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ISOLATION, IDENTIFICATION AND CHARACTERIZATION OF PATHOGENIC BACTERIA FROM FOOD AND SURFACES OF SELECTED RESTAURANTS IN IRAQ

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ABSTRACT

The current research work was undertaken to identify, isolate and characterization of microbial quality of food, dining table surface and kitchen surface of ten popular restaurants in various location of Baghdad city in Iraq during January to December 2016 period. Total 50 samples, seven categories of food and surface samples were collected and cultured in selected medium to isolate the bacterial count and identified as per ht morphological and biochemical characteristics. The result revealed that 14 types of bacterial species including seven gram positive and seven gram negative bacterial isolates namely *Acinetobacter coloaceticus, Bacillus cereus, Bacillus mycoides, Bacillus mycoides, Campylobacter Spp, Clostridium perfringens, E. coli , Enterococcus fecalis, Klebsiella pneumonia, Proteus mirabilis, Pseudomonas aeruginosa, Salmonella Spp, Staphyl coccus capre* and *Staphylococcus aureus* respectively identified from food samples (Cake-9 sample, Chicken Curry-3 sample, Dal-7 samples, Rice-6 samples and Ruti -1 sample) and surface samples (Dining Table Top/Kitchen surfaces-24 samples). The antibiotics susceptibility pattern results showed all of these isolates were resist to most traditional antibiotics but in different ratios. DNA was isolated from each organism isolated from the current study and was amplified by PCR followed by purification and DNA sequencing. The resulting sequences then analyzed to allow for identification of the bacteria isolate. The study concluded that preventive and disinfectant plans should be considered to ensure contamination free restaurants for the better health of all consumers.

Keywords: Characterization of pathogenic bacteria, food quality, Iraq

INTRODUCTION

Epidemiological information demonstrates that irresistible diseases remain all around a danger signal for public health [1]. Pathogenic microorganisms can be isolated and identified much all over the place; from where they straightforwardly to get brought into food. Food is the absolute most critical product in urban people rundown of merchandise and enterprises used, representing about 55% of aggregate family consumption [2]. Improper cleaning of stores and unclean utensils leads to cause contamination of crude and cooked sustenance [3]. Public are more pulled in to non-home made sustenance's like, restaurant nourishments and road side foods. In creating nations like Iraq, India etc., are higher than high salary earner. So essentially client stack is higher in mid-level restaurants as great quality foods are less expensive than top of the line restaurants.

Distinctive investigations were performed to study the microbial nature of road nourishment and house made sustenance and a few examinations on road sustenance, natively constructed and restaurant food items. The causes of microbial contamination in food benefit incorporate environment, the nourishment specialist, the wellspring of food and food itself [4]. Kitchens surfaces and fixture nozzle might be noteworthy wellspring of cross contamination [5]. Most pathogenic microorganisms in food items are intestinal origin; some are found in nasal entries, in the throat, on hair, and on skin [6]. Subsequently, food servers are major sources of contamination and cross-contamination. *Staphylococci, Salmonella, and Campylobacter* are effectively exchanged from chicken to an assortment of kitchen surfaces, utensils, hands, and other nourishment things [7]. The microbial yield from inspecting samples is additionally reliant on different elements e.g. surface material and geography, consistency of microbial soil, sum and time of organisms. Bacterial food borne diseases happen when nourishment that is sullied with microorganisms is eaten and the microscopic organisms keep on developing in the digestion tracts, setting up a contamination which causes sickness. *Salmonella, Campylobacter, hemorrhagic E. coli, Staphylococci* and *Listeria* all bacteria leads to diseases[8]. Additionally, contamination of food at restaurants, with pathogenic microbes is for the most part because of preparing, taking care of, and unhygienic conditions. For instance, microorganisms may spread to nourishment by hands that are not washed subsequent to utilizing the can. They may spread to crude meat amid preparing so it is defiled when brought into the kitchen. Along these lines,

it is critical to ensure hands and working surfaces are completely washed after contact with crude meat, fish and poultry and before working with nourishments that require no further cooking [9]. The past research [10] demonstrates that nourishment security isn't a factor which impacts the general population's decision while choosing an eating foundation [11], the discovery and portrayal of pathogens in food is imperative in the control and counteractive action of food harming outbreaks [12].

The aim and objective of this research is to access the level of bacterial contamination, isolation and characterization of bacterial load among selected food items available and dining table/Kitchen surfaces at various restaurants located in Al-dourah, Al-rubaiee street, Al-karradah, Al-aamyriiah of Iraq. The bacterial isolates obtained in this investigation were classified on the basis of their cultural characteristic, cell morphology, Gram staining reaction and their biochemical properties as described by Barrow and Feltham (1993) [13].

EXPERIMENTAL

Study area and Sampling collection

Total fifty samples (23 samples from dining top/Kitchen surfaces and 27 food Samples like Rice, Chicken Curry, Dal, Cake, Ruti) of ten restaurants located at Al-dourah, Al-rubaiee street, Al-karradah, Al-aamyriiah in Baghdad city, Iraq (Table 1) collected in 2016 and 2017; all samples labeled appropriately and were transported to the laboratory within an prescribed period for culture and treated according to standard method[14]. The food samples (each 25 grams were taken from restaurant in sterile plastic bags in Ice-Box, according to Chessbrough (1984) [15]. Kitchen and dining table samples collected by sterilized cotton swab stick was moisture in a normal saline and swabbed by gentle rolling the swab stick at different part of the surfaces. Caution was taken not contaminate the swab, and it was place back in to its container and sealed with adhesive tape and labeled.

Microscopic examination

One drop of emersion oil was placed on these dried smears on the glass slide and observed under x100 resolution. Gram reaction and morphological characteristics of the organism were observed and recorded observed results were given in Figure 1.

Total viable count (TVC)

Various selective and deferential media used to describe the TVC, enteric microbial count like *Staphylococci* spp. Medias are the Nutrient agar (NA), Mannitol Salt Agar (MSA), Eosine Methylene Blue Agar (EMBA) and HiCrome Agar (HCA) respectively. Final results were tabulated in Table 2.

Bacterial isolation

NA, EMBA, MSA and HiCrome culture Media used to the isolation of bacterial colonies. Nutrient agar medium (NA) is the common microbiological growth medium used for growth of non-fastidious bacteria. This media used for total bacterial count. EMBA media is the best one to identify the microorganism *E.coli* isolated within two days of incubation period at 37°C based on metallic green color. MSA media is a specific medium to identify various gram positive and negative bacteria like *Staphylococcus aureus* at 37°C within two days of incubation period. HiCrome Agar is used to identify the selective microorganism like *Klebsiella* Spp, *Enterococci* Spp, *Escherichia Coli (E.Coli),* etc based on specific color on media after specific period of incubation.

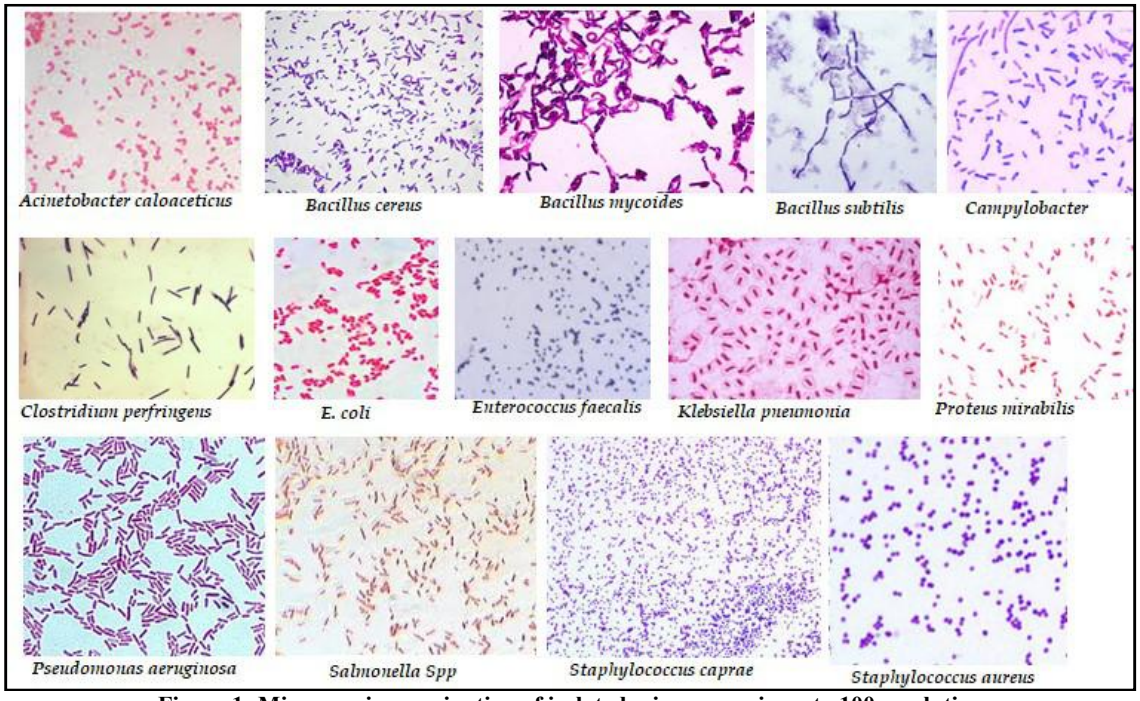

Figure 1: Microscopic examination of isolated micro organism at x100 resolution

Characterizations and Identification of microbes

The bacterial isolates obtained in this investigation were classified on the basis of their cultural characteristic, cell morphology, Gram staining reaction and their biochemical properties as described by method proposed by Barrow et al., (1993) [13]. Biochemical tests used to identify the microorganism were Oxidase, Catalase, Triple Sugar Iron test, Motility Indole Urease, Nitrate Reduction Test, Simmons Citrate Test, Carbohydrate utilization test, Methyl Red and Voges-Proscaure Test, Starch hydrolysis test, Lecithinase test, Hemolysis test, indol test, bile solubility test and citrate utilization test. Along with these traditional methods, Analysis of Bacterial Identification Software (ABIS) open source software is

also used to identify the probable bacteria name based on their morpho-biochemical characters, cultural characteristics, growth conditions, ecology and pathogenecity data.

Antibiotic susceptibility test

The control bacteria isolates were obtained from the National Medical Research Institute, Baghdad. The antimicrobial drugs used were obtained from Sigma Aldrich company. Culture media was prepared by reconstituting commercial powder in distilled water and sterilized at 121ºC for 15 minutes in an autoclave per the manufacturer's instructions [16]. The isolated microbial species were cultured on Hicrome agar Media. The isolates were tested by disc diffusion method for drug susceptibility according to National Committee for Clinical Laboratory Standards guidelines [17]. This was then impregnated with antimicrobial sensitivity discs using sterile forceps and then gently pressed down onto the agar. The antibiotic disc sensitivity was done using Ceftazidime (20µg), Ceftazidime (20µg), Ampicillin (20µg), Ceftriaxone (20µg) Cefotaxime (20µg) and Erythromycin (20µg), obtained results were tabulated in table 4. The criteria to select the antimicrobial agents were based on availability, CLSI guideline, the organisms' Gram reaction, and frequent prescription of drugs for the management of postnatal infections.

DNA extraction, PCR purification and sequencing

The Isolated bacterial cultures were plated on Hekton Enteric Agar and cultured 24 hours in a 37^{o}C in incubator, these obtained bacterial cultures used to DNA extraction. The Prep man Isolation kit was used for isolation of bacterial DNA method developed by Anonymous (2000)[18].

The PCR mix consist of 1µL of the bacterial DNA in a clean 500 µL micro centrifuge tube 1 µL each (20 picomoles) rP2 primer 5'-ACGTTAAAGGC-CTTAGGGCCCAAATT-3' and fD1 primer 5'-CGAGGGCCCTTAAATTGGCCAA TAAG-3. 12.5 µL HotStar Taq master PCR mix (Shimadzu) and 9.5 µL of distilled water was added to the reaction mixture was place in a PCR machine, which was programmed according with manual. The PCR product was run in a 2.5% agarose electrophoresis get and the results were given in figure 3. Samples showing an appropriate size band were saved for purification and DNA sequencing. PCR products were purified using the QIAquick PCR purification Kit (Qiagen Valencia, CA) and eluted with water. DNA sequences were analysed by submission to the ribosomal database project website [19] for identification finally tabulated in Table 5.

Table 1: Sample location, type, composition and date of collection

S.No	Sampling location/City Name	Name of the Sample	Total No. of samples collected (n)	Ingredients in selected food items	Sample Collection date
1	Al-ghannam restaurant/Al-dourah	Cake	1	Prepared with egg, flour sugar, milk,	12/6/2016
		Dining Table Top/Kitchen	2	-	12/6/2016
		Dal	1	boiled lentil with onion, oil, chilly, turmeric,	12/6/2016
2	Al_kassab restaurant/Al-dourah	Rice	1	Boiled white rice	19/7/2016
		Dining Table Top/Kitchen	2	-	19/7/2016
		Dal	1	boiled lentil with onion, oil, chilly, turmeric,	19/7/2016
3	Dadan restaurant/Al-dourah	Cake	2	Prepared with egg, flour sugar, milk,	13/6/2016
		Dining Table Top/Kitchen	2	-	13/6/2016
		Chicken Curry	1	Boiled chicken, oil, turmeric, chilly and other	13/6/2016
4	Blue-sky restaurant/Al-rubaiee street	Cake	2	Prepared with egg, flour sugar, milk,	12/7/2016
		Dining Table Top/Kitchen	3	-	12/7/2016
		Rice	1	Boiled white rice	12/7/2016
5	Kadduri restaurant/Al-karradah	Chicken Curry	1	Boiled chicken, oil, turmeric, chilly and other	17/6/2016
		Dining Table Top/Kitchen	2	-	17/6/2016
		Rice	1	Boiled white rice	17/6/2016
6	Zarzoor restaurant/Al-karradah	Dal	2	boiled lentil with onion, oil, chilly, turmeric,	25/6/2016
		Dining Table Top/Kitchen	2	-	25/6/2016
		Cake	2	Prepared with egg, flour sugar, milk,	25/6/2016
7	Habaibna restaurant/Al-karradah	Dal	1	boiled lentil with onion, oil, chilly, turmeric,	28/6/2016
		Dining Table Top/Kitchen	3	-	28/6/2016
		Rice	1	Boiled white rice	28/6/2016
8	Al-atshan restaurant/Al-karradah	Dal	2	boiled lentil with onion, oil, chilly, turmeric,	30/6/2016
		Dining Table Top/Kitchen	2	-	30/6/2016
		Ruti	1	Dough of flour	30/6/2016
9	Shaikh Mandii restaurant/Al-karradah	Rice	1	Boiled white rice	17/6/2016
		Dining Table Top/Kitchen	3	-	17/6/2016
		cake	2	Prepared with egg, flour sugar, milk,	17/6/2016
10	Hajji Mohammed restaurant/Al-aamyriiah	Chicken Curry	1	Boiled chicken, oil, turmeric, chilly and other	21/6/2016
		Dining Table Top/Kitchen	3	-	21/6/2016
		Rice	1	Boiled white rice	21/6/2016

Table 2: Comparison of Total Viable count (TVC) of micro-organism load on different Media

S.No	Sampling location/City Name	Name of the food item	Nutrient Agar (NA) Test CFU/ml	Eosine Methylene Blue Agar(EMBA) test CFU/ml	Mannitol salt Agar (MSA test CFU/ml	HiCrome Agar (HCA) test CFU/ml
1	Al-ghannam restaurant/Al-dourah	Cake	$2.5x10^6$	$2.4x10^6$	$2.4x10^6$	$2.1x10^6$
		Dining Table Top/Kitchen surface	$6.2x10^6$	$7.1x10^6$	$6.87x10^6$	$6.47x10^6$
		Dal	$3.4x10^7$	$3.0x10^7$	$3.1x10^7$	$3.3x10^7$
2	Al_kassab restaurant/Al-dourah	Rice	$1.2x10^2$	$1.7x10^2$	$1.6x10^2$	$1.4x10^2$
		Dining Table Top/Kitchen surface	$6.8x10^6$	$6.9x10^6$	$6.3x10^6$	$6.0x10^6$
		Dal	$3.0x10^4$	$3.7x10^4$	$3.5x10^4$	$3.1x10^4$
3	Dadan restaurant/Al-dourah	Cake	$5.1x10^5$	$5.2x10^5$	$5.3x10^5$	$5.2x10^5$
		Dining Table Top/Kitchen surface	$5.81x10^6$	$6.0x10^6$	$6.1x10^6$	$6.0x10^6$
		Chicken Curry	$3.4x10^6$	$3.3x10^6$	$3.3x10^6$	$3.2x10^6$
4	Blue-sky restaurant/Al-rubaiee street	Cake	$8.1x10^4$	$6.4x10^4$	$8.4x10^4$	$8.1x10^4$
		Dining Table Top/Kitchen surface	$7.07x10^6$	$7.0x10^6$	$7.2x10^6$	$7.3x10^6$
		Rice	$2.5x10^6$	$2.4x10^6$	$2.4x10^6$	$2.2x10^6$
5	Kadduri restaurant/Al-karradah	Chicken Curry	$3.4x10^7$	$3.0x10^7$	$3.1x10^7$	$3.0x10^7$
		Dining Table Top/Kitchen surface	$6.2x10^6$	$6.87x10^6$	$6.6x10^6$	$6.1x10^6$
		Rice	$2.5x10^3$	$2.8x10^3$	$3.0x10^3$	$3.2x10^3$
6	Zarzoor restaurant/Al-karradah	Dal	$5.0x10^4$	$5.1x10^4$	$5.3x10^4$	$5.0x10^4$
		Dining Table Top	$6.4x10^6$	$6.1x10^6$	$6.8x10^6$	$6.2x10^6$
		Cake	$6.0x10^4$	$6.4x10^4$	$6.5x10^4$	$6.1x10^4$
7	Habaibna restaurant/Al-karradah	Dal	$2.4x10^6$	$3.0x10^6$	$3.1x10^6$	$3.4x10^6$
		Dining Table Top/Kitchen surface	$3.67x10^6$	$4.21x10^6$	$5.01x10^6$	$5.51x10^6$
		Rice	$4.2x10^4$	$4.4x10^4$	$4.4x10^4$	$4.5x10^4$
8	Al-atshan restaurant/Al-karradah	Dal	$2.1x10^2$	$2.1x10^2$	$2.1x10^2$	$2.6x10^2$
		Dining Table Top/Kitchen surface	$5.4x10^6$	$5.3x10^6$	$5.5x10^6$	$5.7x10^6$
		Ruti	$4.4x10^7$	$4.0x10^7$	$4.1x10^7$	$4.7x10^7$
9	Shaikh Mandii restaurant/Al-karradah	Rice	$2.2x10^2$	$2.7x10^2$	$2.0x10^2$	$2.3x10^2$
		Dining Table Top/Kitchen surface	$5.9x10^6$	$5.8x10^6$	$5.8x10^6$	$5.1x10^6$
		cake	$3.7x10^4$	$3.4x10^4$	$3.9x10^4$	$3.7x10^4$
10	Hajji Mohammed restaurant/Al-aamyriiah	Chicken Curry	$3.7x10^5$	$4.0x10^5$	$4.5x10^5$	$4.0x10^5$
		Dining Table Top/Kitchen surface	$7.0x10^6$	$7.2x10^6$	$7.1x10^6$	$7.0x10^6$
		Rice	$3.2x10^2$	$3.0x10^2$	$3.4x10^2$	$3.2x10^2$

Table 3: Name of the Bacterial Isolated from the collected samples of various restaurants in Baghdad city, Iraq

S.No	Sampling location/City Name	Name of the food item	Name of th ebacteria Found in the Sample			
1	Al-ghannam restaurant/Al-dourah	Cake	*E.coli*	*Pseudomonas aeruginosa*	-	-
		Dining Table Top/Kitchen Surface	*E. coli*	*Bacillus subtilis*	*Staphylococcus aureus*	*Pseudomonas aeruginosa*
		Dal	*Staphylococcus aureus*	*Staphyl coccus capre*	-	-
2	Al_kassab restaurant/Al-dourah	Rice	*E.coli*	-	-	-
		Dining Table Top/Kitchen Surface	*E. coli*	*Pseudomonas aeruginosa*	*Bacillus subtilis*	*Salmonella Spp*
		Dal	*Enterococcus fecalis*	*E.coli*	-	-
3	Dadan restaurant/Al-dourah	Cake	*Bacillus cereus*	*E.coli*	*Pseudomonas aeruginosa*	-
		Dining Table Top/Kitchen Surface	*E. coli*	*Salmonella Spp*	*Staphylococcus aureus*	*Clostridium perfringens*
		Chicken Curry	*Staphylococcus aureus*	*Salmonella Spp*	*Clostridium perfringens*	-
4	Blue-sky restaurant/Al-rubaiee street	Cake	*E. coli*	*Bacillus cereus*		-
		Dining Table Top/Kitchen Surface	*E. coli*	*Staphylococcus aureus,*	*Bacillus subtilis,*	*Salmonella Spp*
		Rice	*Bacillus mycoides*	*Staphylococcus aureus*		-
5	Kadduri restaurant/Al-karradah	Chicken Curry	*Staphylococcus aureus*	*Salmonella Spp*	*Clostridium perfringens*	-
		Dining Table Top/Kitchen Surface	*Salmonella Spp,*	*Staphylococcus aureus,*	*E. coli,*	*Bacillus subtilis*
		Rice	*Klebsiella pneumonia*	*Enterococcus fecalis*	-	-
6	Zarzoor restaurant/Al-karradah	Dal	*Enterococcus fecalis*	*Staphyl coccus capre*	-	-
		Dining Table Top/Kitchen Surface	*Salmonella Spp*	*Bacillus subtilis*	*E.Coli*	*Campylobacter*
		Cake	*Acinetobacter coloaceticus*	*Bacillus cereus*	-	-
7	Habaibna restaurant/Al-karradah	Dal	*E.coli*	*Staphyl coccus capre*	-	-
		Dining Table Top/Kitchen Surface	*Pseudomonas aeruginosa,*	*Staphylococcus aureus,*	*Clostridium perfringens*	-
		Rice	*Staphylococcus aureus*	*Bacillus subtilis*	*Pseudomonas aeruginosa*	-
8	Al-atshan restaurant/Al-karradah	Dal	*Staphylococcus aureus*	*Enterococcus fecalis*	-	-
		Dining Table Top/Kitchen Surface	*Salmonella Spp*	*Bacillus subtilis*	-	-
		Ruti	*Proteus mirabilis*	*Staphylococcus aureus*	-	-
9	Shaikh Mandii restaurant/Al-karradah	Rice	*Bacillus mycoides*	*Enterococcus fecalis*	-	-
		Dining Table Top/Kitchen Surface	*Pseudomonas aeruginosa*	*E. coli*	-	-
		cake	*Acinetobacter coloaceticus*	*E.coli*	-	-
10	Hajji Mohammed restaurant/Al-aamyriiah	Chicken Curry	*Campylobacter spp*	*Clostridium perfringens*	*Staphylococcus aureus*	-
		Dining Table Top/Kitchen Surface	*E. coli*	*Staphylococcus aureus*	*Bacillus subtilis*	*Pseudomonas aeruginosa*
		Rice	*Proteus mirabilis*	*Klebsiella pneumonia*	*Staphylococcus aureus*	

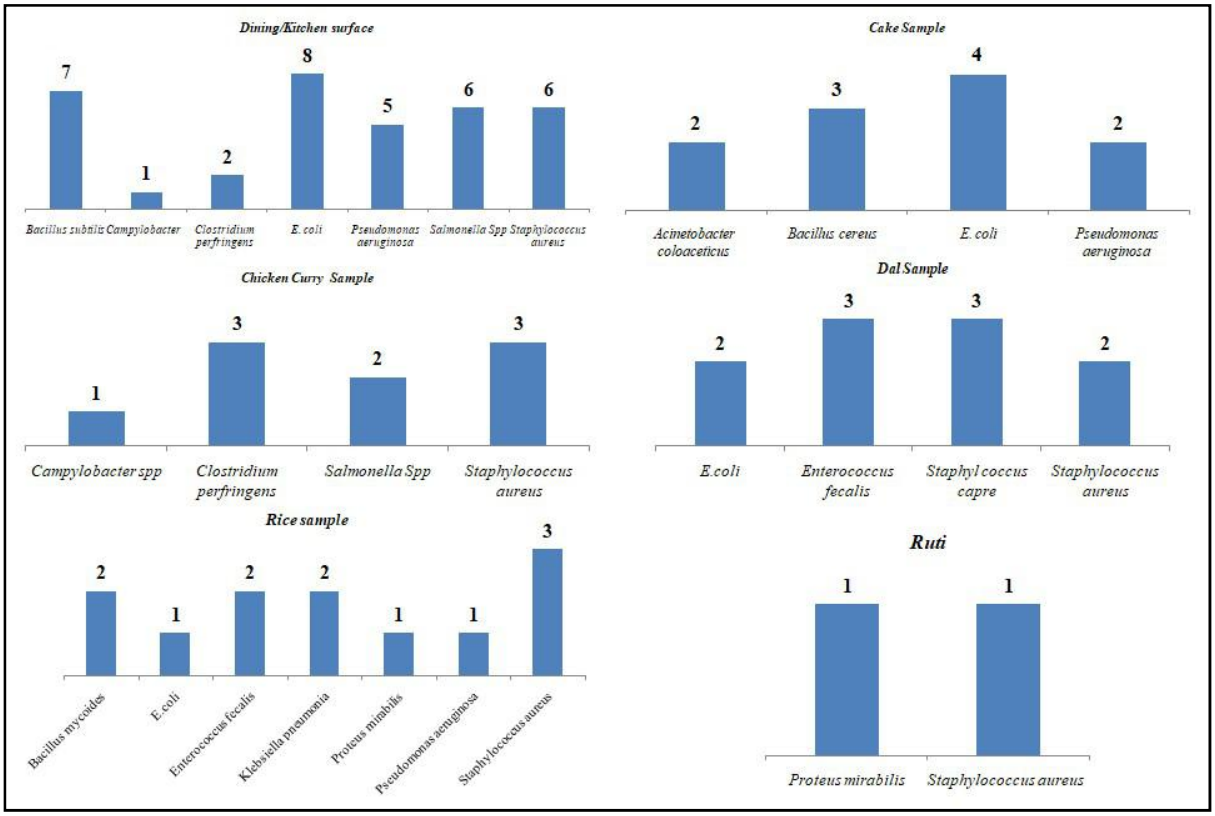

Figure 2: Individual Bacterial count found in various samples collected in the study area

Table 4: The existence of micro organism (Bacteria) Isolated from 50 samples

Bacteria Isolated	Total Positive	Gram stain Reaction	Contamination rate in %
Acinetobacter calcoaceticus	2	Gram (-)ve	2.2
Bacillus cereus	3	Gram (+)ve	3.7
Bacillus mycoides	2	Gram (+)ve	2.25
Bacillus subtilis	9	Gram (+)ve	11
Campylobacter	2	Gram (-)ve	2.5
Clostridium perfringens	5	Gram (+)ve	6.25
E. coli	15	Gram (-)ve	18.5
Enterococcus faecalis	5	Gram (+)ve	6.2
Klebsiella pneumonia	2	Gram (-)ve	2.45
Proteus mirabilis	2	Gram (-)ve	2.5
Pseudomonas aeruginosa	8	Gram (-)ve	10
Salmonella Spp	8	Gram (-)ve	10
Staphylococcus caprae	3	Gram (+)ve	3.7
Staphylococcus aureus	15	Gram (+)ve	18.7
Total:	**81**		**100%**

RESULTS AND DISCUSSION

In the current study total 50 samples were collected and tested for the presence of bacterial isolates from Baghdad restaurant in Iraq. Collected samples (in the form of Swab) cultured onto various agar media and incubated aerobically at 37^{o}C for 48 hours and observed that bacterial growth drastically. The examination of restaurant samples in this study revealed the presence of eleven genre including seven Gram(+)ve (*Bacillus cereus, Bacillus mycoides, Bacillus subtilis , Clostridium perfringens, Enterococcus faecalis , Staphylococcus caprae, and Staphylococcus aureus*) and seven Gram(-)ve (*Acinetobacter calcoaceticus , Campylobacter, E. coli, Klebsiella pneumonia, Proteus mirabilis, Pseudomonas aeruginosa & Salmonella Spp*) bacterial aerobes. From the table 2, it was identified that major number of microbe identified in the swabs collected from surfaces of dining table and kitchen samples. In these samples total 35 positives and six types of bacteria (*Bacillus subtilis, Campylobacter, Clostridium perfringens , E. coli, Pseudomonas aeruginosa , Salmonella Spp & Staphylococcus aureus*) were identified. It was equal to 43.5% of positive samples. So major contamination found in these samples only.

The quantities and qualitative analysis (Table 2 and Table 3) identified bacterial contamination is *Acinetobacter coloaceticus* in 2 samples (2.25%); *Bacillus cereus* in 3 samples (3.75%); *Bacillus mycoides* in 2 samples (2.25%); *Bacillus mycoides* in 9 samples (11.25%); *Campylobacter spp* in 2 samples (2.25%); *Clostridium perfringens* in 5 samples (6.25%); *E. coli* in 15 samples (18.50%); *Enterococcus fecalis* in 5 samples, (6.25 %), *Klebsiella pneumonia* in 2 samples (2.25%), *Proteus mirabilis* in 2 samples (2.25%), *Pseudomonas aeruginosa* in 8 samples (10%), *Salmonella Spp* in 8 samples (10%), *Staphyl coccus capre* in 3 samples (3%) and *Staphylococcus aureus* in 15 samples (18.75%) (Figure 2 and table 3). Bacterial count of restaurant's food and surface samples from various places vary because of different reasons. in the current investigation study, the variation of bacterial loads in seven types of samples are graphically represented in figure 2. It was observed form the figure 3, it can be stated that Dining/Kitchen surfaces 7 bacterial isolates, Cake, Chicken Curry and Dal samples 4 isolates each, rice sample 7 and Ruti samples only 2 bacterial isolates were found.

Total viable count identified form table 1 cake samples have shown $2.5x10^{6}$ to $8.4x10^{4}$ CFU/ml, chicken curry samples from $4.5x10^{5}$ to $3.4x10^{7}$ CFU/ml, Dal samples $2.1x10^{2}$ to $3.1x10^{7}$ CFU/ml, in surface samples $3.67x10^{6}$ to $7.1x10^{6}$

CFU/ml, Rice samples $1.7x10^2$ to $2.4x10^6$ CFU/ml and in Ruti sample $4.0x10^7$ to $4.4x10^7$ CFU/ml respectively observed on NA, EMBA, MSA & HiCrome Agar media. According to the obtained results (Table 4) a high contamination with *Escherichia coli* (18.5%) and *Staphylococcus aureus* (18.7%) were found in samples collected from different restaurants distributed at the Baghdad, Iraq.

A major concern in kitchen microbiology is the potential transmission of pathogens from raw food items brought into the kitchen. The most common culprits of bacterial food-borne illnesses in industrialized nations are *Pseudomonas aeruginosa,.*, *Salmonella spp*., *Clostridium perfringens* and various strains of *E. coli* [20]. These genera were, however, widely distributed in the four kitchens examined, even in areas where direct contact with raw food is unlikely. The relative abundance of Campylobacter, for example, was greatest on surfaces above counter tops, including upper cabinet handles and the microwave panel. Because Campylobacter contamination in kitchens is typically attributed to raw poultry [21], one possible explanation for these patterns is that contamination occurred from the hands of an individual who had handled raw poultry. Distribution of the contamination level of each restaurants shows that restaurants surfaces (kitchen/dining table) have the highest percentage of contamination 43.5% on total investigation. This indicated that food handlers working in these lack good personal hygiene and proper environmental sanitation [22].

As shown in Tables 4, the rate of bacterial isolates resistant (%) to selected antibiotics Ceftazidime, Ceftazidime, Ampicillin, Ceftriaxone, Cefotaxime and Erythromycin was *Acinetobacter coloaceticus* (50%), *Bacillus cereus* (50%), *Bacillus mycoides* (32%), *Bacillus subtilis* (56%), *Campylobacter* (59%), *Clostridium perfringens* (61%), *E. coli* (72%), *Enterococcus fecalis* (19%), *Klebsiella pneumonia* (32%), *Proteus mirabilis* (51%), *Pseudomonas aeruginosa* (73%), *Salmonella Spp* (55%), *Staphyl coccus capre* (59%) and *Staphylococcus aureus* (82%) respectively on average.

PCR results and gene sequencing

Gel analysis of PCR products showed 15 bands along with reference sample (Figure 3) for each of the 14 isolates with the expected size between 100 to 1200bp. Approximate sized DNA bands were also obtained following PCR form of 14 bacterial isolates of restaurant samples. Sequencing food samples yielded inconsistent results finally it was matches with database organisms (table 5).

Table 4: Antibiotic resistance patterns of isolated strains (14) obtained from food restaurants

Bacteria Isolated	Total resistant strains (%)						
	Ceftazidime (20µg),	Ceftazidime (20µg),	Ampicillin (20µg),	Ceftriaxone (20µg)	Cefotaxime (20µg)	Erythromycin (20µg)	Average
Acinetobacter coloaceticus	56	57	58	59	40	27	50
Bacillus cereus	54	52	50	48	48	48	50
Bacillus mycoides	31	32	32	33	32	32	32
Bacillus subtilis	70	67	65	62	41	28	56
Campylobacter	57	58	59	60	60	60	59
Clostridium perfringens	67	64	62	59	58	57	61
E. coli	81	83	84	86	58	39	72
Enterococcus fecalis	20	19	18	18	18	18	19
Klebsiella pneumonia	31	32	32	33	32	32	32
Proteus mirabilis	64	61	59	57	38	25	51
Pseudomonas aeruginosa	70	71	73	74	74	74	73
Salmonella Spp	60	58	55	53	52	51	55
Staphyl coccus capre	67	68	70	71	48	32	59
Staphylococcus aureus	89	85	82	79	79	79	82

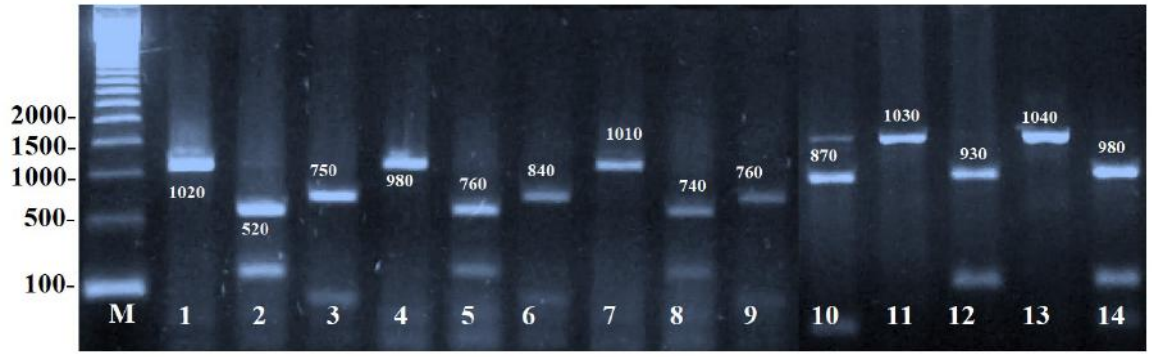

Figure 3: PCR results of isolated 14 bacterial strains for conforming of *Acinetobacter calcoaceticus*(1020bp), *Bacillus cereus*(520bp) , *Bacillus mycoides*(750bp), *Bacillus subtilis*(980bp) , *Campylobacter*(760bp), *Clostridium perfringens*(840bp), *E. coli* (1010bp) , *Enterococcus faecalis*(740bp), *Klebsiella pneumonia*(760bp), *Proteus mirabilis*(870bp), *Pseudomonas aeruginosa*(1030bp), *Salmonella Spp*(930bp), *Staphylococcus caprae* (1040bp) *and Staphylococcus aureus* (980 bp) based o bp balues

Table 5: Sequence data for obtained bacterial isolate

Bacteria Isolate	5'-3' sequence
Acinetobacter coloaceticus	AGGAATTCCCTTTAACCCAACCTAAATTTCCCAAAGGGCCCTGCTCCCATAA CCCATTCAAGGGATTTACAAGGGCCCC
Bacillus cereus	CGGGCCCCAAAAGGGAATTCCCTTTAACCCAACCTAAATTTCCCAAAGGGCC CCTTAAACCCTTGGGAAATTCCCAAAT
Bacillus mycoides	CCGGGCCCAAAAGGGAATTCCCTTTAACCCAACCTAAATTTCCCAAACCGGG CCCAAAAGGGAATTCCCCTTTAACCCAA
Bacillus subtilis	CCTAAATTTCCCAAAAAAGGGAATTCCCTTTAACCCAACCTAAATTTCCCAA AGGGCCCTGCTCCCATAACCCATTCAAG
Campylobacter	AATTTCCCAAACCGGGCCCAAAAGGGAATTCCCAAATTTCCCAAACCGGGCC CAAAAGGGAATTCCCTTTAACCCAACCT
Clostridium perfringens	CAAGGGATTTACAAGGGCCCTTTAACCCAACCTAAATTTCCCAAACCGGGCC CAAAAGGGAATTCCCTTTAACCCAACCT
E. coli	CCCAACCTAAATTTCCCAAACCGGGCCCCAAAAGGGAATTCCCTTTCCCGGG AAATTTCCCAAAGGGGTAACCCAACCT
Enterococcus fecalis	CTTTAACCCAACCTAAATTTCCCAAAGGGCCCTGCTCCCATAACCCATTCAA GGGATTTACAAGGGCCCCTTTAACCCAA
Klebsiella pneumonia	GCTCCCATAACCCATTCAAGGGATTTACAAGGGCCCCTTTAACCCAACCTAA ATTTCCCAAACCGGGCCCCAAAAGGGA
Proteus mirabilis	CCCAACCTAAATTTCCCAAACCGGGCCCCAAAAGGGAATTCCCTTTAACCCA ACCTAAATTTCCCAAAGGGCCCCTTAA
Pseudomonas aeruginosa	TAAATTTCCCAAAGGGCCCTGCTCCCATAACCCATTCAAGGGATTTACAAGG GCCCTTTAACCCAACCTAAATTTCCCAA
Salmonella Spp	ACCTAAATTTCCCAAACCGGGCCCAAAATTTAACCCAACCTAAATTTCCCAA ACCGGGCCCAAAAGGGAATTCCCAAATT
Staphyl coccus capre	CCCTTTAACCCAACCTAAATTTCCCAAACCGGGCCCAAAACAACCTAAATTT CCCAAACCGGGCCCAAAAGGGAATTCC
Staphylococcus aureus	CCAAAGGGCCCTGCTCCCATAACCCATTCAAGGGATTTACAAGGGCCCTTTA ACCCAACCTAAATTTCCCAAACCGGGCC

CONCLUSION

From this research it is evident that eating of food items available in the selected restaurants is a health risk even though as yet there having not been any report of any outbreak assigned to its consumption in the selected city areas. However, to ensure the safety and health of their customers, food restaurants should inculcate food safety practices and habits in their staff and food processing. The critical control points to preventing food borne illness such as preventing cross contamination from the raw products Food handlers should also be trained on hygienic food handling practices and safety. In the current research PCR experiment proved to be consistent way of identifying bacteria.

REFERENCES

[1] WHO. 2001. WHO Surveillance programme for control of foodborne infections and intoxications in Europe, Seventh report 1993-1998. Berlin, Germany

[2] Hauwa Tahir, Zuwaira Danyaro,Halima Ladan, Sanusi Magaji, Isolation And Identification Of Bacteria Involved In The Contamination Of Some Selected Fast Foods Sold Within Wuntin Dada Area, Bauchi,International Journal of Advances in Science Engineering and Technology, Vol4 (4), Spl. Issue-2, 2016, 99-101

[3] Davis, CE. and Samuel, C. Detecting Bacterial Contamination. Bio Tech international, Inc, 31, 1997, 364-396.

[4] Beuchat, C. R., Pathogenic microorganisms associated with fresh produce. J. of Food Protection, 59 (2), 1995, 204-216.

[5] Chen, Y., Jackson, KM., Chea, FP., and Schaffner, DW. Quantification and Variability Analysis of Bacterial Cross-Contamination Rates in Common Food Service Tasks. J. of Food Protect, 64(1), 2001, 72-80

[6] Farooq S, Hashmi I, Qazi IA, Qaiser S, Rasheed S. Monitoring of coliforms and chlorine residual in water distribution network of Rawalpindi, Pakistan. Environ Monit Assess, 140, 2008, 339–347

[7] De Boer E, Hahne M. Bacteriological analysis of air of kitchens in Zrural and Urban areas of Panipat district in Haryana (India). Int J Pharm Bio Sci , 2, 2011, 248–256

[8] Kaclikova E, Kuchta T, KayT, Gray D., Separation of Listeria from Cheese and enrichment media using antibodycoated microbeads and centrifugation. J. microbial. Methods, 46, 2001, 63–67.

[9] Nidham M. Jamalludeen,Isolation Of *Staphylococcus Aureus* And *Escherichia Coli*; The Important Food Borne Pathogens From Several Restaurants In Basrah City, Iraq, Basrah Journal of Veterinary Research,Vol.15, No.3,2016, Proceeding of 5th International Scientific Conference, College of Veterinary Medicine, University of Basrah, Ira

[10] Ciftcioglu,G., Arun ,O.O., Vural, A. A. Aiydin and Aksu,H., Survival of *E.coli* 0157:H7 in minced meat and Hamburger patties, Journal of Food, Agriculture and Environment, 6(1), 2008, 24-27

[11] Cowan, S.T., and Steel K.J. Manual for identification of bacteria 2nd Ed. (London: Cambridge University Press,1965, pp163-169

[12] Sudad Jasim Mohammed , Quality and quantity microbial assessment of the mobile restaurants (caravans) in Baghdad, J. Pharm. Sci. & Res. Vol. 10(9), 2018, 2354-2355

[13] Barrow, G.I. and Feltham, R.K., In Cowan and Steel's Manual for Identification of medical Bacteria. 3rd Ed. Cambridge, 1993.

[14] Sherman. C, Biochemical Activities of Microorganisms. Microbiology a Laboratory Manual 7th Ed., 2009, 143-203

[15] Chees brough M, Microbiological examination of specimens and biochemical testing of microorganisms in: Medical laboratory manual of tropical countries. 1st edition. Volume 2. Tropical Health Technology, Butterworth Heinemann Ltd. Printed in Great Britain At University Press, Cambridge, 1984, pp. 26-39, 57-69s

[16] Onyango et al., In vitro Salmonella Drug Susceptibility, J Infect Developing Countries 2(2), 2008, 106-111

[17] Cormican M., Whyte T., Hanahoe B., Antimicrobial susceptibility testing in Ireland: An introduction to the methods of the National Committee for Clinical Laboratory Standards (NCCLS). Department of Medical Microbiology, University of College Hospital Galway and Department of Bacteriology. National University of Ireland Galway.2001.

[18] Anonymous, Prepman Ultra Sample preparation reagent quick card, Applied Biosynthesis, Foster City, CA, 2000.

[19] http://rdp.cme.msu.edu/

[20] Mead, P.S., Slutsker, L., Dietz, V., McCaig, L.F., Bresee, J.S., Shapiro, C., et al. Food-related illness and

death in the United States. Emerg Infect Dis 5, 1999, 607–625.

[21] Cogan, T.A., Bloomfield, S.F., and Humphrey, T.J., The effectiveness of hygiene procedures for prevention of cross-contamination from chicken carcases in the domestic kitchen. Lett Appl Microbiol 29, 1999, 354–358

[22] Jibrin Yusuf Dahiru, Firdausi Aliyu Abubakar, Hamza Idris and Saratu Abubakar Abdullahi. Bacterial Contamination of Food Handlers at Various Restaurants in Kano State Metropolis, Kano Nigeria. Int. J. Curr. Microbiol. App. Sci. 5(5), 2016, 165-170.