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TO KNOW THE IMPORTANCE OF FOOD MICROBIOLOGY IN COMMERCIAL KITCHENS: A STUDY OF CRUISE LINES AND HOTELS

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Abstract

Food microbiology is a vital component in ensuring the safety and stability of the food that we eat. Like all technical subjects, it is full of jargon that can appear complex and difficult to non-microbiological staff. Understanding the basics of food microbiology and its importance in maintaining food safety is vital. United states of America has very strict rules and regulations regarding the food hygiene and safety and so my basis for this research started way back when I was working in one of the cruise liners at USA and further after working in many five star hotels, I managed to gather food samples and thorough microbiological evaluation was done in the microbiology lab of the cruise liners and hotels. The hotels executive chef and the microbiologist was a big help in allowing taking the food samples and using the microbiology lab equipments in order to get the food sample results. Interviews were conducted and data collected on their knowledge of risk perception, food handling practices, temperature control, food borne pathogens, and personal hygiene.

The food tests were conducted to know and gather knowledge about Salmonella and staphylococcus aureus. The lack of current knowledge of food hygiene and safety among hotels and restaurant staff highlights increased risk associated with food in India.

Keywords: restaurants, food safety, food borne pathogens, hygiene, cross-contamination.



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Introduction

Numerous microbiological hazards and risks are associated with different areas of the food industry (Todd, 2003). According to Clayton et al. (2002) the number of food handlers in hotels that receive food hygiene education is high, yet a high proportion of food poisoning outbreaks still occur due to poor food handling practices. Good Hygiene Practices describes "all practices regarding the conditions and measures necessary to ensure the safety and suitability of food at all stages of the food chain", which is an important process that eventually leads to the safety in the kitchen (FAO, 2012). Food is a part and parcel of every body's life. But if food is contaminated while cooking so may spread food borne infection and thus a serious public health concern. Food poisoning or food borne infection are the illnesses caused by consumption of food containing bacteria, viruses or other pathogens (Humphrey et al. 2007, Tribe et al. 2002). Several health agencies and scientists who have worked on food related diseases have reported because of unhygienic food consumption there are high chances of getting different serious diseases of prime concern e.g. diarrhoea (Wilson 2005, Argudín et al. 2010). Diseases owing to having microbial contaminated food accounts for 5000 deaths, 325000 hospitalization (Mead et al. 1999) and 76 million illnesses in the United States only (Imee et al. 2007). In the recent past as the demand for meals at restaurants had significantly increased so that the concerns about hygiene issues also got importance speedily (Imee et al. 2007). Studies on food borne disease outbreaks show that eating food prepared in restaurants is an important source of infection (Angulo and Jones, 2006). These data suggest a critical need for action that is focused on preventing disease transmission within the hotel industry (Angulo and Jones, 2006). This study aims to enhance the knowledge of kitchen staff on food safety as well as kitchen hygiene in various five star hotels of India.

Materials And Methods

Research location

The study was undertaken in many five star hotels in India and data was also collected from the cruise liner industries ,USA. The cruise liners and hotels selected have intense patronage throughout the year.

Ethical consideration

The data was collected after a consent was obtained from the Executive Chef of the Hotel and chef de cuisine of the cruise lines and the study was undertaken under their able guidance in the microbiology lab of the hotels and cruise liners with careful supervision of the microbiologist.

Sample size and sampling method

Data was obtained by taking samples of cooked food namely mutton seekh kebab, chicken tikka masala, cashewnut gravy, raw chicken and fish from the butchery and by doing hand swab test of seven chefs along with various colour coded chopping boards.

Statistical analysis

The following chart gives us the crisp information regarding the various microbiological test on food samples collected at different outlets of the kitchen.

Figure 1. shows the microbiological evaluation report of cooked food samples

Figure 2.shows the microbiological evaluation report of uncooked/raw food samples

	Microbiologi	ical Evaluatio	n And Report C	of Food Sample	es	
Results Are Expressed Per Gram Of Sample						
Sample Specification	Location	Tymc 50/Gm	Total Viable Count 1000/Gm	Coliform Count 50/Gm	E.Coli 50/Gm	Quality Grade
Mutton Seekh Kebab	Chutney	Nil	Tntc	Nil	4	A
Chicken Tikka Masala	Chutney	Nil	Tntc	2	Nil	A
Cashewnut Gravy	Chutney	Nil	Tntc	Nil	Nil	A
Tomato Gravy	Chutney	Nil	Tntc	Nil	Nil	A
	Quality Grade					
	A - Excel					
	B - Accep					
	C - Un Acce	еріавіе				
Remarks:- Bacte	 rial Counts Were Ui Samples.	nder The Lim	it For All			
Figure 1.						

Figure 3.shows the microbiological report of hand swab test

Microbiological Evaluation And Report Of Food Samples						
Results Are Expressed Per Gram Of Sample						
Sample Specification	Location	Тутс	Total Viable Count	Coliform Count	E.Coli	Quality Grade
Chicken Raw	Butchery	3	Tntc	53	58	В
Fish	Butchery	39	Tntc	36	48	В
	Quality Grade					
	A – Excellent					
	B – Acceptable					
	C - Un Accep	C - Un Acceptable				
Remarks:- Bacterial Counts Were Under The Limit For All Samples.						

Figure 2.

Name	Location	Coliform Count Nil/Gm	E.Coli 5/Gm	Quality Grade
Irfan	Chutney	6	1	C
Jasveer	Chutney	2	1	C
Rakesh	Chutney	2	Nil	В
Pradeep	Chutney	2	Nil	В
Sajjan	Chutney	2	Nil	В
Sunil	Chutney	1	1	В
Nitesh	Continental	1	1	В
Green Chopping	Chutney	Nil	Nil	A
White Chopping	Chutney	10	3	C
Brown Chopping	Chutney	5	7	C
Red Chopping	Chutney	7	10	C
	Quality	Grade		

Figure 3.

B-Acceptabe

C - Un Acceptable

Results And Discussions		
Media preparation (form the base of	the petridish which is	used for holding food sample under
test)		
Conical flask for 100ml	•	
a) Nutrient agar – for bacteria	l counts	
b) Potato dextrose agar – for y	east and yould count	
c) EMB agar – for E.coli		
d) $V rb - for coliform$		
AUTOCLAVE all solutions in autocla	ve for sterilization @	
(equipment)	121	°C @ 15 lbs.
(once stays at 121° for 15 mins switch	h off and let the pressu	e come down to normal)
1. Sampling process		
Sterilization of petri dish		
in oven – at 160°C for 2	hrs wrapped in silver	foil
autoclave – wrapped in	butter paper	
laminar (equipment)		
disinfect using isopropyl solution (@ 90% concenteration	
clean base/ floor & swollen on UV	/ lights for 30 mins.	
2. Incubation process / time: Please	follow chart for salmo	nella and staphylococcus.
AUTOCLAVE		
	LAMINAR	INCUBATOR1 & 2

Yeast and mould no. 1 @ 25° + /- 2° C for 3 days.

Bacteric no. 2 @ 37°C +/- 2 °C for 3 days

Some basic information is thus needed to understand the nature of bacteria salmonella on the various food stuff.

Salmonella

Scope: This method is applicable for the determination of presence or absence of salmonella in foodstuffs.

Eqiupment & Glassware-

- 1. Sterile, 250ml wide mouth bottle flasks
- 2. Balance, wih weights, 2000gm capacity, sensitivity of 0.1gm
- 3. Incubator, $37\pm2^{\circ}$ C
- 4. Refrigerated incubator or laboratory or laboratory refrigerator, 4 ± 2 °C.
- 5. Water bath, circulating, thermostatically-controlled, 42 ± 0.2 °C
- 6. Sterile spoons/ spatula or other appropriate instruments for transferring food samples
- 7. Sterile culture dishes, 15× 100mm, glass or plastic
- 8. Sterile pipets, 1ml, with 0.01ml graduations, 5 and 10ml, with 0.1ml graduations
- 9. Inoculating needle and inoculating loop (about 3mm id), nichrome, platinum- iridium, chromel wire, or sterile plastic
- 10. Sterile test or culture tubes, 16×150mm and 20×150mm, serological tubes, 10×75mm or 13×100mm
- 11. Test or culture tube racks
- 12. Sterile forceps
- 13. Fisher or Bunsen burner
- 14. pH meter

Media And Reagent

- 1. Lactose broth
- 2. Selenite cystine (SC) broth
- 3. Tertrathionate (TT) broth
- 4. Rappaport vassiladis (RV)medium. NOTE. RV medium mus be made from its individual ingredients. Commercial formulations are not acceptable.
- 5. Xylose lysine desoxycholate (XLD) agar
- 6. Hektoen enteric (HE) agar

- 7. Bismuth sulfite (BS) agar
- 8. Triple sugar iron agar (TSI)
- 9. Tryptone (tryptophane) broth
- 10. MR-VP roth
- 11. Simmons citrate agar
- 12. Urea broth
- 13. Malonate broth
- 14. Lysine decarboxylase broth
- 15. Buffered peptone water
- 16. Ethanol 70%
- 17. Kovac's reagent
- 18. Voges-proskauer (VP) test reagents
- 19. Methyl red indicator
- 20. Sterle distilled water
- 21. Salmonella polyvalent somatic (O) antiserum
- 22. Salmonella polyvalent flagellar (H) antiserum

Procedure

Determination of salmonella takes place in 4 stages

Pre -enrichment in non -selective medium:

Aseptically weight 25 gr of sample in case of rasgulla crushed it into small particles by means of sterilized pistal mortar & dispense it into 225 ml of pre-sterilized peptone water/lactose broth. Incubate at 37° C for 16 to 20 hrs.

Enrichment in selective medium:

In a test tube take 10 ml of selenite cystine broth /tetra thionate broth & inoculate it with 10 ml of the above culture obtained after incubation. Now incubate it for 24hrs at 37°C. In a another test tube take approximately 10 ml of stirlised magnesium chloride/malachite green medium and inoculate it with 0.1 ml of the culture obtained after inoculation and incubation. Incubate it at 42°C for 24 hrs.

Plating out & Identification: By means of streaking inoculate phenol red/ brilliant green agar with the culture. Obtained in magnesium chloride/malachite green medium after incubation.

Inoculate the two other selective media by means of streaking from the culture obtained from selenite cystine broth/tetra thionate broth.

Invert the dishes& incubate at 37°C for 20-24 hrs. After incubation examine the dishes for typical salmonella colony. Typical salmonella colonies grow on phenol red/ brilliant green agar causes the colour of the medium to change from pink to red.

Confirmation: For confirmation take five colony suspected/typical from each solid selective media. Streak the selected colonies onto nutrient agar. Incubate the inoculate plate at 37^oc for 24 hr. Use this culture for biochemical and serological confirmation.

TSI agar: Streak the gar slope surface and stab the butt and incubate at 37°c for 24 hrs. Typical salmonella culture show alkaline slant (red) with gas production & acid (yellow) butt ,with formation of H2S (blackening).

UREA AGAR:- Streak the agar slope surface and incubate at 37°C for 24 hours. If the reaction is positive splitting of urea liberate ammonia which change the phenol red into rose pink and latter to deep cerise. The reaction is often apparent to 2 to 4 hours.

VOGES PROSKAUER (V.P):- Suspend a loopful of colony in a sterile tube containing 0.2ml of VP medium; incubate at 37°C for 24 hours.

After incubation add two drops of ceratine solution, three drops of ethanolic solution of 1-N Nephthol and two drops of potassium hydroxide, shake after the addition of each reagent.

The formation of bright pink to red colour within 15 minutes, indicates positive reaction.

Voges Proskaur (V.P Medium): Dissolve 7.0 gr of peptone 5.0 gr glucose,5.0gr di-potassium hydrogen phosphate (K2HPO4) in 100 ml of water by heating. Adjust the pH level 6.9. Transfer 3ml of medium into each of several tubes, sterilized by autoclave.

Manolate Broth: Transfer 3mm loopful of 24h tryptone broth culture to manolate broth culture to manolate broth. Since occasional uninoculated tubes of malonate broth turn blue (Positive test) on standing, include uninoculated tube of this broth as control. Incubate 46±2h at 35°C,but examine after 24 h. Most *Salmonella* species.

Perform Methyl Red Test As Follows: To 5ml of 96 h MR-VP broth, add 5-6 drops of methyl red indicator. Read results immediately. Most *salmonella* cultures give positive test, indicated by diffused red colour in medium. A distinct yellow colour is negative test. Discard, as not *Salmonella* Cultures that give positive KCN and VP test and negative methyl red cultures give negative test (green or unchanged colour) in this broth.

Indole Reaction: Incubate the tube containing 5ml of tryptone/trytophane medium with a suspected colony.

Incubate at 37°C for 24hrs. After incubation add 1ml of kovac reagent formation of red ring indicate positive reaction & yellow ring indicate a negative reaction.

Tryptone/trytophane: dissolve 5gr Nacl 1gr tryptophane in 100ml of water, dissolve by heating & adjust pH 7.5, dispense 5ml of medium into test tubes, sterilized by autoclave.

Kovac reagent: Dissolve 5gm of 4-dimethyl amino benzyldehyde in 25ml Hcl & 75ml of 2 methyl butan2ol.

Serological Confirmation: Elimination of auto agglutinable strains: place one drop of saline solution (* > % gr in 1000ml of water) onto carefully cleaned glass slide. Dissolve in this drop a part of colony to be tested & form a homogenous & turbid suspension. Rock the slide gently for 30-60 sec & observe the result against the dark back ground preferably with the add of magnifying glass.

If the bacteria have clumped into two or more distinct units, the strain is considered autoagglutinable & shall not be submitted to the further test as the detection of antigen is impossible.

Examination of 'O' 'VI' & 'H' antigen: Use only colony recognized as non-auto-agglutinable place one drop of anti 'O' serum onto a cleaned slide, if agglutination occur the reaction is considered positive.

Examination of 'VI' antigen: Use only colony recognized as autoagglutinable place one drop of anti 'H' serum onto a cleaned slide, if agglutination occurs the reaction is considered positive.

Biochemical Reaction: TSI – Acid & gas production

H2S production – positive

Urea splitting – negative

Lysine decarboxylation – positive

B galactoside reaction – negative

Voges proskaur reaction – negative

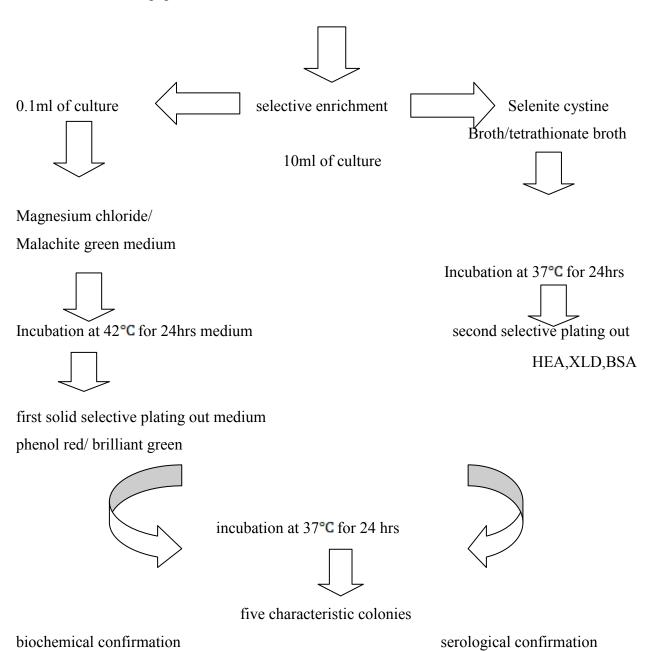
Indole reaction – negative

Diagrammatic Scheme For The Analysis Of Salmonella:

Test portion 25gram

+

Pre enrichment with peptone water or lactose broth, incubation at 37°C for 24hrs



interpretation of results

Ref: Is 5887 (Part 3) Method For Detction Of Bacteria Responsible For Food Poisoning.

Table 1: Biochemical And Serological Reactions Of Salmonella

Test of	substrate	Positive result	Negative result	Salmonella species reaction (a)
1.	Glucose (TSI)	Yellow butt	Red butt	+
2.	Lysine decarboxylase (LIA)	Purple butt	Yellow but	+
3.	H2S (TSI and LIA)	Blackening	No blackening	+
4.	Urease	Purple red color	No color change	-
5.	Lysine decarboxylase broth	Purple color	Yellow color	+
6.	Phenol red dulcitol broth	Yellow color and /or gas	No gas, no color change	+ (b)
7.	KCN broth	Growth	No growth	-
8.	Malonate broth	Blue color	No color change	- (c)
9.	Indole test	Violet color at surface	Yellow color at surface	-
10.	Polyvalent flagellar test	Agglutination	No agglutination	+
11.	Polyvalent somatic test	Agglutination	No agglutination	+
12.	Phenol red lactose broth	Yellow color and/or gas	No gas/no color change	- (c)
13.	Phenol red sucrose broth	Yellow color and/or gas	No gas,no color change	-
14.	Voges proskauer test	Pink to red color	No color change	-
15.	Methyl red test	Diffuse red color	Diffuse yellow color	+
16.	Simmons citrate	Growth blue color	No growth,no color change	V

a ,+ 90% or more positive in 1 or 2 days; - 90% ormore negative in 1 or 2 days; v, variable

TABLE 2: Criteria for discarding non-salmonella cultures

Test or substrate	Results
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b Majority of S.arizonae cultures are negative.

c Majority of S.arizonae cultures are positive.

1. Urease	Positive (purple-red color)
2. Indole test and polyvalent flagellar (H) test	Positive (violet color at surface) Negative (no agglutination)
Or indole test and spicer- Edwards flagellar test	Positive (violet color at surface) Negative (no agglutination)
3. Lysine decarboxylase and KCN broth	Negative (yellow color) Positive (growth)
4. Phenol red lactose broth	positive (yellow color and or gas) (a),(b)
5. Phenol red sucrose broth	Positive (yellow color and/ or gas) (b)
6. KCN broth, voges -proskauer test, methyl red test	Positive (growth) Positive (pink to red color) Negative (diffuse yellow color)

- a Test malonate broth positive cultures further to determine if they are S.arizonae
- b do not discard positive broth cultures if corresponding LIA culture gives typical salmonella reactions, test further to determine if they are salmonella species.

Ref: Bacteriological Analytical Manual(Usfda)

Stayphylococcus Aureus: Staphylococcus aureus is highly vulnerable to destruction by heat treatment and nearly all sanitizing agents. Thus the presence of bacterium or its enterotoxins in processed foods or on food processing equipment is generally an indication of poor sanitation. Staureus can cause severe food poisoning.

Scope: This method is applicable for the isolation, identification & enumeration of S.aureus in food stuff

Equipment & glassware:

- 1. Sterile, 250ml wide-mouth bottle flasks.
- 2. Balance, with weights, 2000gm capacity, sensitivity of 0.1gm.
- 3. Incubator, $37 \pm 2^{\circ}$ C.
- 4. Refrigerated incubator or laboratory refrigerator, 4 ± 2 °C.
- 5. Water bath, circulating, thermostatically- controlled, 42 ± 0.2 °C.
- 6. Sterile spoons/ spatula or other appropriate instruments for transferring food samples.
- 7. Sterile culture dishes, 15× 100 mm, glass or plastic.
- 8. Sterile pipets, 1ml,5 and 10ml with 0.1ml graduations.

- 9. Inoculating needle and inoculating loop (about 3mm id) nichrome, platinum- iridium, chromel wire or sterile plastic.
- 10. Sterile test or culture tube s, 16×150 mm and 20×150 mm, serological tubes, 10×75mm or 13×100 mm.
- 11. Test or culture tube racks.
- 12. Sterile forceps.
- 13. Fisher or Bunsen burner
- 14. pH meter.

Procedure : For each dilution to be plated, aseptically transfer 1ml sample suspension to 3 plates of Baird-parker agar. Spread inoculum over the surface of agar plate using bent glass streaking rod. Retain plates in upright position until inoculums is absorbed by agar (about 10min on properly dried plates). If inoculums is not readily adsorbed, place plates upright in incubator for about 1hr. Invert plates and incubate 45-48hr at 35°C. Colonies of S.aureus are circular, smooth, convex, moist, 2-3mm in diameter on uncrowded plates, grey to jet-black, frequently with light colored.

Count and record colonies. If several types of colonies are observed which appear to be S.aureus on selected plates, count number of colonies of each type and record counts separately. When plates of the lowest dilution contain <20 colonies, they may be used. If plates containing >200colonies with the typical appearance of S.aureus and typical coloniesv do not appear at higher dilutions, use these plates for the enumeration of S.aureus, select >1 colony of each type counted and test for coagulase production.

Coagulase test:

Slide method: Emulsify a portion of suspect colony in a normal saline or water. Mix this with straight wire dipped in human or rabbit plasma. Coagulase positive S.aureus produce visible clumping immediately.

Tube method: emulsify a single suspect colony from a 24hr growth of blood agar medium in 1ml citrated rabbit plasma diluted 1 in 5 in 0.85 percent saline. This test is usually carried in a narrow tube. Place in an incubator or in a water bath at 37°C. Observe every hour to note down clotting of plasma. Reading should be carried out for as long as possible, preferably avoiding over night incubation.

Test Catalase: Use growth from TSA slant test on glass slide or spot plate, and illuminate properly to observe production of gas bubbles.

Anaerobic utilization of glucose: Inoculate tube of carbohydrate fermentation medium containing glucose (0.5%). Immediately inoculate each tube heavily with wire loop. Make certain inoculums reaches bottom of tube. Cover surface of agar with layer of sterile paraffin oil at least 25mm thick. Incubate 5 days at 37°C. Acid is produced anaerobically if indicator changes to yellow throughout tube, indicating presence of S.aureus. Run controls simultaneously (positive and negative cultures and medium controls).

Anaerobic utilization of mannitol: Repeat 2, above, using mannitol as carbohydrate in medium. S.aureus is usually positive but some strains are negative. Run controls simultaneously. Lysostaphin sensitivity: Transfer isolated colony from agar plate with inoculating loop to 0.2ml phosphate-saline buffer, and emulsify. Transfer half of suspended cells to another tube (13×100mm) and mix with 0.1ml phosphatise- saline buffer as control. Add 0.1ml lysostaphin (dissolved in 0.02 M phosphatise-saline buffer containing 1% NaCl) to original tube for concenteration of 25µg lysostaphin/ml. Incubate both tubes at 35°C for not more than 2hr. If turbidity clears in the mixture, test is considered positive. If clearing has not occurred in 2hr, test is negative. S.aureus is generally positive.

Thermostable nuclear production: This test is claimed to be as specific as the coagulase test but less subjective, because it involves a color change from blue to bright pink. It is not a substitute fot the coagulase test but rather is a supportive test, particularly for 2+ coagulase reactions. Prepare microslides by spreading 3ml toluidine blue-deoxyribonucleic acid agar on the surface of each microscope slide. When agar has solidified, cut 2mm diameter wells (10-12 per slide) in agar and remove agar plug by aspiration. Add about 0.01ml of heated sample (15min in boiling water bath) of broth cultures used for coagulase test to well on prepared slide. Incubate slides in moist chamber 4hr at 35°C. Development of bright pink halo extending at least 1mm from periphery of well indicates a positive reaction.

Table 1. Typical characteristic of S.aureus, S.epidermidis and Micrococci.

Characteristics	S.aureus	S.epidermidis	Micrococci
Catalase Activity	+	+	+
Coagulase Production	+	-	-
Thermonuclease Production	+	+	-
Lysostaphin Senstivity	+	+	-
Anaerobic Utilization of Glucose	+	+	-
Mannitol	+	-	-

a +, Most (90% or more) strains are positive, - Most (90% or more) strains are negative.

Diagram Of Procedure For The Analysis Of S.Aureus

99ml of sterilised buffer in screw capped bottle

Aseptically take 11 gm of sample

Shake well to mix the content properly

Aseptically take 1 ml or its desired dilution in petridish

Solid baired parker agar medium plate

Spread inoculums over surface of agar plate using sterile bent glass streaking rod

Retain plates in upright position until inoculums absorbed by the agar

After the inoculums properly absorbed invert the plates and incubate at 35 degrees C for 24 hours

Count circular, smooth, convex, moist.2-3mm in diameter on uncrowded plates ,gray to jet black colonies with clearing zone

Ref: Usfda (Bam) Bacteriological Identification Manual 2001

Conclusion

Food safety includes food hygiene, hazard related to food and its risks become important issues if we are preparing and handling safe foods. Microbiological hazard is the most considerable and increasing interest in the food safety programmes as the outbreaks are worldwide and also can be a trans- national issue. Chemical hazard is less crucial, but it needs a special attention especially in developing countries, where most of the food processors and food handlers quite often misused those chemicals in foods. The excessive amount of permitted chemicals and the nonfood grade chemicals are still found in foods especially in developing countries. Knowledge and attitudes related to food safety are critical among the hospitality industry managers who will supervise their food handlers in preparing foods in their food outlets. The food legislation and education with emphasis on the later can give a food safety assurance. However it should involve the behavioural change and enable people to set and implement their own food safety agenda. Therefore integrated education, training, behavioural change, food legislation and the consciousness of food handlers, government officers in charge with food safety, educators in hospitality studies and consumers are necessary together to minimize the unintended consequences from the technological development and its hazard and risks related to food. So how will this information contribute in food safety assurance in hospitality industry in the future and what can we hope to achieve? The answer is that in meeting the huge challenge in food safety in the 21st century, all people who are concerned and related to safe food should be encouraged to obtain and maintain a current food safety knowledge.

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