Culture dependent and independent studies on emerging food-borne pathogens *Cronobacter sakazakii, Klebsiella pneumoniae* and *Enterococcus faecalis* in Indian food

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Abstract

Emerging food-borne pathogens *Cronobacter sakazakii, Klebsiella pneumoniae* and *Enterococcus faecalis* may cause food-borne diseases in neonates, elderly and immunocompromised patients. Data on prevalence of emerging pathogens in food from India is lacking. Sixty food samples were analyzed from local markets in Mumbai for the presence of *C. sakazakii, K. pneumoniae* and *E. faecalis*. Present study shows that incidence of *C. sakazakii* and *K. pneumoniae* was highest in sprouts and least in milk (Table 1). Total 35% and 85% of food samples were positive for *C. sakazakii* and *K. pneumoniae* respectively. Culture independent species specific PCR based method was better than conventional culture based method in detecting these pathogens. E. faecalis was not detected in any of the food samples. All the isolates of *C. sakazakii* and *K. pneumoniae* were resistant to ampicillin. *C. sakazakii* was also resistant to cephalothin and *K. pneumoniae* was resistant to many other beta-lactam antibiotics like amikacin, aztreonam and amoxicillin-clavulanic acid. Our results suggest that conventional method should be carried out in combination with molecular methods for the accurate identification of these pathogens. Increase in drug resistance amongst emerging pathogens is also a concern.

Introduction

Emerging pathogens are those causing illnesses that have only recently appeared or have been recognised in a population or that are well recognised but are rapidly increasing in incidence (Morse, 2004). Cronobacter is an opportunistic pathogen. It causes food-borne diseases in neonates and infants and is therefore classified in hazard identification category A by World Health Organization/Food and Agriculture Organization (FAO/WHO, 2004). The primary reservoirs of Cronobacter are not well defined due to the ubiquitous nature of this bacterium. Cronobacter strains have been isolated from a wide range of foods including milk, cheese, dried foods, meats, water, vegetables, rice, tea, herbs and spices, and various food production environments (Friedemann, 2007; Chen *et al.*, 2016).

Klebsiella pneumoniae is known as neonatal pathogen and is included in hazard identification category B (FAO/WHO, 2004). It has been isolated from raw vegetables, street foods in Malaysia, India and Africa (Haryani *et al.*, 2007; Tambekar *et al.,* 2011). Moreover, food-borne nosocomial outbreaks due to ESBL-Kp (Extended Spectrum Beta lactum *Klebsiella pneumoniae*) have also been reported from Spain (Calbo *et al.*, 2011).

Enterococci are natural inhabitants of the gastrointestinal tract of humans, other mammals and birds. They are frequently found in many foods, including those of meat, dairy and vegetable origin (Gundogan *et al.*, 2013). *E. faecalis* possesses a broad spectrum of natural and acquired antibiotic resistance. Vancomycin resistance has become a clinical problem and a majority of vancomycin resistant enterococcus (VRE) infections are due to the multidrug resistant vancomycin resistant *E. faecalis*. But the global epidemiology of vancomycin resistant *E. faecalis* is not well understood (Peter *et al.*, 2012). *K. pneumoniae* and *E. faecalis* have been isolated from fried rice, salad and tomato stew in Ghana (Manu *et al.*, 2010) as well as hidden fresh vegetables and fruits (Kharousi *et al.*, 2016).

The changing lifestyle and urbanization have increased the demand of processed food and associated risks of foodborne infections. Ensuring food safety requires due attention during harvest, transport, processing, storage and finally during food preparation and storage by consumers. Processed, frozen or ready-to-eat food is gaining popularity in recent years due to changing food habits, product diversification, busy lifestyle and mass production practices. In urban settings, there is a growing tendency to buy meat, milk and vegetables on the

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weekend and store these items in the freezer or refrigerator. Microwave ovens are often used for reheating of food. However, while using a refrigerator and microwave are part of daily life in urban settings, most users and food handlers rarely have a chance to learn how to safely store and reheat food (FAO, 2015). Thus, inspite of the ubiquitous nature of these emerging foodborne pathogens worldwide; data regarding the prevalence and the antibiotic sensitivity of *C. sakazakii, K. pneumoniae* and *E. faecalis* from Indian foods is lacking. Our objective in the present study was to determine the incidence of *C. sakazakii, K. pneumoniae* and *E. faecalis* in milk powder (processed food), ready to eat raw sprouts and fish. Antibiotic sensitivity pattern of these isolates were also evaluated.

Materials and Methods

Samples

Total 60 samples comprising of milk products such as powdered milk, infant formula, cheese and paneer - 20 samples, sprouts comprising of mung, matki, wal, channa, watana, mixed sprouts -20 samples , fish – 20 samples were purchased from local markets of Mumbai, brought in ice and processed within 1 h after bringing to laboratory.

Chemicals and media

The media and the antibiotic discs used for the experiment were acquired from HiMedia Laboratories, Mumbai, India. Taq polymerase enzyme, dNTP and primers were from Merck, India. The *C. sakazakii* was previously isolated in our laboratory from mung sprout sample; while the cultures *K. pneumoniae* MTCC 109 and *E. faecalis* MTCC 439 were obtained from the Microbial Type Culture Collection, Chandigarh, India.

Isolation of the emerging pathogens by conventional method

C. sakazakii was isolated as per the standard methods adopted from the online Bacteriological Analytical Manual U. S. Food and Drug Administration (2012) for milk or milk products with minor modifications; 0.1 ml of aliquot from pre-enriched 10% homogenate in buffered peptone water (BPW) were spread on Hi Chrome *Enterobacter Sakazakii* Agar plates. Typical bluish green colonies were picked up for standard biochemical characterisation viz: Gram staining, motility, oxidase, catalase, IMViC, aesculin, dulcitol, and sucrose fermentation,arginine, ornithine and lysine decarboxylation and DNase test.

The isolation of *K. pneumoniae* was carried as per Haryani *et al* (2007). The sample (25 g) of was homogenised in 225 ml lactose broth and incubated at 37°C for 24 h. 1.0 ml of the pre-enriched sample was enriched in tetrathionate broth incubated at 37°C for 24 h. 0.1 ml of the aliquots were spread plated on Bismuth Sulphite agar and Hi Chrome *Klebsiella* selective agar. Typical black and purple magenta coloured, mucoid colonies from Bismuth Sulphite agar and Hi Chrome *Klebsiella* isolation agar respectively were picked up for biochemical characterization using Hi25 Enterobacteriaceae Identification Kit (HiMedia, Mumbai, India).

E. faecalis was isolated as per Valenzuela *et al.* (2010) with minor modification. 25 g of the sample was homogenized in 225 ml of sterile saline; settled on ice for 10 min and spread plated (0.1 ml) on Slantez and Bartely agar. After incubation for 48 h at 44±1°C the typical red or maroon colonies were streaked on Bile esculin agar and *Enteococcus* confirmatory agar and incubated for 24 h at 37°C.

Detection by culture independent PCR method and confirmation of typical/atypical colonies from selective plate by species specific PCR.

DNA was isolated using boiling method (Saroj *et al.*, 2008). In brief, 1 ml pre-enriched sample or food homogenate was centrifuged at 10621 g and the pellet obtained was suspended in 100 µl of distilled water. DNA was extracted by boiling for 5 min in water bath and centrifuged at 10621 g. Similarly the DNA from typical colonies from selective plates was also isolated by boiling method. Supernatant was used as a template for PCR. The PCR was carried out in a 25 µl volume containing 10 mM Tris/HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 1 µl primer pairs (10 pmol each), 0.5 µl (1.5 U) Taq polymerase, 0.2 mM dNTPs (Merck, Banglore, India) and 1.0 µl genomic DNA . C. sakazakii specific PCR (151 bp product) was carried out by using gyr B gene specific primers [F1 (5′-AGGTAAAATCCACCAGCAAAC-3′)) and R1 (5′-CAGAATATCGTATTCAAACTC-3′))] as described by Huang *et al.* (2013). *K. pneumoniae* specific 130 and 260 bp PCR product was obtained using primers pf (5′-ATT TGA AGA GGT TGC AAA CGA T-3′)/pr1 (5′-TTC ACT CTG AAG TTT TCT TGT GTT C-3′) and pf/pr2 (5′-CCG AAG ATG TTT CAC TTC TGA TT-3′) respectively as described by Liu *et al.* (2008). *E. faecalis* specific 733 bp PCR product was obtained using primers E1 (5′ TCA ACC GGG GAG GGT 3′) and E2 (5′ ATT ACT AGC GAT TCC GG as described by Deasy *et al.* (2008).The amplified DNA products were separated by agarose gel electrophoresis (1.5% w/v), stained with ethidium

Table 1. Incidence of different emerging pathogens in food samples by different methods

Food samples	C. sakazakii				K. pneumoniae			
	A	B	C	D	A	B	C	D
Sprouts (20)	1	2	8	9	1	8	7	4
Fish (20)	1	4	4	11	0	6	14	0
Milk (20)	1	0	1	18	2	11	2	5
Total (60)	3 (5%)	6 (10%)	13 (22%)	38 (63%)	3 (5%)	25 (42%)	23 (38%)	9 (15%)

E. faecalis was not detected in the study
A- Samples positive only by standard conventional method
B- Samples positive by only PCR method
C- Samples positive by both methods
D- Samples negative by both methods

bromide and visualised by UV trans-illumination (Figure 1). All DNA samples were also tested with universal 16s rRNA gene primers to rule out PCR inhibition by food component (Nossa *et al*., 2010).

Antibiotic sensitivity test

C. sakazakii (16) and *K. pneumoniae* (24) isolates were screened for Antibiotic Sensitivity using different antibiotics by agar diffusion method as described by National Committee for Clinical and Laboratory Standards Institute (2014). The type and concentration of the antibiotics used were as follows: amikacin 30 µg; ampicillin 10 µg; cephalothin 30 µg; chloramphenicol 30 µg; ciprofloxacin 10 µg; gentamycin 10 µg; tetracycline 30 µg; trimethoprim 5 µg; vancomycin 30 µg for C. sakazakii and amikacin 30 µg; ampicillin 10 µg; amoxicillin-clavulanic acid 30 µg; aztreonam 30 µg; ceftazidimie 30 µg; cefepime 30 µg; ciprofloxacin 5 µg; cephotaxime 30 µg; imipenem 10 µg; pipercillin-azobactum10 µg for *K. pneumoniae*.

Results

A total of 60 food samples (milk products, fish and sprout) were analysed for *C. sakazakii, E. faecalis* and *K. pneumoniae* by both Standard Culture (SC) method and culture independent species specific PCR method. This analysis showed four different possibilities. Samples positive by both SC and PCR based method or samples negative by both the methods. Samples positive for pathogens by only SC method or samples pathogens positive by only PCR method (Table 2). Our results show that incidence of *C. sakazakii* and *K. pneumoniae* was highest in sprouts and least in milk (Table 1). Total 35% of samples were positive for C. sakazakii irrespective of the method analysed (Table 1). However, when each method of detection was analysed separately for

C. sakazakii, 5% samples were positive only by SC method but negative by PCR method; 10% samples were negative by SC method but only positive by PCR method. Twenty two percent samples were positive by both the methods. However, both culture dependent and independent methods failed to detect 63% of the samples. Similarly, for *K. pneumoniae,* 85% of samples were positive irrespective of the method analyzed (Table 1). However, when each method of detection was analyzed separately for *C. sakazakii,* 5% samples were positive only by SC method but negative by PCR method; 42% samples were negative by SC method but only positive by PCR method. Also, 38% samples were positive by both the methods and 13% samples were negative for both detection methods (Table 1, Table 2). All food samples tested were negative for *E. faecalis* by both methods.

In the present study, all the isolates of *C. sakazakii* tested were resistant to ampicillin and were moderately resistant to cephalothin (93.3%), vancomycin (46.6%) and trimethoprim (20%). In case of *K. pneumoniae* all the isolates were resistant or showed intermediate resistance to aztreonam, cephotaxime and chloramphenicol while 95.8% isolates were resistant to ampicillin and cefuroxime. High resistance was also observed for amoxicillin-clavulanic acid (87.5%) and pipercillin-tazobactam (70.83%) while only 16.6%, 12.5% and 8.3% isolates showed intermediate resistance to imipenem, ceftazidime and cefepime respectively (Table 3).

Discussion

High incidence of *C. sakazakii* and *K. pneumoniae* was observed in Indian food. This may be due to ubiquitous nature of these pathogens and also due to poor hygienic handling and distribution of the food. Sprouts have been identified as a problem because

Table 2. Sample wise distribution of emerging pathogens[a] by different methods of detection

Food sample	Samples positive only by SC method		Samples positive by only PCR method		Samples positive by both methods		Samples negative by both methods	
	C. sakazakii	K. pneumoniae	C. sakazakii	K. pneumoniae	C. sakazakii	K. pneumoniae	C. sakazakii	K. pneumoniae
Fish1						+	-	
Fish2						+	-	
Fish3				+			-	
Fish4				+			-	
Fish5			+	+			-	
Fish6			+			+	-	
Fish7			+				-	
Fish8			+	+			-	
Fish9						+	-	
Fish10	+					+		
Fish11					+	+		
Fish12					+	+		
Fish13						+		
Fish14						+		
Fish15				+			-	
Fish16						+	-	
Fish17						+	-	
Fish18						+		
Fish19					+	+		
Fish20				+		+		
Total	1/20	0/20	4/20	6/20	4/20	14/20	11/20	0/20
Sp1	+					+		
Sp2					+	+		
Sp3						+	-	
Sp4						+	-	
Sp5				+			-	
Sp6				+			-	
Sp7				+	+		-	
Sp8				+			-	
Sp9				+			-	
Sp10		+					-	
Sp11				+	+	+	-	
Sp12				+			-	
Sp13				+	+			
Sp14				+	+			-
Sp15				+	+			-
Sp16							-	-
Sp17				+	+		-	
Sp18				+	+			
Sp19			+				-	
Sp20			+					
Total	1/20	1/20	2/20	11/20	8/20	7/20	9/20	4/20
Mp1		+					-	
Mp2	+						-	
Mp3				+			-	
Mp4				+			-	
Mp5				+			-	
Mp6				+			-	
Mp7				+			-	
Mp8							-	-
Mp9							-	-
Mp10							-	-
Mp11							-	
Mp12				+			-	
Mp13	+				+			
Mp14							-	
Mp15							-	-
Mp16							-	
Mp17							-	
Mp18				+		+		
Mp19						+		
Mp20			+					
Total	1/20	2/20	0/20	8/20	1/20	2/20	18/20	5/20
Grand Total	3/60 (5%)	3/60 (5%)	6/60 (10%)	25/60 (42%)	13/6 0 (22%)	23/60 (38%)	38/60 (63%)	9/60 (15%)

[a] *E. faecalis* was not detected in any of the samples
Sp- sprout, Mp –milk product

of the potential for pathogen growth during the sprouting process (Nguyen and Carlin, 1994). Sprouts have been implicated in several foodborne outbreaks (Kim *et al*., 2012). Our present study also supports these findings. Baumgartner *et al*. (2009) reported that 61% samples of sprouts and fresh herbs/salads were contaminated with *Cronobacter* species. Kim *et al*. (2009) have reported significantly high incidence of *C. sakazakii* in mixed sprouts from retail shops in Seoul, Korea. In a study on fish and fish products, Kim *et al*. (2007) have shown that dried shrimp had the maximum contamination of *C. sakazakii*. Though, *K. pneumoniae* is known as nosocomial pathogen, it has been very often reported from food samples (Al-Mutairi, 2011; Puspanandan *et al*., 2012). Park and Sanders (1990) have reported that 40% of the alfalfa seeds and 67% of the mung bean samples were positive for *K. pneumoniae*. Interestingly none of the samples were positive for *E. faecalis*. However, incidence of *E. faecalis* from foods like French

cheeses, Egyptian fresh raw milk cheese products have been reported (Jamet *et al*., 2012; Hammad *et al*., 2015). Milk products are hygienically processed and also are low water activity product. Therefore, comparatively low incidence of *C. sakazakii* and *K. pneumoniae* was observed.

All the three pathogens evaluated in this study are emerging pathogens unlike the other known food-borne pathogens such as *Salmonella* and *Vibrio cholerae*. The detection methods developed for these three pathogens may not be effective. Our results clearly demonstrate that, SC method or molecular method alone would be insufficient in detecting these pathogens. There is a high probability of obtaining false negative results. The false negative results from culture dependent method is higher than culture independent PCR based detection method. Efficacy of culture independent PCR based method in detecting both *C. sakazakii* and *K. pneumoniae* was better than SC method for all food samples except *K.*

Table 3. Antibiotic sensitivity patterns of *C. sakazakii* /*K. pneumoniae*

Name of the Antibiotic[a]	Percentage of organisms resistant	
	C. sakazakii (%)	*K. pneumoniae* (%)
Amikacin	nd*	20.8
Ampicillin	100	95.8
Aztreonam	nd	100
Amoxicillin-Clavulanic acid	nd	87.5
Cefepime	nd	8.3
Ceftazidime	nd	12.5
Cefuroxime	nd	95.8
Cephalothin	93.3	nd
Cephotaxime	nd	100
Chloramphenicol	0	100
Ciprofloxacin	0	nd
Imipenem	nd	16.6
Gentamycin	0	nd
Pipercillin-Tazobactam	nd	70.8
Trimethoprim	20	nd
Tetracyclin	0	nd
Vancomycin	46.6	nd

nd*- not done; only relevant antibiotics for respective pathogens were tested.
[a] The antibiotic disc concentration is given in material and methods.

pneumoniae in fish samples (Table 1). For example, more than 50% milk product samples were positive by PCR for *K. pneumoniae* and all these samples were negative for *K. pneumoniae* by SC method. There could be two possibilities; one is detection of dead bacteria, which is not probable; since genomic DNA from 10^3 to 10^4 CFU/ml is needed for PCR detection (Gorski and Csordas, 2010). Second possibility is that these cells are in dormant or VBNC states, which were unable to grow on a rich medium without appropriate resuscitation step (Li *et al.*, 2014). The detection speed and accuracy of culture dependent method was improved by confirmation of typical and atypical colonies on selective plate by species specific PCR method. This reduced the time of detailed biochemical tests and accurately identified the positive samples (Figure 1). Typical colonies which gave negative PCR were rechecked with conventional biochemical tests. Cawthorn *et al.* (2008) showed the inconsistencies in the detection of *C. sakazakii* by different methods. Therefore, a combination of phenotypic and genotypic methods enhances accuracy of detection.
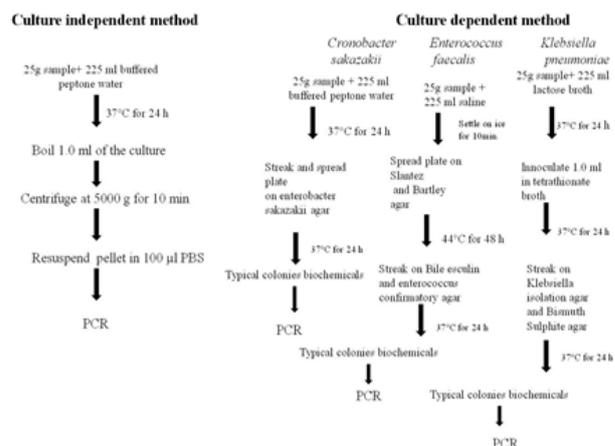
Figure 1. Flowchart for culture independent and culture dependent methods for detection of *C. sakazakii, E .faecalis* and *K. pneumoniae*.

Resistance of *C. sakazakii* to ampicillin and cephalothin has been reported (Kim *et al.* 2008). However, our isolates were also resistant to vancomycin and trimethoprim in contrast to the report by (Li *et al.* 2014); which reports resistance only to ampicillin and susceptibility to trimethoprim and vancomycin. The results show that *K. pneumoniae* were resistant to third generation cephalosporins – aztreonam, cefotoxime, and cefuroxime along with ampicllin as well as combination of clavulanic acid–amoxicillin and pipercillin-tazobactum probably due the presence of ESBL enzymes as well as AmpC (Kaur *et al.* 2013). Similar results on multi drug resistance for both the organisms have been reported in Africa (Zhou *et al.* 2011).

Conclusion

There is a high prevalence of *C. sakazakii* and *K. pneumoniae* in Indian food samples. *E. faecalis* was not found in any food samples. The combination of culture independent PCR and standard microbial culture method should be used for detection of *C. sakazakii* and *K. pneumoniae* for effective detection. Both pathogens detected in the food were resistant to many antibiotics; both *C. sakazakii* and *K. pneumoniae* being opportunistic pathogen, this is of concern.

References

Al-Kharousi, Z. S., Guizani, N., Al-Sadi, A. M., Al-Bulushi, I. S. and Shaharoona B. 2016. Hinding in fresh fruits and vegetables: opportunistic pathogens may cross geographical barriers. International Journal of Microbiology. 2016: 1-14.

Al-Mutairi, M. F. 2011. The incidence of enterobacteriaceae causing food poisoning in some meat products.

Advance Journal of Food Science and Technology 3: 116-121.

Baumgartner, A., Grand, M., Liniger, M. and Iversen, C. 2009. Detection and frequency of *Cronobacter* spp. *(Enterobacter sakazakii)* in different categories of ready-to-eat foods other than infant formula. International Journal of Food Microbiology 136: 189-192.

Calbo, E., Freixas, N., Xercavins, M., Riera, M., Nicolás, C., Monistrol, O., Solé, M. D. M., Sala, M. R., Vila, J. and Garau, J. 2011. Foodborne nosocomial outbreak of SHV1 and CTX-M-15–producing *Klebsiella pneumoniae:* Epidemiology and Control. Clinical and Infectious Diseases 52: 743-749.

Cawthorn, D.M., Botha, S. and Witthuhn, R. C. 2008. Evaluation of different methods for the detection and identification of *Enterobacter sakazakii* isolated from South African infant formula milks and the processing environment. International Journal of Food Microbiology 127: 129 –138.

Chen, W., Yang, J., You, C. and Liu Z. 2016. Diversity of *Cronobacter* spp. isolates from the vegetables in the middle-east coastline of China. World Journal of Microbiology and Biotechnology 32: 90-104.

Clinical and Laboratory Standards Institute (CLSI) 2013. (January 2013). Performance standards for antimicrobial susceptibility testing Twenty-third informational supplement, M100-S23, 34: 1 Retrieved on August 4, 2014 from CLSI Website: *http://reflab. yums.ac.ir/uploads/clsi_m100-s23 2013.pdf*

Deasy, B. M., Rea, M. C., Fitzgerald, G. F., Cogan, T. M. and Beresford, T. P. 2000. A rapid PCR based method to distinguish between *Lactococcus* and *Enterococcus.* Systemic and Applied Microbiology 23: 510-522.

Friedemann, M. 2007. Enterobacter sakazakii in food and beverages (other than infant formula and milk powder. International Journal of Food Microbiology 116: 1–10.

Gorski, L. and Csordas, A. 2010. Molecular detection: Principles and methods. In Dangyou, L. (Ed). Molecular detection of Food borne Pathogens, p. 3-4. United Kingdom: Taylor and Francis.

Grand, M., Liniger, M. and Iversen, C. 2009. Detection and frequency of *Cronobacter* spp. *(Enterobacter sakazakii)* in different categories of ready-to-eat foods other than infant formula. International Journal of Food Microbiology 136: 189-192.

Gundogan, N. Ataol, O. and Ozturk, F. 2013. Determination of virulence factors in *Staphylococcus aureus, Enterococcus faecalis* and *Enterococcus faecium* isolated from meat and milk products. Journal of Food Safety 33: 387–393.

Hammad, A. M., Hassan, H. A. and Shimamoto, T. 2015. Prevalence, antibiotic resistance and virulence of *Enterococcus* spp. in Egyptian fresh raw milk cheese. Food Control 50: 815-820.

Haryani, Y. Noorzaleha, A. S., Fatimah, A. B., Noorjahan, B. A., Patrick, G. B., Shamsinar, A. T., Laila, R. A. S. and Son, R. 2007. Incidence of *Klebsiella pneumonia* in street foods sold in Malaysia and their characterization by antibiotic resistance, plasmid

profiling, and RAPD–PCR analysis. Food Control 18: 847–853.

Huang, C. H., Chang, M. T. and Huang, L. 2013. Use of novel species-specific PCR primers targeted to DNA gyrase subunit B (gyrB) gene for species identification of the *Cronobacter sakazakii* and *Cronobacter dublinensis.* Molecular and Cellular Probes 27:15-18.

Food and Drug Administration (FDA) March 2012. Online bacteriological analytical manual revised edition. 8th ed. Arlington: U. S. Food and Drug Administration Retrieved on December 24, 2014 from FDA Website: *www.cfscan.fda.gov/ebam/bam-mm.html*

Food and Agriculture Organization/ World Health Organization (FAO/WHO) February 2004. Workshop on *Enterobacter sakazakii* and other microorganisms in powdered infant formula, Geneva. Retrieved on July 2, 2014 from WHO Website: *www.who.int/foodsafety/ micro/jemra/meetings/feb2004/en/.*

Jamet, E., Akary, E., Poisson, M. A., Chamba, J. F., Bertrand, X. and Serror, P. 2012. Prevalence and characterization of antibiotic resistant E*nterococcus faecalis* in French cheeses. Food Microbiology 31(2): 191-198.

Kaur J., Chopra, S., Sheevani and Mahajan, G. 2013. Modified double disc synergy test to detect ESBL production in urinary isolates of *Escherichia coli* and *Klebsiella pneumoniae.* Journal of Clinical and Diagnostic Research 7: 229-233.

Kim, H., Lee, Y., Beuchat, L. R., Yoon, B. J. and Ryu, J. H. 2009. Microbiological examination of vegetable seed sprouts in Korea. Journal of Food Protection 72: 856-859.

Kim, K., Jang, S. S., Kim, S. K., Park, J. H., Heu, S. and Ryu, S. 2008. Prevalence and genetic diversity of *Enterobacter sakazakii* in ingredients of infant foods. International Journal of Food Microbiology 122: 196-203.

Kim, S. A., Kim, O. M. and Rhee, M. S. 2012. Changes in microbial contamination levels and prevalence of foodborne pathogens in alfalfa *(Medicago sativa)* and rapeseed *(Brassica napus)* during sprout production in manufacturing plants. Letters in Applied Microbiology 56: 30-36.

Li, L., Mendis, N., Trigui, H., Oliver, J. D. and Faucher, S. P. 2014. The importance of viable but non-culturable state in human bacterial pathogens. Frontiers in Microbiology 5: 1-20.

Li, Y., Chen, Q., Zhao, J., Jiang, H., Lu, F., Bie, X. and Lu, Z. 2014 Isolation, identification and antimicrobial resistance of *Cronobacter* spp. isolated from various foods in China. Food Control 37: 109-114.

Liu, Y., Liu, C., Zheng, W., Zhang, X., Yu, J., Gao, Q., Hou, Y. and Huang, X. 2008. PCR detection of *Klebsiella pneumoniae* in infant formula based on 16S-23S internal transcribed spacer. International Journal of Food Microbiology 125: 230–235.

Morse, S. 2004. Factors and emergence of disease emergence.Scientific and Technical Review of the Office International des Epizooties (Paris) 23: 443-451.

Nguyen, C. and Carlin, F. 1994.The microbiology of minimally processed fresh fruits and vegetables. Critical Reviews in Food Science and Nutrtion 34: 371–401.

Nossa, C. W., Oberdorf, W. E., Yang, L., Aas, J. A., Paster, B. J., DeSantis, T. Z., Brodie, E. L., Malamud, D., Poles, M. A. and Pei, Z. 2010. Design of 16S rRNA gene primers for 454 pyrosequencing of the human fore gut microbiome. World Journal of Gastroenterology 16: 4135-4144.

Park, C. E. and Sanders, G. W. 1990. Source of Klebsiella pneumoniae in alfalfa and mung bean sprouts and attempts to reduce its occurrence. Canadian Institute of Food Science and Technology Journal 23: 189–192.

Peter, A., Radhakrishnan, E. K., Mathew, J. and Zacharia, S. 2012. Characterization of vancomycin resistant *Enterococcus faecium* from clinical and chicken sources. Asian Pacific Journal of Tropical Biomedicine S1738-S1741.

Puspanadan, S., Afsah-Hejri, L., Loo, Y. Y., Nillian, E., Kuan, C. H., Goh, S. G., Chang, W. S., Lye, Y. L., John, Y. H.T., Rukayadi, Y., Yoshitsugu, N. ,Nishibuchi, M, and Son, R. 2012. Detection of *Klebsiella pneumoniae* in raw vegetables using Most Probable Number-Polymerase Chain Reaction (MPN-PCR). International Food Research Journal 19: 1757-1762.

Saroj, S.D., Shashidhar, R., Dhokane, V., Hajare, S., Sharma, A. and Bandekar, J. R. 2006. Microbiological evaluation of sprouts marketed in Mumbai, India, and its suburbs. Journal of Food Protection 69: 2515-2518.

Saroj, S. D., Shashidhar, R., Karani, M. and Bandekar, J. R. 2008. Rapid, sensitive, and validated method for detection of *Salmonella* in food by an enrichment broth culture-Nested PCR combination assay. Molecular and Cellular Probes 22: 201–206.

Tambekar, D. H., Kulkarni, R.V., Shirsat, S. D. and Bhadange, D. G. 2011. Bacteriological quality of street vended food panipuri: a case study of Amravati city (MS). India Bioscience Discovery 2: 350-354.

Turton, J. F., Perry, C., Elgohari, S. and Hampton, C. V. 2010. PCR characterization and typing of *Klebsiella* pneumoniae using capsular type-specific, variable number tandem repeat and virulence gene targets. Journal of Medical Microbiology 59: 541–547.

Valenzuela, A. S., Benomar, N., Abriouel, H., Canamero, M. M. and Galvez, A.2010. Isolation and identification of Enterococcus faecium from seafoods: Antimicrobial resistance and production of bacteriocin-like substances. Food Microbiology 27: 955-96.

World Health Organization April 2015. Food Safety: What you should know, Regional Office South East Asia Reterived on August 3, 2016 from WHO Website:h*ttp://www.searo.who.int/entity/world_health_day/2015/whd-what-you-should-know/en/*

Yeboah-Manu, D., Kpeli, G., Akyeh, M. and Bimi, L. 2010. Bacteriological Quality of Ready-to-Eat Foods Sold on and Around University of Ghana Campus. Research Journal of Microbiology 5: 130-136.

Zhou, X., Gao, J., Huang, Y., Fu, S. and Chen, H. 2011. Antibiotic resistance pattern of *Klebsiella pneumoniae* and *Enterobacter sakazakii* isolates from powdered infant formula. African Journal of Microbiological Research 5: 3073-3077.