

Bacterial and *Aspergillus spp.* Contamination of Domestic Kitchens in Riyadh, Saudi Arabia

Suaad S. Alwakeel

Botany Department, Girls College of Education, Scientific Section, P.O. Box 27104, Riyadh 11417, Saudi Arabia,
Email: Dr.zenah@yahoo.com Tel: +966-1-470-6790 Fax: +966-1-470-6790

Abstract

A randomized sampling of 50 households in Riyadh City, Saudi Arabia was conducted to determine microbial and *Aspergillus spp* contaminants in domestic kitchens between May and June 2006. Samples were taken from open air in the kitchen and from used kitchen sponges. Inoculation procedures were varied from direct inoculation of the sponge into the medium to dilution of a cut portion of the sponge. A total of 200 samples were taken from which, 700 culture plates were done (BAP and Nutrient agar). Identification by the API system of identification (Analytical Profile Index, BioMerieux) revealed *Pseudomonas aeruginosa*, *Klebsiella pneumonia*, *Staphylococcus epidermidis*, *Enterobacter cloacae*, *Diphtheroids* and *Bacillus cereus*, *Aspergillus spp.* was isolated and identified microscopically. Among the isolates, *Staphylococcus epidermidis*, *Staphylococcus aureus* was isolated in 90% of the plates followed by *Pseudomonas aeruginosa* (83%), *Klebsiella pneumonia*; *Bacillus cereus* (63%) and *Aspergillus spp* (15%) These opportunistic pathogens may be harmful especially in immunocompromised hosts. In this setting, there is a constant risk of contamination and transfer to willing hosts, thus appropriate measures should be implemented such as the use of disposable sponges.

Key words: (Contamination - Domestic kitchens - Riyadh - Sponge - Bacterial).

Introduction

Pathogenic organisms continuously enter the home with foods (foodborne) or through water (waterborne), through foods prepared in the home by an infected person (person-to-person spread), through the air (airborne), by insects or via pets (Beumer *et al.* 1999). These are considered as the primary sources of potential harmful microorganisms in the home.

In the domestic environment, the kitchen is particularly important in spreading infectious diseases. (Bryan 1988) indicated that a colonized person handling the implicated food was the most frequently identified factor that contributed to staphylococcal food poisoning, shigellosis and typhoid fever. Several studies on bacterial contamination in the kitchen were carried out in the past decades (Finch *et al.* 1978; Speirs *et al.* 1995). Bacterial load of hand towels, dishcloths, tea towels, steel sinks and working surfaces were implicated to be the frequent

sites (Finch *et al.* 1978; Borneff *et al.* 1988; Josephson *et al.* 1997; Ikawa and Rossen 1999; Kusumaningrum *et al.* 2002).

Foodborne diseases associated with foods prepared in contaminated kitchen include *Salmonella* as the most common culprit (Holah and Thorpe 1990; Dufrenne *et al.* 2001; Kusumaningrum *et al.* 2004). Some other bacterial infections associated with contaminated kitchen environment are caused by *Campylobacter*, *Listeria*, *Staphylococcus aureus*, *Bacillus cereus* and *Escherichia coli* (Dufrenne *et al.* 2001; Regnath *et al.* 2004).

Fungi such as *Aspergillus niger* has also been implicated to cause heavy environmental contamination in the kitchen (London *et al.* 1996).

These studies have revealed that domestic kitchen sites have been found repeatedly contaminated with a variety of bacterial contaminants. Wet areas including sponges, dishcloths and sink drains continually appear to

act as a reservoir that harbours and encourages the growth of potential pathogens (Josephson *et al.* 1997; Enriquez *et al.* 1997; Hilton and Austin 2000; Beumer *et al.* 1996).

In Saudi Arabia, very few literatures have identified bacterial contaminants in the domestic kitchens. This study therefore aimed to investigate and identify the bacterial flora and fungi in kitchens of 50 different homes in Riyadh, Saudi Arabia.

Materials and Methods

Setting: Randomly selected 50 different homes in Riyadh City, Saudi Arabia were surveyed for potentially harmful pathogens in the domestic kitchen between May and June 2006.

Collection of samples and Methodology

We collected 50 samples of used kitchen sponges from these 50 different households from different districts of Riyadh City. Each collected sample was placed in a sterilized plastic bag. Sampling was done in four parts as follows:

Part I. Samples were taken from the open air from 50 kitchens. Two kinds of culture media, Blood Agar Plate (BAP) and Nutrient Agar Plate (NA) two replicas were placed on top of the kitchen sink and wash area, left open for one hour, then incubated for 24 hours at 28°C.

Part II. Fifty kitchens were sampled by taking their sponges used in cleaning the dishes and wash area. A small sample (5 cumm.) was taken from each sponge and was individually placed in 50 ml. distilled water, shaken and mixed well. The mixture was allowed to settle well and sample was taken from the mixture and inoculated into a BAP, then incubated for 24 hours at 28°C.

Part III. Fifty loops were inserted into each kitchen sponge from 50 different homes for at least one hour. After one hour, each inserted loop was inoculated into BAP by streak method and two replicates of the plates were incubated for 24 hours at 28°C.

Part IV. Another piece (5 cumm.) of the kitchen sponge from 50 different kitchens were taken, cut and placed directly on to the center of the BAP. The two replicates of the plates with the sponge sample were incubated for

24 hours at 28°C.

Isolation and Identification of microbes

After 24-48 hours of incubation, the colonies that appeared visually dissimilar were chosen, counted and subcultured to fresh BAP and incubated at 37°C for 24 hours. Identification of microorganisms did not commence until it was evident that a pure culture had been obtained.

Colonies were observed for size, texture, color and hemolytic reactions. Colonies are Gram stained and individual bacterial cells were observed under the microscope. The bacteria were speciated using these isolated colonies (Beumer *et al.* 1996).

Further identification of enteric organisms was done using the API 20E system (Analytical Profile Index, BioMerieux, Durham, NC, USA). Colonies from BAP were harvested and mixed with 0.5 ml McFarland standard until turbidity of the solution and a bacterial suspension was obtained. Using a sterile pipette, the bacterial suspension was inoculated to rehydrate each of the wells making sure that the end of the pipette touched the end of the cupule, allowing capillary action to draw the fluid into the well as bulb was slowly squeezed. Inoculation of specific test wells was done according to the manufacturer's instructions. The strips were incubated for 18 to 24 hours at 37°C. Test results were logged to an API 20E chart to determine the bacterial code which was compared to the API 20E Codebook for accurate identification of the organism.

Fungi was grown and cultured in Nutrient Agar plates for 18 to 24 hours at 28°C and inoculated into Potato Dextrose agar (PDA) for identification. Fungal isolates were identified microscopically.

Many other additional tests were done for further identification of the microorganism. Also done were API 20 Staph, API 20 Strep, API 20 Anaerobes and many traditional morphological, biochemical and physiological tests were selected for the study. The API system was then used with the additional tests to collect necessary data for the exact identification of the microorganisms.

Results

The following results were obtained and summarized in table 1:

Part IA—Isolated microorganism on BAP from open

air sampling revealed growth of 4 microorganisms. Gram negative *Klebsiella pneumonia* and *Pseudomonas aeruginosa* were identified by the API system of identification. *Staphylococcus epidermidis* was identified by the API 202Staph and coagula negative test. *Diphtheroids* were identified by the API 20E system. Among these 4 isolates, *Pseudomonas aeruginosa* accounted for 96% of isolates (48 colony types from 50 plates), followed by *Staphylococcus epidermidis* (90%), *Klebsiella pneumonia* (74%) and *Diphtheroids* (60%).

Part IB–Isolated microorganisms when grown on nutrient agar from open air sampling of kitchen sinks and wash area revealed *Klebsiella pneumonia* and *Pseudomonas aeruginosa*. *Aspergillus* spp. was identified through its microscopic morphological characteristics. *Pseudomonas aeruginosa* accounted 90% (40 colony types from 50 plates) whereas *Klebsiella pneumonia* accounted 60% of colony types from 50 plates and *Aspergillus* spp accounted for 30% (15 colony types from 50 plates).

Part II–Isolated microorganism grown on BAP from a kitchen sponge diluted in 50 ml. distilled water revealed *Klebsiella pneumonia*, *Pseudomonas aeruginosa* and *Staphylococcus epidermidis*. All organisms were identified by the API system. *Staphylococcus epidermidis* accounted for 90% (45 colony types from 50 plates), *Pseudomonas aeruginosa* (60%) and *Klebsiella pneumonia* (56%).

Part III. Isolated microorganisms grown on BAP taken from the loop inserted into the kitchen sponge

revealed *Bacillus cerus* and *Diphtheroids*. *Bacillus cerus* accounted for 36% (18 colony types from 50 plates) and *Diphtheroids*, 30% (15 colony types from 50 plates).

Part IV. Isolated microorganisms grown on BAP taken from kitchen sponge samples directly placed into the culture media revealed *Pseudomonas aeruginosa* and *Enterobacter cloacae*. *Pseudomonas aeruginosa* colonies accounted 96% from 50 plates whereas *Enterobacter cloacae* accounted 20%.

Discussion

Domestic kitchen environments are potential places for harboring and spreading pathogenic bacteria including *Pseudomonas aeruginosa*, *Klebsiella pneumonia*, *Bacillus* spp, *Diphtheroids*, *Enterobacter cloacae* and *Staphylococcus epidermidis*. According to the (Kusumaningrum *et al.* 2002; Tumwine *et al.* 2003; Borneff *et al.* 1985,1989) these pathogens survive on surfaces for hours or days, depending on the species. They also stated that wiping of surfaces (physical removal) tends to transfer and spread microorganisms from one surface to the other (Ojima *et al.* 2002a) bacteria are readily spread from cloths and sponges during wiping (Cogan *et al.* 2002; Ojima *et al.* 2002b; Gorman *et al.* 2002).

Enterobacter cloacae are frequently encountered as microflora of household kitchen sponges. They are isolated up to 55% of kitchen sponges (Beumer *et al.* 1996). This is because *E. cloacae* is less susceptible to chlorination. *Enterobacter* species better survive

Table 1. Microorganisms isolated and identified from 50 domestic kitchens in Riyadh, Saudi Arabia.

Culture Medium	No. of genus isolated	Sampling	Microorganisms identified	No. of colonies / 50 plates (%)
BAP	4	Open air	1. <i>Staphylococcus aureus</i> 2. <i>Diphtheroides</i> 3. <i>Pseudomonas aeruginosa</i> 4. <i>Klebsiella pneumonia</i>	45 (90%) 30 (60%) 48 (96%) 37 (74%)
NA	3	Open air	1. <i>Pseudomonas aeruginosa</i> 2. <i>Klebsiella pneumonia</i> 3. <i>Aspergillus</i> spp.	40 (90%) 30 (60%) 15 (30%)
BAP	3	5 mm ³ sponge diluted in 50 ml distilled water	1. <i>Pseudomonas aeruginosa</i> 2. <i>Klebsiella pneumonia</i> 3. <i>Staphylococcus epidermis</i>	30 (60%) 28 (56%) 45 (90%)
BAP	2	Loop inserted into sponge	1. <i>Bacillus cereus</i> 2. <i>Diphtheroids</i>	18 (36%) 15 (30%)
BAP	2	5 mm ³ sponge directly into media	1. <i>Pseudomonas aeruginosa</i> 2. <i>Enterobacter cloacae</i>	48 (96%) 10 (20%)

Note: BAP-blood agar plate, NA-nutrient agar plate.

than other non-sporeforming bacteria and become the predominant microflora in used sponges at room temperature. Fortunately, *Enterobacter* species rarely cause disease in a healthy individual. However, this opportunistic pathogen possesses an endotoxin and plays a major role in the pathophysiology of sepsis and its complications. Individuals at risk are those who stay in the hospital (especially in ICU), with malignancies, those using foreign devices (catheters) and the immunocompromised individuals. They can cause pneumonia, which can be fatal.

Pseudomonas aeruginosa, an opportunistic pathogen causes UTI, respiratory tract infection, dermatitis, soft tissue infection, bacteremia, bone and joint infection, gastrointestinal infections and a variety of systemic infections. Ragnath *et al* showed that *Pseudomonas aeruginosa* can also be found in household drains of showers and in kitchens (Regnath *et al.* 2004). Its predilection to moist environment makes it more possible to exist in kitchen surfaces and used sponges. Once infection with *Pseudomonas* is established, it is hard to control since this organism is frequently resistant to many commonly used antibiotics (Qarah *et al.* 2006; Humphrey 2001).

Generally, *Bacillus* species are neither morphologically nor phylogenetically indistinguishable from each other. Though most of the members of this genus is considered contaminants, there are 2 members which are of significant medical importance, *B. anthracis* and *B. cereus*. *B. anthracis* causes anthrax and *B. cereus* causes food poisoning (Cunha 2006).

Corynebacterium and diphtheroid infections usually causes toxic manifestations involving the heart, kidneys and nerves brought about by the toxins released by the bacteria to the bloodstream. Mortality rates are very high especially if it affects the very young and the very old (Frassetto *et al.* 2006).

Klebsiella pneumonia can cause pneumonia, septicemia, wound infection, burn infection, UTI and ankylosing spondylitis. Like *Pseudomonas*, it is an opportunistic pathogen. Pneumonia caused by *Klebsiella* has around 50% mortality, due to the underlying disease but may reach 90% in untreated cases (Umeh and Berkowitz 2006).

Staphylococcus epidermidis, *Staphylococcus aureus* causes infections from use of foreign materials like

catheters and prosthesis. Though it is a normal flora of the skin and mucous membranes and was regarded as a contaminant, invasion of this organism may cause severe infection and sometimes can be very fatal (Herchline 2006).

Aspergillus spp. are molds found in organic matter transmissible via inhalation. It can cause a broad spectrum of disease in humans, ranging from hypersensitivity reactions to direct angioinvasion. It primarily affects the lungs causing bronchopulmonary aspergillosis, necrotizing pneumonia, aspergilloma and invasive aspergillosis which is a rapidly progressive, often fatal infection (Harman and Szwed 2006).

Our study showed that these potentially harmful pathogens are easily accessible to every individual through contaminated sources such as a kitchen sponge and wash cloths. Aside from the previously reported *Enterobacter* species that are very common inhabitants of the kitchen, particularly *Pseudomonas* and *Klebsiella pneumonia* are present too. Though these microorganisms are opportunistic, they may somehow cause infection to unaware members of the household.

Our study revealed similar results with those of (Josephson and Rubino 1997; Scott *et al.* 1982; De Boer and Hahne 1990). Enterobacteriaceae like *Klebsiella* and *Enterobacter*; other species like *Pseudomonas* and *Staphylococcus* were similarly reported (Finch *et al.* 1978; Speirs *et al.* 1995).

Conclusion

Domestic kitchen environments can potentially spread bacteria. These disease outbreaks maybe related to or associated to use of contaminated kitchen sponges and other household practices which harbor these pathogens. There is constant risk of contamination transfer from the used surfaces; disposable sponges should be considered for use whenever possible. Reusable sponges should be dried after use or immersed in boiling water for 5 min. Furthermore, hygienic measures and precautions in the kitchen should be well maintained to reduce harmful bacterial levels.

References

- Beumer RR, Bloomfield S, Exner M, Fara GM, Scott E. 1999. The need for home hygiene. Policy and Guidelines on home hygiene. *Ann Ig.* 11: 11-26.

- Beumer RR, Te Giffel MC, Spoorenberg E, Rombouts FM. 1996. *Listeria* species in domestic environments. *Epid and Infection*. 117: 437-42.
- Borneff J. 1989. Efficient hygiene precautions in the household today. *Zentralbl Bakteriol Mikrobiol Hyg (B)*. 187 (4-6): 404-13.
- Borneff J, Hassinger R, Wittig J, Edenharder R. 1988. The distribution of microorganisms in household kitchens I. Problems, experiments, results. *Zentralbl Bakteriol Mikrobiol Hyg* 186: 1-29.
- Borneff J, Wittig JR, Borneff M, Hartmetz G. 1985. Occurrence of enteritis-causing agents in private households—a pilot study. *Zentralbl Bakteriol Mikrobiol*. 180, (2-3): 319-34.
- Bryan FL. 1988. Risks of practices, procedures and processes that lead to outbreaks of foodborne diseases. *J. of Food Protection*. 57: 663-73.
- Cunha BA. *Bacillus* infections. www.emedicine.com. 2006.
- Cogan TA, Slader J, Bloomfield SF, Humphrey TJ. 2002. Achieving hygiene in the domestic kitchen: the effectiveness of commonly used cleaning procedures. *J. of Applied Microbiology*. 92: 885-92.
- De Boer E, Hahne M. 1990. Cross-contamination with *Campylobacter jejuni* and *Salmonella* spp. from raw chicken products during food preparation. *J. of Food Protection*. 53: 1067-8.
- Dufrenne J, Ritmeester W, Deffgou-van Asch E, Van Leuden F, de Jonge R. 2001. Quantification of the contamination of chicken and chicken products in the Netherlands with *Salmonella* and *Campylobacter*. *J. Food Protection*. 64: 538-41.
- Enriquez CE, Enriquez-Gordillo R, Kennedy DI, Gerba CP. 1997. Bacteriological survey of used cellulose sponges and cotton dishcloths from domestic kitchens. *Dairy Food & Environ Sanitation* 17: 2-24.
- Finch JE, Prince J, Hawksworth M. 1978. A bacteriological survey of the domestic environments. *J. of Applied Bacteriology*. 45: 357-64.
- Frassetto LA *et al.* 2006. *Corynebacterium* infections. www.emedicine.com.
- Gorman R, Bloomfield S, Adley CC. 2002. A study of cross-contamination of food-borne pathogens in the domestic kitchen in the Republic of Ireland. *Int. J. Food Microbiol*. 76 (1-2): 143-50.
- Harman EM and Szwed T 2006. *Aspergillosis*. www.emedicine.com.
- Herchline T. 2006. *Staphylococcal* infections. www.emedicine.com.
- Hilton AC, Austin E. 2000. The kitchen dishcloth as a source and vehicle for foodborne pathogens in a domestic setting. *Int. J. Environ. Health Res*. 10: 257-61.
- Holah JT, Thorpe RH. 1990. Cleanability in relation to bacterial retention on unused and abraded domestic sink materials. *J. of Applied Bacteriology*. 599-608.
- Humphrey T. 2001. The spread and persistence of *Campylobacter* and *Salmonella* in the domestic kitchen. *J. of Infection*. 43: 50-3.
- Ikawa JY, Rossen JS. 1999. Reducing bacteria in household sponges. *J. Environ. Health*. 62: 18-22.
- Josephson KL, Rubino JR, 1997. Pepper IL. Characterization and quantification of bacterial pathogens and indicator organisms in household kitchens with and without the use of a disinfectant cleaner. *J. of Applied Microbiology*. 83: 737-50.
- Kusumaningrum HD, van Putten MM, Rombouts FM, Beumer RR. 2002. Effects of antibacterial dishwashing liquid on foodborne pathogens and competitive microorganisms in kitchen sponges. *J. of Food Protection*. 65: 61-5.
- Kusumaningrum HD, van Asselt ED, Beumer RR, Zwietering MH. 2004. A quantitative analysis of cross-contamination of *Salmonella* and *Campylobacter* spp via domestic kitchen surfaces. *J. Food. Prot.* 67 (9): 1892-903.
- London KW, Coke AP, Burnie JP, Shaw AJ, Oppenheim BA, Morris CQ. 1996. Kitchens as a source of *Aspergillus niger* infection. *J. Hosp. Infect.* 32 (3): 191-8.
- Ojima M *et al.* 2002 b. Hygiene measures considering actual distributions of microorganisms in Japanese households. *J. of Applied Microbiology* 93 (5): 800-9.
- Ojima M *et al.* 2002 a. Bacterial contamination of Japanese households and related concern about sanitation. *Environ Health Res* 12 (1): 41-52.
- Qarah *et al.* *Pseudomonas* infections. www.emedicine.com, 2006.
- Regnath T, Kreutzberger M, Illing S, Oehme R, Liesenfeld O. 2004. Prevalence of *Pseudomonas aeruginosa* in households of patients with cystic fibrosis. *Int. J. Hyg Environ. Health* 207 (6): 585-8.
- Scott E, Bloomfield SF, Barlow CG. 1982. An investigation of microbial contamination in the home. *J. Hyg. (Lond)*. 89 (2): 279-93.
- Speirs JP, Anderson A., Anderson JG. 1995. A study of microbial content of domestic kitchen. *Int. J. of Environ. Health*: 5 (13): 109-22.
- Tumwine J, Thompson J, Katui-katua M, Mujwahuzi M, 2003. Johnstone N, Porras I. sanitation and hygiene in urban and rural households in East Africa. *Int. J. Environ. Health Res*. 13 (2): 107-15.
- Umeh O and Berkowitz LB. 2006. *Klebsiella* infections. www.emedicine.com.

التلوث البكتيري والفطري لجنس الاسبرجلس في بعض المطابخ المنزلية في مدينة الرياض في المملكة العربية السعودية

سعاد صالح الوكيل

قسم النبات ، كلية التربية للبنات ، الأقسام العلمية ، ص.ب ٢٧١٠٤ ، الرياض ، ١١٤١٧ ، المملكة العربية السعودية

الملخص

نفدت هذه التجربة لمعرفة مدى التلوث و لتحديد المحتوى البكتيري في المطبخ العائلي بين شهري أيار و حزيران عام ٢٠٠٦ م ، تم فيها أخذ عينات عشوائية من (٥٠) منزل في مناطق مختلفة في مدينة الرياض ، المملكة العربية السعودية ، تم فحص عينات من الهواء في المطبخ ، كما أنه تم أخذ عينات من إسفنج المطبخ المستعمل . تم التلقيح بطرق متعددة منها الطريقة المباشرة وهي التي تم فيها أخذ عينة من الأسفنج مباشرة و تم أخذ عينة بواسطة ابرة التلقيح من داخل وخارج الاسفنجة، ٢٠٠ عينة عملت و ٧٠٠ مزرعة لقحت بطريقة التخطيط واستعملت طريقة التخفيف لعزل الميكروبات منها ايضا. ظهر في النتائج بعد زراعتها في بيئة (BAP) و تعريفها من قبل نظام (API) للتعريف أنواع متعددة من البكتيريا منها:

Pseudomonas aeruginosa , klebsiella pneumonia ,Staphylococcus epidermidis, Staphylococcus aureus Enterobacter cloacae, Diptheroids, Bacillus cereus., and Aspergillus spp.

من خلال العزل اتضح أن نسبة *Staphylococcus.aureus, Staphylococcus epidermidis* ٩٠% من مجموعة الأطباق تبعتها *Pseudomonas aeruginosa* ٨٣% و *Bacillus cereus, klebsiella pneumonia* ٦٣% وفطر الاسبرجلس بنسبة ١٥% هذه الأنواع من البكتيريا والفطر قد تكون خطرة خاصة في المطابخ و في هذه الحالة قد تكون هناك مخاطرة دائما للتلوث البكتيري لهذا يجب أخذ طرق سليمة و صححيه في التعامل مع الإسفنج المتكرر الاستعمال وتنقية الهواء في المطبخ.