF FOOD BORNE BACTERIAL CONTAMINATION IN SOME EGYPTIAN FOOD

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ABSTRACT

This study was undertaken to investigate the prevalence and antibiotic resistance of food borne bacterial contamination in some Egyptian food. Total viable bacteria and total coliform bacteria were isolated from different sources of food; carbohydrates (bread, flour and basbousa), vegetables (outer and inner tissues of potato and outer and inner tissues of cucumber) and proteins (minced meat, cheese and milk). The study resulted in maximum value of total viable bacteria found in outer tissue of potato $68X10^4 \pm 1.0$, while the minimum value found in inner tissues of potato and cucumber. The study resulted in total coliform was maximum value in minced meat $6.4X10^3 \pm 0.3$. Basbousa and inner tissue of potato and cucumber were free from coliforms. The ability of isolates to producing proteolytic enzymes was tested, we found that 326 isolate (63.92%) from all isolates had this ability, thus we selected most 2 potent proteolytic isolates. The two isolates were identified as *Bacillus cereus* and *Escherichia coli.* The identification confirmed by microlog 34.20 system and 16SrRNA for two isolates and the same result was founded. Sensitivity tested for the most potent proteolytic species to 12 of the most commonly used antibiotics in the Egyptian pharmacy. The results showed that all species were sensitive to most of antibiotics, except *B. cereus* which was strongly susceptible to azteronam and ceftazidim. The data showed that raw meat, cooked food products, and raw milk were most commonly contaminated with foodborne pathogens and many pathogens were resistant to different antibiotics. The study provided useful information for assessment of the possible risk posed to consumers, which has significant public health impact.

- Keywords: foodborne pathogens, *Bacillus cereus*, *Escherichia coli*, proteolytic enzymes, 16SrRNA -

INTRODUCTION

It has been estimated that as many as 30% of people in industrialized countries suffer from a food borne disease each year and in 2000 at least two million people died from diarrheal disease worldwide (WHO, 2002a). Foods are not only of nutritional value to those who consume them but often are ideal culture media for microbial growth, chemical reactions that cause offensive and sensory changes in foods are mediated by bacteria that use food as carbon and energy source. Some of the major bacterial genera which cause food born infection and intoxication (PUNDIR and JAIN, 2011). Contamination of food can affect a large number of populations. About 2.5 million people die every year from water born diseases. More than 40% of total population of Indonesia & 60% in Thailand suffered from gastroenteritis per year. A total of 32.7% outbreak was involved with restaurant catering bakery products (NAZIR and ISLAM, 2007).

Fruits and vegetables carry microbial flora while passing from the farm to the table. The produce is exposed to potential microbial contamination at every step including cultivation, harvesting, transporting packing, storage and selling to the final consumer (FDA, 2000). Sources of environmental microbial contamination include raw materials, processing equipment, manufacturing activities, sanitation and maintenance practices, workers, waste, animal and insect pests, and microbial growth niches embedded in equipment and in structural components of the building. The survival and growth of microorganisms in a food-processing environment may lead to contamination of the finished product that may, in turn, result in a reduction of microbiological safety and quality. Most food plants have locations that can promote the growth of pathogens and spoilage microorganisms that may be transferred directly on to product or carried into additional niches. The origins of these growth habitats are mainly unhygienic design, construction, and maintenance and repair activities that prevent easy cleaning and disinfection. The presence of water and nutrients (food product) is required to form a microbial growth niche and the chemical composition of the food and conditions of water activity, pH, temperature, etc., will select the "normal" organisms that can grow there. Microbial growth niches may be established when water is used to clean dry processing environments not designed for wet cleaning and not all points in the equipment are promptly and completely dried (JAY, 1996, TESFAYE *et al.*, 2011). VANDERZANT and SPLITTSTOESSER, (1992) demonstrated that the microbial growth on equipment for processing perishable foods is governed mainly by the ecology of the food, the process, packing room temperature, presence of food residue on the equipment, and efficacy of cleaning and disinfection. Recontamination of a biocidaly treated food may increase the risk of foodborne illness if the food is not heated to destroy pathogens before consumption. Perishable foods that do not receive a biocidal treatment in the final container may be decontaminated by spoilage microorganisms before packing (VANDERZANT and SPLITTSTOESSER, 1992, HAILESELASSIE *et al.*, 2013).

The research project will deal with investigate the prevalence and antibiotic resistance of *Bacillus cereus*, *Escherichia coli* contamination in some Egyptian food.

2. MATERIALS AND METHODS

2.1. Food samples

All food samples were collected from Mansoura city, Egypt. They collected from different shops, super market, groceries and butchers. Ten samples were taken from each of bread, flour, basbousa, potatoes, cucumber, minced meat, cheese and milk.

2.2. Preparation of samples

Twenty five grams of each of the following samples bread, flour, basbousa, outer tissue and inner tissue of potatoes and cucumber, minced meat, cheese and ten ml of raw milk (unboiled/unpasteurized) was homogenized in 225 ml sterile physiological saline solution (0.85% NaCl) in 500 ml conical flask using a plender for 1-2 minutes, then decimal dilutions were prepared.

2.3. Isolation of bacteria

One ml of appropriate dilution was inoculated on both of Nutrient agar medium and MacConkey agar medium; the plates were incubated aerobically for 24h at37°C. Total viable bacteria (T.V.B) were enumerated on Nutrient agar medium using pour plate technique. Total coliform (T.C) bacteria were counted on MacConkey agar medium by using pour plate technique also. The plates were incubated aerobically for 24h at 37°C.

2.4. Purification

After the incubation period (24h), the growing colonies were enumerated for counting. After counting a sterile wire loop was used to pick the isolate from the plate and was streaked on freshly prepared nutrient agar medium then inoculated for 24h at 37°C in order to get pure culture. The growing colonies were purified and examined by using cultured morphological appearance and Gram reaction.

2.5. Proteolytic assay

Proteolytic activity was carried out according to Casein - Pholine method (RAMALAKSHMI *et al.*, 2012). Culture media was Centrifugated at 7200 rpm for 10 min and supernatant was used as enzyme source. However, 1% casein (in 0.1 M phosphate buffer and pH 7.0) was used as substrate. 1 mL each of enzyme and substrate was incubated at 50°C for 60 min. The reaction was terminated by adding 3 mL of Trichloroacetic acid (TCA). One unit of protease activity was defined as the increase of 0.1 unit optic density at 1 h incubation period. Then it was centrifuged at 5000 rpm for 15 min. From this, 0.5 mL of supernatant was taken, to this 2.5 ml of 0.5M sodium carbonate was added, mixed well and incubated for 20 min. Then it was added with 0.5 ml of folin phenol reagent and the absorbance was read at 660 nm using Spectrophotometer. The amount of protease produced was estimated and expressed in microgram of tyrosine released under standard assay conditions. Based on the tyrosine released the protease activity was expressed in microgram of tyrosine released by 1 mL of enzyme in 30 minutes at 300C on tyrosine equivalent.

2.6. Identification of bacterial isolates

2.6.1. Conventional methods

The appearance of cultures, cell morphological characteristics and physiological characteristics of the purified selected identified isolates were studied. Media and reagent were prepared according to standard and procedures as described by (MACFADDIN, 1980). The identification was carried out by traditional characters and biochemical tests for isolates according to (Krieg *et al.,* 1994) and confirmed out by biolog micolog 34.20 system for most potent proteolytic bacterial species. Characterization of the most potent isolates were completed and confirmed by Biolog Microlog 34.20 system at the Unit of Identification of Microorganisms and Biological Control Unit of Agriculture Reasearch Center, Giza, Egypt.

2.6.2. Molecular method

The polymerase chain reaction (PCR) methods based on 16S rRNA gene for identification of isolates were used. Genomic DNA was extracted and purified by using Qiagen kit (Qiagen Company). The purity was assessed from the A260/A280 ratios: Cultures of bacteria were streaked on tryptic soy agar medium and incubated at 37°C for 24 h. A single colony of each pathogen was grown in (LB) broth medium in Erlenmeyer flask and incubated at 37°C for 24 h. Culture was harvested by centrifugation at 4°C for 10 min, DNA was extracted from pellets according Qiagen kit instructions. Full length 16S rRNA (1500 bp) were amplified from isolates by PCR using universal forward primer 518F (5'–CCAGCAGCCGCGGTAATACG-3') and 800R (5'-TACCAGGGTATCTAATCC-3'). Optimum conditions (denaturation 94-1 min, annealing 63-45 s and extension 72-2 min, 35 cycles). Amplified 16S rRNA was purified from 0.8% melting point agarose gel. Bands obtained from PCR product were eluted and purify by (Qiagen elution kit) PCR instructions, DNA band desired was excised from ethidium bromide stained agarose gel with a razor blade, transferred to Ependorf tube. DNA was sequenced directly using specific primer with concentration 20 pmol Macrogen Sequencing Company, Korea.

2.7. Antibiotic susceptibility test

2.7.1. Antibiotic disks

Antimicrobial susceptibility profile of identified bacterial species, *Bacillus cereus* and *E.coli* against different antibiotics ampicillin, aztreonam, cefadroxil, ceftazidime, chloramphenicol, ciprofloxacin, erythromycin, imipenem, neomycin, norfloxacin, streptomycin and vancomycin were studied. The antibiotic discs used in this research were purchased from Oxoid LtD., England.

2.7.2. Disc diffusion agar method

Antibiotic susceptibility test for the bacterial isolates was carried out by disc diffusion technique according to BAURE *et al.*, (1966). The technique was done by inoculation of pure colonies of the tested organism into 5 mL of sterile nutrient broth and incubation at 37°C for 24h. Then 0.1ml of bacterial suspension (0.5 McFarland turbidity) was spreading by sterile swabs on nutrient agar plates. Duplicate plates were prepared for the strain. Antibiotic discs were applied to the surface of plates at constant distances. The plates were incubated at 37°C for 24h. At the end of incubation period zones of inhibition were measured as (mm). The entire diameter of the zone was measured including the diameter of the disc. The end point of the reading was taken as complete inhibition of the growth to the naked eye. Our (+++) or (++++) indicate high inhibitory effect (large diameter of clearing zone) and (-) indicate no inhibitory effect (good growth).

2.8. Statistical analysis

The variations between experiments were estimated by standard deviations, and statistical significance of changes was estimated by student's t-test. Only the probability $P \leq 5\%$ was regarded as indicative of statistical significance.

Table 1 - Counts of total viable bacteria (T.V.B) and total coliform (T.C.B) & Log10 cfu/ ml for samples of food collected from Al-Mansoura city, Egypt.

T.C.B	T.V.B		Types of Food	
	Counts (cfu/ml)	Log 10 (cfu/ml)	Counts	Log 10
I-Carbohydrates				
Bread	$40.5x10^3 \pm 0.5$	4.61	$5.3x10^3 \pm 0.4$	3.72
Flour	$35.3x10^3 \pm 0.9$	4.55	$5.4x10^3 \pm 0.4$	3.73
Basbousa	$1.1x10^3 \pm 0.1$	3.03	0.0	0.0
II-Vegetables				
Potato				
Outer tissue	$68x10^4 \pm 1.0$	5.83	$3.2x10^3 \pm 0.3$	3.51
Inner tissues	$0.1x10^3 \pm 0.1$	1.98	0.0	0.0
Cucumber				
Outer tissue	$52.7x10^4 \pm 2.5$	5.72	$1.8x10^3 \pm 0.2$	3.26
Inner tissues	$0.1x10^3 \pm 0.0$	2.0	0.0	0.0
III-Proteins				
Minced meat	$28x10^3 \pm 3.5$	4.44	$6.4x10^3 \pm 0.3$	3.80
Cheese	$42.7x10^4 \pm 2.3$	5.63	$2.1x10^3 \pm 0.2$	3.32
Milk	$39.7x10^4 \pm 0.6$	5.60	$2.33x10^3 \pm 2.5$	3.37

All values are the means of triplicate plate.

3. RESULTS

3.1. Isolation of total viable bacteria from different types of food

All growing isolates were enumerated, collected, purified and tabulated. All growing isolates were collected from investigated types of food. Table 1 includes the isolates numbers and sources of collected isolates. Total viable bacterial counts (TVB) and total coliform bacterial count (TCB) of three main groups of food were tabulated in Table 4. In carbohydrates, the highest count 40.5x103cfu/gm was recorded in bread. The total viable bacterial count in flour was $35.3x10^3$cfu/gm. The lowest count $1.1x10^3$ cfu/gm was recorded in Basbousa. Both Fig. 1 and Table 4 show these results. The count of total coliform bacteria (TCB) was $5.4x10^3$ and $5.3x10^3$ cfu/gm in flour and bread respectively. This group of bacteria (TCB) was not recorded in Basbousa (Table 1). The counts of T.V.B were $68x10^4$ and $52.7x10^4$ cfu /ml in the epidermis of both of potato and Cucumber respectively. While the count was reduced to a lowest count $1.0x10^2$ cfu /mL of the inner tissues of both potato and Cucumber. It

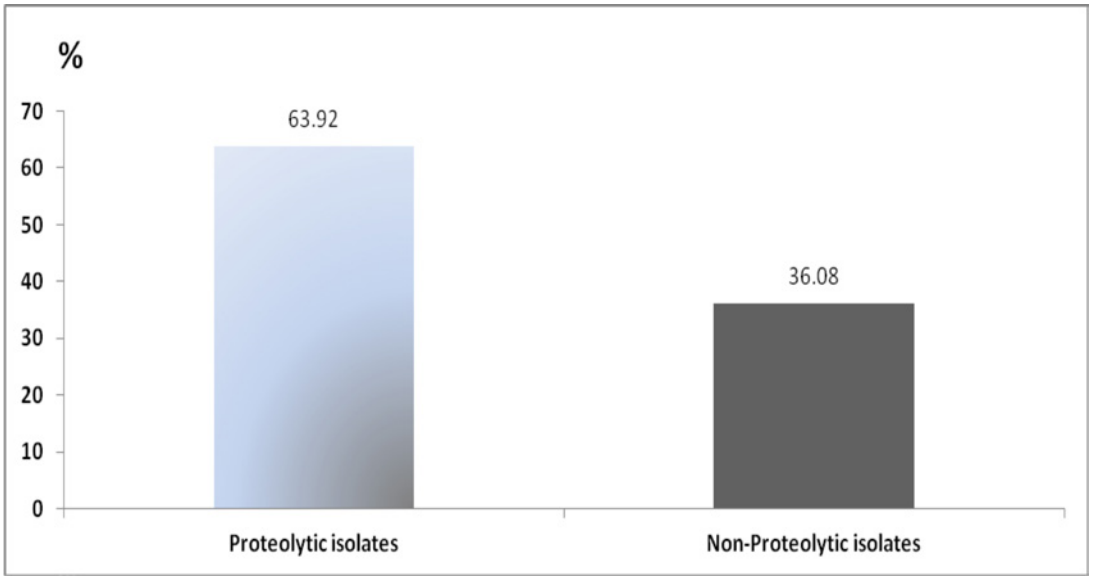

Fig. 1 - The percentage of proteolytic and non-proteolytic isolates from different kinds of food is

is important to notice that TCB was not recorded in inner tissues of potato and Cucumber. The counts of TCB were $3.2x10^3$ and $1.8x10^3$ cfu/mL from the outer tissue of potato and Cucumber respectively. The total viable bacterial count in milk was $39.7x10^4$cfu/mL while the TCB was $2.33x10^3$ cfu/mL. From Table 1 we notice that the highest count of total viable bacteria was $42.7x10^4$ in cheese. And also the lowest count of TCB was $2.1x10^3$cfu/mL in cheese. From Table 4, the count of T.V.B was $28x10^3$ cfu/mL, while the count of TCB was $6.4x10^3$ cfu/mL.

3.2. Screening test for detection of most potent proteolytic bacterial species

Fig. 1 showed the potency of proteolytic activities of all purified isolates. The proteolytic isolates were 326 isolates (63.92%), while the non-proteolytic isolates were 184 isolates (36.08%). It also shows that the largest clearing zone was 21 mm in case of isolates 62 and 412 (Fig. 2). This indicates that these isolates were the most potent of proteolytic activity.

3.3. Presumptive and confirmation identification

The cultural study, morphological appearance, Gram reaction and physiological characteristics of four most potent proteolytic isolates were studied according to KRIEG *et al.* 1994. These results were tabulated in Table 2. The presumptive identification of the two most potent proteolytic activities. Isolate numbers 62 and 410 were identified as *Bacillus cereus* and *Escherichia coli* respectively. The most potent bacterial isolates were confirmed by using Biolog Microlog 34.20 system for identification. 16S rRNA gene bands which were detect by specific primer at 1500 bp. The 16S rRNA sequences for two isolates were blasted with genebank sequence

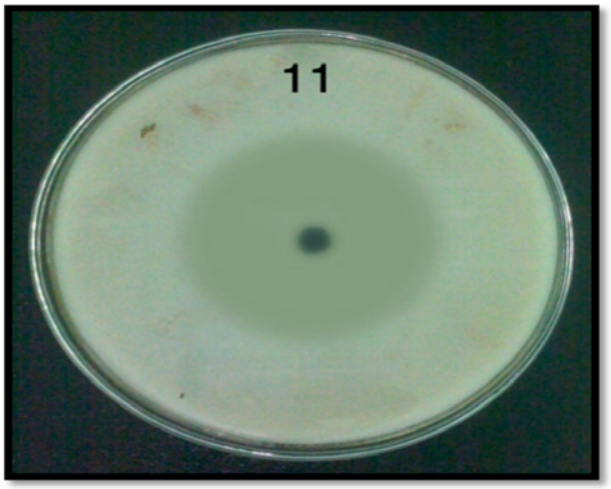

Fig. 2 - Example of proteolytic activity of isolated species.

Table 2 - Morphological and biochemical features of strains 62 and 412.

Character and biochemical tests Code of Isolate	62	412
Growth	Aerobic or Facultative anaerobic	Facultative anaerobic
Morphology of colony	Colony smooth, convex, circular and creamy in color	Colony smooth, convex, circular and creamy in color
Gram stain	+	-
Cell shape	Straight rods, arranged in paires	Straight rods, arranged singly or in pairs
Motility	Motile	Motile
Flagella arrangement	Peritrichous	Peritrichous
Oxidase		--
Gelatine hydrolysis	+	-
D-Glucose, acid production	+	+
D-Glucose, gas production	-	+
Nitrate reduction	+	+
Catalase production	+	+
Oxidation- fermentation	F	F
Voges-Proskaure	NT	-
Arginine dihydrolase	NT	-
Acid production:		
L-Arabinose	-	+
Lactose	NT	+
Maltose	NT	+
Trehalose	NT	+
D-Mannitol	-	+
D-Xylose	-	+
Indole production	NT	+
Methyle red	NT	+
Citrate (Simmons)	NT	-
H_2S production	NT	-

NT= not tested for this isolate because of results of another biochemical tests, F= fermentative.

Table 3 - BLAST analysis of 16S rRNA sequences of the representative isolates.

Isolate	Closest Validly Described Species		Identities		
	Description	Accession number	Match	Total	% Similarity
62	*Bacillus cereus* KM007-1	KF055368	481	485	99
412	*Escherichia coli* Xuzhou21	CP001925	1465	1469	99

database (Table 3) and found closet to the same isolates identified by conventional methods.

3.4. Antibiotic susceptibility test of different antibiotics

The antibiotic susceptibility test was obtained on the bacterial isolates by using 12 different antibiotics by disc diffusion method (Table 4). *Bacillus cereus was* resistant (non susceptible) to Aztreonam and ceftazidime. *Escherichia coli* (gram negative bacteria) were resistant (non susceptible) to vancomycin. While gram positive organisms *Bacillus cereus* was non susceptible (resistant) to vancomycine. *Bacillus cereus* was resistant to ampicillin, while *Escherichia coli* was susceptible (sensitive) to ampicillin. *Bacillus cereus* was resistant to ampicillin while *Escherichia coli* was non susceptible (sensitive) to ampicillin. The studied bacterial species both gram positive and gram negative were sensetive to 8 antibiotics (chloramphenicol, cephadroxil, erythromycin, norfloxacin, imipenem, neomycin, ciproloxacin and streptomycin). *Escherichia coli* was sensitive to three more (ampicillin, aztreonam and ceftazidime) i.e. it was sensitive to 11 antibiotics, thus *Escherichia coli* was the most sensitive bacterial species. *Bacillus cereus* was sensitive to one more vancomycin and ampicillin respectively i.e. each of them was sensitive to 9 antibiotics. The smallest inhibition zone was 8mm which was recorded in *Escherichia coli* due to the effect of erythromycin and ceftazidime respectively.

Table 4 - Antimicrobial susceptibility profile of studied microorganisms against different antibiotics expressed as diameter of clearing zones.

Antibiotics (Conc.)		*Bacillus cereus*	*Escherichia coli*
Ampicillin	**AM10**	0	10
Aztreonam	**ATM30**	0	17
Cefadroxil	**CFR30**	22	16
Ceftazidime	**CAZ30**	0	8
Chloramphenicol	**C30**	23	24
Ciprofloxacin	**CIP5**	20	20
Erythromycin	**E15**	23	13
Imipenem	**IPM10**	37	25
Neomycin	**N30**	20	17
Norfloxacin	**NOR10**	21	20
Streptomycin	**S10**	20	15
Vancomycin	**VA30**	17	0

4. DISCUSSION

The bacterial count is considered an index of quality that gives an idea about the hygienic measures during processing and helps in the determination of keeping quality of the product (ABERLE *et al.*, 2001). This work will emphasize on the counts and characteristics of bacterial genera that is considered to be important in healthy foods giving an attention to their classification and identification. The main scope of this work is to count the different bacterial species found in the different food sources and study the antibiotic susceptibility patterns of these bacterial species which are isolated from Al-Mansoura city, Egypt. Bacterial counts of foods includes T.V.B and T.C.B are similar with the results of PRADNYA and SONALI (2008) who found the counts of T.V.B and T.C.B were in range 9-10 log cfu/mL for local open market in India. All isolated bacterial species are common components of the bacterial flora of mammals, birds, insects' reptiles and are commonly found in soil, on plants, water and foods as normal flora (GILMORE *et al.*, 2013).

These groups of bacteria were isolated by PRADNYA and SONOLI, 2008; NAZIR and ISLAM, 2007; EASA, 2010; OLUFEMI and AKINYERA, 2011; KUDJAWU *et al.*, 2011. Presence of *Escherichia coli* and T.C.B in food usually indicates lack of hygiena in handling and post process contamination, therefore *Escherichia coli* & T.C.B enumeration are used as food quality parameter (GONZALZ *et al.*, 2003). The present study was initiated by collection of food samples. All isolates were selected and purified and initial morphologically identified (cocci 46.7% and rods 53.3%) and (Gram's stain as gram positive rods & cocci 60.3% and Gram negative rods 39.7%). For some species, the range is wide and the growth occurs in a variety of substrates (as a true for coliform bacteria) but for others (e.g. many of pathogens) can grow in limited kinds of substrates. Thus, the bacteria found in food differ according to their ability of utilization of energy. The foods that are most often involved in Staphylococcal food poisoning differ widely from one country to another (BENNETT and LANCETTE, 1995). The present study was concerned with isolation of the bacterial content of different food samples, which collected from open markets in Al-Mansoura city, Egypt. All isolates were selected, purified and initial morphological-

ly identified by shape and gram stain as 46.7% cocci and 53.3% rods and gram positive cocci and rods 60.3% and gram negative rods 39.7%.

16S rRNA gene sequencing will continue to be the gold standard for the identification of bacteria, and the automation of the technique could enable it to be used routinely in clinical microbiology laboratories, as a replacement of the traditional phenotypic tests. Modern technologies have made it possible to construct a high density of oligonucleotide arrays on a chip with oligonucleotides representing the 16S rRNA gene sequence of various bacteria. Such a design will facilitate automation of the annealing and detection of the PCR products of 16S rRNA gene amplification, and hence routine identification of most clinical isolates will be possible. The use of 16S rRNA gene sequencing has several advantages. First, the turnaround time is short. Because amplification of the 16SrRNA gene takes only four to six hours, and the annealing and detection of PCR products takes only another few hours, theoretically the identification can be completed within one day. Second, it can be used for slow growing bacteria, unlike most commercially available kits that are based on phenotypic tests that require the detection of growth of the organism in the presence of certain specific substrates, and hence the slow growing bacteria are usually "unidentified" when the growth control shows a negative result. Third, the problem of "unidentifiable strains" will be overcome and there would be minimal misidentification – the identification of a clinical strain is clearly defined by the number of base differences between it and the existing species. Fourth, oligonucleotides representing all bacterial species, including those rarely encountered clinically, can be included in the array, making it easy to identify the rare species. Lastly, such a technique will be applicable not only to pyogenic bacteria, but also to other organisms such as mycobacteria (EL-HADEDY and ABU EL-NOUR, 2012).

Antibiotic resistance (Gram positive and Gram negative bacterial species) from food sources are important and serious problem in clinical field (EL-AIDY, 2007). The antibiotic sensitivity against bacteria is assayed by disc-diffusion method and in our study as well (SELIM, 2011; SELIM *et al.*, 2012; 2013)). In this study, the antibiotics susceptibility patterns of potent proteolytic bacterial species (*Bacillus cereus* and *Escherichia coli*) against 12 different antibiotics were investigated. Antibiotics include ampicillin (AM), aztreonam (ATM), cefadroxil (CFR), ceftazidime (CAZ), chloramphenicol (C), ciprofloxacin (CIP), erythromycin (E), imipenem (IPM), neomycin (N), norfloxacin (NOR), streptomycin (S) and vancomycin (VA). In this study *Bacillus cereus* was resistant to 3 antibiotics (ampicillin, azactam and cerazdime). Beta-lactam antibiotics also bind to inhibit the action of other cytoplasmic proteins that had a role in peptidoglycan synthesis and turn over (ABIGAL and DIXE, 1994). Transpeptidation reactions that cross links the peptide side chain of polysaccharide peptidoglycan back bone. Transpeptidase and other proteins were called "penicillin binding protein". The net result of beta- lactam binding to this protein was to stimulate endogenous enzyme that degrade peptidoglycan (ABIGAL and DIXE, 1994). The inhibitory effect of vancomycin was similar to the effect of Beta-lactam antibiotic.

Our results showed that *Bacillus cereus* and *Escherichia coli* were sensitive to cefadroxil, chloramphenicol, ciprofloxacine, erythromycin, imipenem, neomycin, norfloxacin and streptomycin. Neomycin, aminoglycoside antibiotic; erythromycin, as macrolid antibiotic have the ability to bind to the 50S or 30S ribosomal subunit (inhibit protein synthesis). Also, inhibitory effect of ciprofloxacin and norfloxacin, as quinolones antibiotic, may be due to having the ability to inhibit bacteria by interfering with their ability to make DNA with diverse targets DNA gyrase, this inhibition effect leads to preventation of multiply of bacteria. The present results showed that all tested *Escherichia coli* strains were resistant to vancomycin antibiotic, while Srinivasona *et al.*, (2007) who found that all tested *Escherichia coli* strains were resistant to two or more antimicrobial used in veterinary medicine. *Bacillus cereus* was resistant to aztreonam and ceftazidime.

Antibiotic resistance can be categorized in three types: natural or intrinsic resistance; mutational resistance and extrachromosomal or aquired resistance. The resistance of isolates to beta-lactam antibiotic may be due to drug inactivation: i.e. AmpC cephalosporinase (beta lactamase enzyme that open the Beta-lactam (ring) as an intrinsic resistance. Target site modification (i.e. change in PBPs- penicillin binding proteins-) as mutational resistance represented in drug inactivation (ABIGAIL and DIXIE, 1994). Moreover the resistance of isolates to aminoglycoside antibiotic and erythromycin macrolides antibiotics may be due to inaccessibility of the target as an intrinsic resistance, reduced permeability or uptake as mutational resistance and aquired resistance represented in drug activation (DIAB *et al.*, 2002; 2004). The resistance of isolates to furadantine antibiotic may be due to chromosomal or plasmide mediated and inhibition of nitrofuran reductase. Also the resistance of isolates to fluoroquinolones antibiotics may be due to reduced permeability or uptake as mutational resistance (FANGE *et al.*, 2009). The mode of action of ciprofloxacin and norfloxacine as a quinolones antibiotic as accumulated with the explanation of FANGE *et al.*, (2009).

In our present study, four studied bacterial species which isolated from food sources were investigated to all selected antibiotics. Most of the selected antibiotics represent the following classes ; beta-lactam, aminoglycoside, macrolide and quinlones. Total bacterial counts is con-

sidered an index of quality which gives an idea about the higienic measures during processing and help in the determination of keeping quality of the product (ABERLE *et al.*, 2001). In this study, the identified bacterial species were *Bacillus cereus* (13.72%) and *Escherichia coli* (18.3). These results had a strong support of many researches. These bacterial species are common components of the microbial flora community. Generally, the methods of production, transportation, handling and sale of food entirely unhygienic and entirely depend on the traditional system, such system could pose favorable environment for bacterial contamination. The existences of these bacterial species which isolated in different food sources. These results are agreement with FDA, 2000. Moreover the studied food sources are considered as reservoir for some pathogenic bacteria (MANGES *et al.*, 2006).

The *Bacillus* species are of the soil origin and may contaminate bread through the raw material and bakery requirements used. *Bacillus cereus* is widely distributed in the environment and is found almost everywhere including, dust, water and decaying matter. The microbiotas in dried traditional vegetables sold in open market in parts of Ghana are dominant by aerobic mesophilic bacteria including mainly Bacillus, lactic acid bacteria, coliform and moulds. Many studies provided evidence that *Escherichia coli* is a frequently occurring organism in milk (SOOMRO *et al.*, 2002), this evidence agrees with the obtained results. Presence of *Escherichia coli* and T.C in food usually indicates lack of hygiene in handling, storing food and production inadequate storage and post process contamination. Therefore *Escherichia coli* and T.C enumeration are used as food quality parameter (GANZALEZ *et al.*, 2003). *Escherichia coli* was isolated from the samples and also has been detected in many studies

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