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A STUDY OF BACTERIAL CONTAMINATION IN DIFFERENT PLACES IN HOUSE KITCHENS

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ABSTRACT

In the present study, thirty samples were collected from the surfaces of refrigerators,sinks and dining tables from five house kitchens in the southern Iraqi city of Basra. The identification of bacteria by Vitek II showed the presence of *Staphylococcus vitulinus, Staphylococcus lentus, Staphylococcus warneri, Kocuria rosea, Kocuria kristinae, Klebsiella pneumoniae, Aeromonas hydrophila, Enterobacter cloacae* and *Vibrio cholerae*. The identification of Methicillin-resistant *Staphylococcus aureus* (MRSA) was carried out using the HiChrome Meresa agar base. Most of these bacteria are pathogenic and can cause food deterioration and foodborne diseases. In addition, the susceptibility to antibiotic has been studied by the Vitek II system.

KEYWORDS: Vitek II, MRSA, *Vibrio cholerae*.

INTRODUCTION

The kitchen is probably the most important room in the house and plays a key role in the transmission of infectious diseases. Germs are often found in sponges, kitchen sinks, countertops, cutting boards, kitchen utensils, refrigerators, towels and even stovetops. The growth of unwanted contaminating bacteria causes not only the deterioration of the sensory and organoleptic characteristics of food but can also cause disease. Pathogens in the food are of intestinal origin; however, some are found in the nostrils, throat, skin and hair (Othman, 2015). Uncooked material is the main cause of contamination in the kitchen, although the area near the kitchen could be a source of free-living bacteria (Wolde and Bacha, 2016). Another way to contaminate food during processing and preparation is to manipulate infected foods and unhealthy practices. Also, handling food in unhygienic conditions, especially with dirty hands can cause its contamination (Alum *et al*., 2016). Domestic and industrial kitchens are the primary focus of infection care. In these settings, there are many studies that show that cross-contaminationis one of the main contributors to foodborne diseases (Alsayeqh, 2015; de Oliveira *et al.*, 2014). Pathogens enter the home continuously with food or water, through food prepared at home by an infected person, through the air, insects or pets (Tyagi and Tyagi, 2013).The number of disruptions in food-borne diseases has increased by bacteria. Several possible causes of these outbreaks are storage temperature, inadequate thermal treatment, cross-contamination, poor hygienic conditions of processing plants and food contact with contaminated surfaces (De Vere and Purchase, 2007).

Our interest in hygiene and cleanliness athome is important to prevent infection at its source, very often due to improper cooking practices including cross-contamination. Biological contaminants such as bacteria, viruses, fungi, protozoa, and worms are the most common cause of food poisoning, which mayvary from mild to chronic, sometimes conditions that threaten life, such as cholera, campylobacteriosis, *Escherichia coli* gastroenteritis, salmonellosis, shigellosis, typhoid fever, brucellosis and amoebiasis (Adiga *et al.*, 2012).

The present study aims to identify places in the kitchen that may harbor disease causing bacteria.

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MATERIALS AND METHODS

Collection of samples

A total of 30 samples was collected from different sites from five house kitchens. Samples were obtained from fridge, sink and dining table by using sterile cotton swabs. Specimens were cultured in nutrient agar and incubated at 37 °C for 24 h.

Identification of bacteria

The isolates were characterized morphologically on the basis of gram staining and identified on the basis of biochemical reactions which include catalase, mannitol fermentation, coagulase test (Holt *et al.*, 1994), growth on HI Chrome MeReSa agar base(HI media- India) for MRSA. While the other gram-positive and gram-negative bacteria were identified by the Vitek II systemt (Biomerieux, USA).

Antibiotic susceptibility test with VitekII system

Identification of a total of 125 isolates was performed using Vitek II system, according to the manufacturer's instructions

RESULTS AND DISCUSSION

In the present study, a total of 254 isolates were isolated from different places of house kitchens. Eighty-seven of them were identified as Methicillin-Resistant *Staphylococcus aureus* (Table 1).

Table 1. Biochemical characterization of isolated *Staph. aureus* using HiChrome MeReSa agar base.

Test	Result
Gram stain	+
Shape	cocci
Catalase	+
Mannitol fermentation	+
Growth on MRSA medium	+

While the rest (167 isolates) was identified using the Vitek II system (Table 2). The direct-identification, reporting time of the Vitek II system was 4.5- 10 h after incubation.

From Table 3, *Staph. lentus* was susceptible to all antibiotics, *Staph. aureus* and *Staph. vitulinus* were susceptible to all antibiotics and resistant to tetracycline. While, *Staph. warneri* was susceptible to all antibiotic except tetracycline, rifampicin, rifampicin/sulfamethoxazole and intermediate to nitrofurantoin. *Staph. warneri* may showed different

Table 2. Identification of bacteria from house kitchens using the Vitek II system.

Organism	Correctly identified%
Staph. vitulinus	85%
Staph. lentus	89%
Staph. warneri	85%
Kocuria rosea	92%
Kocuria kristinae	87%
Klebsiella pneumoniae	96%
Aeromonas hydrophila	99%
Vibrio cholerae	98%
Enterobacter cloacae	96%

sensitivity to many antibiotics using Vitek II (Campoccia *et al.*, 2010). Also the accuracy of Vitek II was higher when the strains of *Staph. aureus* were grown on solid media before the susceptibility test, in accordance to flow cytometry (Nuding and Zabel, 2013).

The results of antibiotics for gram-negative bacteria showed that *Klebsiella pneumoniae was* susceptible to many antibiotics and intermediate to ticarcillin, ceftazidime, cefepime and resistant to aztreonam and trimethoprim/sulfamethoxazole. *Aeromonas hydrophila* and *Enterobacter cloacae* were susceptible to all antibiotics and resistant to ticarcillin, ceftazidime, aztreonam and intermediate to minocycline. *Vibrio cholerae* was susceptible to amikacin, gentamycin, tobramycin, ciprofloxacin, and trimethoprim/sulfamethoxazole, while resistant toticarcillin, ceftazidime, aztreonam and intermediate topiperacillin, piperacillin, tazopactum, imipenem, meropenem and minocycline (Table 4).

The VitekII method of high susceptibility to antibiotics was achieved when tests were performed for *K. pneumoniae.* In addition to that, this method has the ability to recognize and interpret resistance mechanisms with high precision and standardization (Karagoz *et al.*, 2015; Livermore *et al.*, 2002).

Blondel-Hill *et al.*, (2003) observed the variability and validity of the AES system used in clinical microbiology laboratories to improve the correction of the results of the sensitivity tests and the clinical importance of the curative recommendations.

Hansen *et al.*, (2002) observed that the identification and susceptibility tests in Vitek II by inoculating plates with Gram-negative bacteria directly from positive blood cultures, match in 85% of the results compared to conventional sensitivity tests on strains grown on solid media after one day

Table 3. Antibiotic resistance profilesusingthe VitekIIsystem (GP bacteria).

Staph. aureus															
FOX	GN	TOB	LEV	MXF	ICR	ERY	CNM	LNZ	TCP	VAN	TEC	TGC	NTF	RFP	SXT
POS (+)	≤0.5(S)	≤1(S)	≤0.12(S)	≤0.25 (S)	NEG(-)	0.5(S)	≤0.25(S)	1(S)	≤0.5(S)	≤0.5(S)	≥16 (R)	0.5(S)	32 (S)	1(S)	20(S)
Staph. vitulinus															
POS (+)	≥0.5(S)	≥1(S)	≤0.12(S)	≤0.25 (S)	NEG(-)	≤0.25(S)	≤0.25(S)	1(S)	≤0.5(S)	≤0.5(S)	≥16 (R)	0.5(S)	32 (S)	1(S)	20(S)
Staph. lentus															
POS (+)	≤0.5(S)	≤1(S)	≤0.12(S)	≤0.25 (S)	NEG(-)	≤0.25(S)	≤0.25(S)	1(S)	≤0.5(S)	≤0.5(S)	≤1(S)	≤0.12(S)	32 (S)	≤0.5(S)	≤10(S)
Staph. warneri															
POS (+)	≤0.5(S)	≤1(S)	≤0.12(S)	≤0.25 (S)	NEG(-)	0.5(S)	≤0.25(S)	2(S)	≤0.5(S)	≤0.5(S)	≥16 (R)	0.5(S)	64 (I)	4(R)	80(R)

FOX = Cefoxitin screen, GN = Gentamycin, TOB =Tobramycin, LEV=Levofloxacin, MXF=Moxifloxacin, ICR=Inducible clindamycin, ERY=Erythromycin, CNM=Clindamycin, LNZ=Linezolid, TCP=Teicoplanin, VAN=Vancomycin, TGC=Tigecycline, NTF=Nitrofurantoin, RFP =Rifampicin, SXT=Trifampicin/sulfamethoxazole, TEC= Tetracycline, Susceptible (S), Intermediate (I), Resistant (R).

Table 4. Antibiotic resistance profiles vitek II system (GN bacteria).

Klebsiella pneumoniae													
TIC	PIP	PIP/TAZO	CAZ	FEP	ATM	IPM	MER	AK	GN	TOB	CIP	MIN	TSX
64(I)	≤4(S)	8(S)	16(I)	16(I)	≥64(R)	≥0.25(S)	≤0.25(S)	≤2(S)	≤ 1(S)	≤ 1(S)	≤ 0.25(S)	2(S)	160(R)
Aeromonas hydrophila													
≥128(R)	8(S)	16(S)	≥64(R)	ND	≥64(R)	≥0.25(S)	1(S)	≤2(S)	≤ 1(S)	≤ 1(S)	≤ 0.25(S)	8(I)	≤ 20(S)
Vibrio cholerae													
≥128(R)	64(I)	64(I)	≥64(R)	ND	≥64(R)	8(I)	8(I)	≤2(S)	≤ 1(S)	≤ 1(S)	≤ 0.25(S)	8(I)	≤ 20(S)
Enterobacter cloacae													
≥128(R)	16(S)	ND	32(R)	16(I)	≥64(R)	0. 5(S)	≤0.25(S)	≤2(S)	≤ 1(S)	≤ 1(S)	≤ 0.25(S)	8(I)	≤ 20(S)

TIC=Ticarcillin,PIP = Piperacillin, PIP/TAZO = Piperacillin Tazopactum, CAZ = Ceftazidime, FEP=Cefepime, ATM= Aztreonam,IPM = Imipenem, MER = Meropenem, AK = Amikacin, GN = Gentamycin, TOB = Tobramycin, CIP = Ciprofloxacin,MIN=Minocycline, TSX=Trimethoprim/Sulfamethoxazole,Susceptible (S), Intermediate (I),Resistant (R).

of incubation.

Due to the large reduction in reporting of errors in the results, interest in the automated Vitek II system has increased. For example, false computer reports of susceptibility to resistant microorganisms can reduce workload and objectivity in the analysis of results. Its determination of the resistance profile as a function of the MIC values is considered superior in comparison with the disc diffusion method (Barry *et al.,* 2003).

Table (5) shows that the most bacteria identified were MRSA, *Staph.vitulinus*, *Staph.lentus*, *Staph. warneri*, *Kocuria rosea*, *Kocuria kristinae*, *Klebsiella pneumoniae*, *Aeromonas hydrophila*, *Enterobacter cloacae* and *Vibrio cholerae*.

On the other hand, the refrigerator was more contaminated in contract to sink surface and dining table (Table 6).

And this may be related to unwashed raw food, leaky containers, dirty surfaces of the containers that are introduced into the refrigerators. These can directly contaminate other stored foods and remain on the interior surfaces. This, in turn, carries the risk of long-term indirect contamination in the subsequent preparation of food (Cogan *et al.,* 2002). Most shop keepers wash vegetables only with water, which is a risk factor in kitchens (da Cunha *et al.,* 2014). (James *et al.,* 2008) showed that most households do not have a constant energy supply, which affects the temperature regime of refrigerators. Most refrigerators do not work well due to power outages or a defective power supply of 4 °C (70.6%), which allows the growth of microorganisms that can be pathogenic and, therefore, increase the risk of food borne diseases.

There is a direct correlation with the opening frequency of the door and the temperature of the refrigerator. As expected, both found that higher temperatures were associated with more door openings (James *et al.,* 2017). (Hassan *et al.,* 2015) found that average refrigerator temperatures during the day (6:00 a.m. to 6:00 p.m.) were approximately a degree higher and were due to a higher frequency of door openings. (Khan and Afroz, 2014) have shown that the frequency of door openings has a significant impact on energy consumption and interior temperature.

Figure (1) shows that MRSA was the most common bacteria in kitchen refrigerators and formed 42%, followed by *Staph. vitulinus* and *Staph. lentus*, while *Kocuria kristinae* and *Staph. Warneri* with low abundance formed (5%, 6%), respectively. Refrigerators are one of the most important kitchen appliances found in homes used to store, conserve

Table 5. Types of bacteria isolated from different places in the kitchen.

Kitchen	Place	Bacteria identified
1	Refrigerator	MRSA
2	Refrigerator, Sinksurface	MRSA, *Staph. vitulinus, Aeromonas hydrophila*
3	Sink surface, Refrigerator, Table	MRSA, *Vibrio cholerae, Enterobacter cloacae Staph. lentus Klebsiella pneumoniae*
4	Refrigerator, Sink surface, Table	*Kocuria rosea, Staph. warneri, Staph. lentus Kocuria rosea, Kocuria kristinae Kocuria kristinae*
5	Refrigerator	MRSA

Table 6. Percentageof different bacteria isolated from different places in the kitchens.

Bacterial Isolates	Number	Fridge	Sink	Dining table	Percentage
MRSA	87	65	22	-	34%
Staph. vitulinus	41	41	-	-	16%
Staph. lentus	32	32	-	-	13%
Staph. warneri	8	8	-	-	3%
Kocuria rosea	24	10	-	14	9%
Kocuria kristinae	22	-	12	10	9%
Klebsiella pneumoniae	16	-	-	16	6%
Aeromonas hydrophila	14	-	14	-	6%
Vibrio cholera	6	-	6	-	2%
Enterobacter cloacae	4	-	4	-	2%
Total	254	156	58	40	

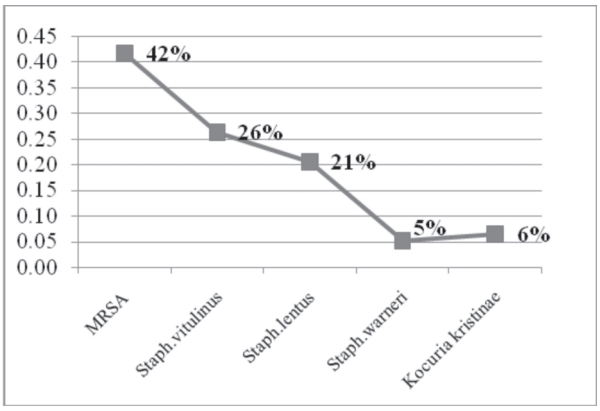

Fig. 1. Bacteria isolated from kitchen fridge

and extend the shelf life of food. Cooling is used to control the rate of certain chemical and enzymatic reactions, as well as the speed of growth of food microorganisms (Srivastava *et al.,* 2006).

The lowest temperature in a limited volume reduces the reproduction rate of bacteria, so that the refrigerator reduces the rate of deterioration. Cooling is a popular food storage technique in many countries and works by reducing the rate of bacterial reproduction. Thus, the apparatus for reducing the rate of deterioration of food is used (Godwin *et al.,* 2006).

Oluwafemi *et al.,* (2015) isolated *Staph. aureus*, *Escherichia coli*, *Bacillus subtilis*, *Enterobacter* spp., *Klebsiella* spp., *Shigella* spp., *Aspergillus flavus*, *A. niger*, *A. fumigatus*, *Saccharomyces cerevisae,* and *Rhizopus* spp. from refrigerators.

Otu-Bassey *et al., (*2017) isolated *Staph.aureus*, *E. coli*, *Shigella spp.*, *Pseudomonas aeruginosa*, *Aeromonas hydrophilia*, *Salmonella typhi*, *Klebsiella pneumonia*, *Streptococcus pyogenes* and *Proteus mirabilis* from house refrigerators. Tesfaye *et al.,* (2015) isolated *Staph. aureus* from washing sponges in percentage 34.3%. Obi and Ndukwu, (2016) isolated *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Aspergillus niger* from sponges.

The presence of pathogenic bacteria in home refrigerators and foods that contaminate directly orindirectly pose a significant risk to the health of consumers in terms of food poisoning. Therefore, knowledge of food safety, focusing on cooking hygiene and prevention of cross-contamination is necessary when the scope and impact of domestic food reduce transmitted diseases. Pathogens from contaminated foods cannot be completely removed from the kitchen, even if the food is stored at the proper temperature. Proper food preservation, preparation methods, regular hygiene routines and disinfection of food contact areas can control the growth, survival and spread of foodborne pathogens.As increasingly depend cooling food, it is important to control the temperature and provide cleaning habits consistent and efficient (Ahmed and Mashat, 2014; Oluwafemi *et al.,* 2015).

Because *Staph. aureus* does not form spores, contamination resulting fromheat treatment of food can be avoided. *Staph. aureus* is able to contaminate food products during processing and preparation and is, therefore,the main cause of foodborne diseases. Staphylococcal food poisoningis reported to be the third most common cause of foodborne illness worldwide (Kumar *et al.,* 2012). Symptoms of staphylococcal poisoning are usually rapid and occur approximately 3 hours after ingestion (range 1-6 hours) (Walderhaug, 2014).

Methicillin-resistant *Staph. aureus* (MRSA) is believed to be derived from the casc chromosome acquisition of mec staphylococci (SCCmec), which carries the mecA gene for methicillin resistance. Intrinsic antibiotic resistance is attributed to the presence of mecA(Mehrotra *et al.,* 2000).

Figure (2) shows thatthe abundant bacteria in sink surface were MRSA (38%) followed by *Aeromonas hydrophila* (24%), *Kocuria rosea* (21%), *Vibrio cholerae* and, *Enterobacter cloacae* (10% and 7%) respectively.

Studies about the domestic environment Josephson *et al.,* (1997); (Rusin, 1998) showed that microorganisms, including some potentially pathogenic species, are often found in all areas of the home environment, such as laundry areas, drains, U-pipes, toilets and diaper loops are more associated with intensive pollution and the emergence of potentially harmful species. Koenig, (2014) found Enterobacteria in kitchen sponges and tea towels. The main reservoir of staphylococci

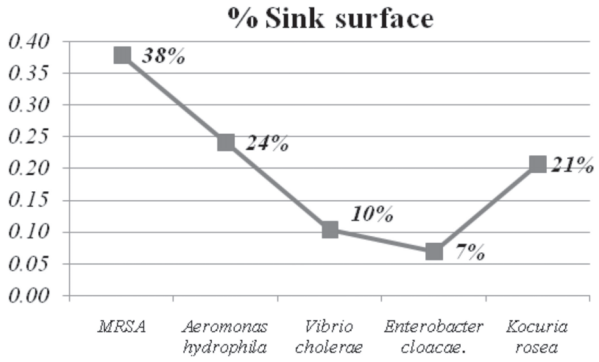

Fig. 2. Bacteria isolated from kitchens table

happens to be humans. Therefore, its transmission can be by direct contact with the hands or bodily fluids of infected people or by indirect contact with contaminated particles (Blanchard *et al.,* 2015; Widerström *et al.,* 2016).

In the dining table, the frequency of bacteria were *Klebsiella pneumoniae* (40%), *Kocuria kristinae* (35%), *Kocuria rosea (*25%) (Fig. 3).

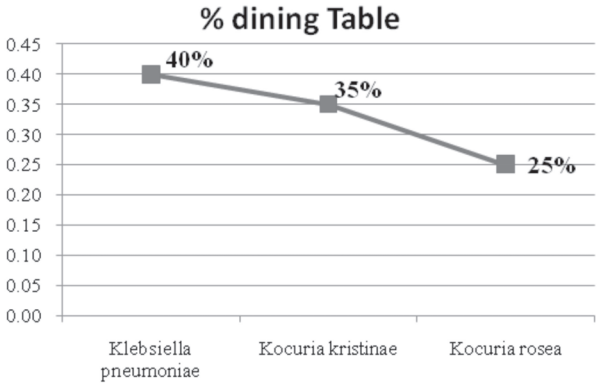

Fig. 3. Bacteria isolated from dining table.

K. Pneumonia can cause lung infections, septicemia, wound infection, burn infection, urinary tract infection, and ankylosing spondylitis. Like *Pseudomonas*, it is an opportunist agent. *Pneumonia* by *Klebsiella* spp. has a 50% mortality due to the underlying disease but can reach 90% in untreated cases (Umeh and Berkowitz, 2002).

The domestic kitchen environmentis a potential site for lodging and multiplication of pathogenic bacteria, including *Pseudomonas aeruginosa*, *K. pneumonia, Bacillus spp., Diptheroids, Ent. cloacae* and *Staph.epidermidis* (Kusumaningrum *et al.,* 2002; Tumwine *et al.,* 2003). Food borne pathogens are the leading cause of disease and death in developing countries, generating billions of dollars in medical and social costs (Nagarajan, 2018).

It has been suggested that, although raw materials are probably the main sources of contamination in the kitchen, the kitchen environment could also provide free living sources for bacterial populations. Sponges and wipes have been identified as potential agents in the spread of microorganisms and bacteria have been shown to persist in these vehicles Shen *et al.,* (2014). Martins *et al.,* (2014) recommended using techniques that include personal care, surface cleaning practices, temperature control, equipment maintenance, work environment improvements and the proper disposal of waste to eliminate contamination. As Rossi *et al.,* (2018) indicated in their search that some of the shopkeepers wore jewelry and nail polish on their fingernails. This practice is inappropriate because it could be a physical or biological contaminant once jewelry or nail polish can fall on food or due to dirt and microorganisms on these surfaces, and can become a source of cross-contamination.

It is important to clean and disinfect all surfaces that come in contact with food, which helps to eliminate some of the bacteria and germs. The use of hot water and detergent cleaning, hygiene in kitchens should be well maintained in order to reduce the content of harmful bacteria. Vegetables should be introduced into the fridge after actual washing. The instruments of kitchens such as garbage containers, sinks, dishwashers, etc., should be cleaned weekly or regularly by disinfectant agents.

CONCLUSION

The present study showed that kitchens contain contaminated pathogenic bacteria which can cross to contribute the food associated with infections; therefore, the regular cleaning of the kitchen areas is important to prevent people from being vulnerable to developing food poisoning

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