

Ready to eat salads in selected retail outlets of Mauritius: an assessment of their hygiene status through enumeration of β -glucuronidase positive *Escherichia coli*

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Article history

Received: 22 August 2015
Received in revised form:
10 February 2016
Accepted: 3 March 2016

Abstract

Minimally processed cut and packaged salads are exposed to a range of conditions during harvest, preparation, and distribution, and these conditions are likely to increase the potential for microbial contamination. The aim of the present study was to assess the microbiological status of ready to eat (RTE) vegetable or fruit salads by using a hygiene indicator, *Escherichia coli*, in selected retail outlets offering such items in Mauritius. A total of 110 samples purchased from 22 stores were examined for *E. coli* using ISO 16649 (2). Contamination of faecal origin was found in 30 % of the samples analysed. The results obtained indicate the need for implementation of food safety assurance programmes in the production chain of RTE salads to improve their microbiological quality.

Keywords

Ready-to-eat salads
Escherichia coli
Microbiological analysis

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Introduction

Ready to eat (RTE) foods are foods that can be used for immediate consumption at the point of sale (Clarence *et al.*, 2009). They can be obtained from fresh products through selection, washing, peeling, cutting, sanitization, rinsing, drying and packaging (De Oliveira *et al.*, 2011) and can be raw or cooked, hot or chilled. They can be consumed without further heat treatment (Francis *et al.*, 1999; Tsang, 2002). Ready meal markets have been major growth sectors in recent decades (Consumers' Union, 2010) as a result of changes in consumer attitudes (Lehto *et al.*, 2011). In particular, consumption of RTE salads has been on the rise (De Oliveira *et al.*, 2011; Anon., 2012). Salads are mixtures of minimally processed ready-to-eat vegetables (that is raw vegetables which have been washed, peeled, sliced, chopped or shredded) combined with other ingredients, with or without dressing (Anon., 2002). In Europe, the sector now accounts for an annual consumption in excess of 480,000 tonnes; for instance, the market has expanded by 20% in the UK over the past five years (Anon., 2012). This is even the case in developing countries (De Oliveira *et al.*, 2011; Oranusi and Olorunfermi, 2011). This trend is associated with healthier eating ('five fruit and vegetables a day' policy, for instance in UK) and rising demand for fresh-cut, convenient, ready-to-eat

produce, and rising numbers of households where all adults work and there is less time available for food preparation (Sagoo *et al.*, 2003; Consumers' Union, 2010; Anon., 2012). Minimally processed leafy vegetables are very attractive RTE products to consumers looking for healthy and convenient meals (De Oliveira *et al.*, 2011). Indeed, leafy greens are an essential part of a healthy diet and as they supply consumers with vitamins, minerals, and fibres (FAO and WHO, 2004).

Food of non-animal origin (FoNAO) is eaten in many forms, and a preponderant component of almost all meals. Although micro-organisms are traditionally associated with products of animal origin (Leifert *et al.*, 2008), recent surveys indicated that a wide range of raw fruits and vegetables can be contaminated with potential human pathogens, and outbreaks of gastrointestinal illness caused by bacteria, viruses and parasites have been reported for different intact or processed vegetables and, to a lesser extent, fruits (Adak *et al.*, 2005; EFSA, 2013). FoNAO can potentially be associated with large outbreaks as seen in 2011 associated with verocytotoxin-producing *E. coli* (VTEC) O104 (EFSA, 2013). Using epidemiological data from 2007 to 2011, EFSA (2013) concluded that: FoNAO were associated with 10% of the outbreaks, 26% of the cases, 35% of the hospitalisations and 46% of the deaths, and from 2008 to 2011, there was a

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rise in the numbers of reported outbreaks, cases, hospitalisations and deaths associated with food of non-animal origin.

Minimally processed cut and packaged salads are exposed to a variety of conditions during harvest, preparation, and distribution, and these conditions can increase the potential for microbial contamination (Sagoo *et al.*, 2003). Vegetables may harbour pathogenic *E. coli* and *Salmonella* spp. enteric bacteria involved in large foodborne outbreaks worldwide, causing symptoms of gastroenteritis, and even chronic infections (Francis *et al.*, 1999; Vakalounakis and Fragkiadakis, 1999; Ligoxigakis *et al.*, 2002; Vakalounakis *et al.*, 2004; D'Aoust, 2007; De Oliveira *et al.*, 2011). Products tagged with the highest risk of enteric pathogen contamination are sprouted seeds and unpasteurized fruit juices (Griffin, 1998). Food borne illness is a major international health problem with heavy economic consequences (Duff *et al.*, 2003). The increased consumption of RTE salads together with the associated risk of disease to which consumers may be exposed, is a matter of great concern (Oranusi and Olofunfemi, 2011).

E. coli, a member of the genus *Escherichia* within the family Enterobacteriaceae, is normal flora in human and animals, their presence in foods are indications of excessive human handling (Adamolekun and Adamolekun, 1992). *E. coli* strains have been isolated from foods implicated in illnesses (Firstenberg and Sullivan, 1997). A subgroup called Enterohemorrhagic *E. coli* (EHEC) can cause food borne illness (Dolores and Doyle, 2001). As pointed out by Hosein *et al.* (2008), *E. coli*, especially serotype O157:H7 is considered as an important food borne pathogen causing gastroenteritis epidemics in North America, Europe, Asia and Africa and the most frequently implicated foods have been undercooked, contaminated ground beef, raw milk, unpasteurised cider and apple juice, bean sprouts or fresh leafy vegetables such as lettuce and spinach (Hosein *et al.*, 2008). Packaged, prewashed, ready-to-eat leafy greens have been of particular concern (Rangel *et al.*, 2005). Recently, *E. coli* O104: H4 isolated from fresh sprouted seeds, has caused several deaths in Europe (Soon *et al.*, 2013). Risk assessment of RTE salads is thus highly relevant.

Indicator organisms are not generally pathogenic and are commonly used by public health practitioners as markers of poor sanitation and bacterial contamination from environmental sources like soil or from animal or human faeces (Anon., 2002). Levels of these organisms often act as an indirect measure of the potential for dangerous faecal

pathogens to be present, of the food quality and of the potential health risk they pose to consumers (Rosmini *et al.*, 2004). *E. coli* is commonly used as surrogate indicator (Busta *et al.*, 2003; Ingham *et al.*, 2004); its presence in food generally indicates direct and indirect fecal contamination (Anon., 2002) and poor hygienic conditions such as poor quality of raw materials or food components, undercooking, cross-contamination (HPA, 2009). *E. coli* is considered to be more suitable than thermotolerant coliforms as indicator of faecal contamination because it better correlates with potential contamination by enteric pathogens (Doyle and Erickson, 2006; De Oliveira *et al.*, 2011). More recently, it has been concluded that generic *E. coli* can act as a suitable indicator for the presence of *Salmonella* and *E. coli* O157:H7 (Ingham *et al.*, 2004; Ceuppens *et al.*, 2014). Ideally, *E. coli* should not be detected and as such a level of less than 3 per gram is considered as satisfactory as and greater than 100 as unsatisfactory in Australia, for example (FSANZA, 2001). The Public Health Laboratory services (UK) consider a level of less than 20 colony forming units (CFU) per ml or g as satisfactory and greater than 100 CFU per g or ml as unsatisfactory (PHLS, 2000).

A number of studies have been carried out in relation to microbiological status of salads and vegetables. A study undertaken in 2001 in UK indicated that the vast majority of retail bagged prepared RTE organic salad vegetables samples analysed were of acceptable microbiological conditions (Sagoo *et al.*, 2001). Another study conducted in the United States (US) in 2010 on 208 packages revealed that *E. coli* was found in only 2.5% of the samples (Consumers' Union, 2010). More recently, a study conducted in Brazil revealed that out of 162 minimally processed samples of leafy vegetables analysed, 53.1% contained *E. coli* (De Oliveira *et al.*, 2011).

Mauritius, a small island of 1,965 square kilometres is a middle-income country and one of the most competitive in Africa (WTO, 2014). Preliminary research carried out by local public laboratories in 1998 (MOAIFS, 1998) in view of threats associated with the consumption of salad vegetables (watercress, lettuce and carrots) indicated that micro-organisms such as *Escherichia coli*, *Aeromonas* spp. as well as yeast and moulds were present. *E. coli*, in particular, was isolated in large numbers from samples analysed. However, the later study focussed on food outlets found in regions where sewage spill-overs are known. But there has been no follow-up and limited information is available on the health challenges from food borne diseases associated with

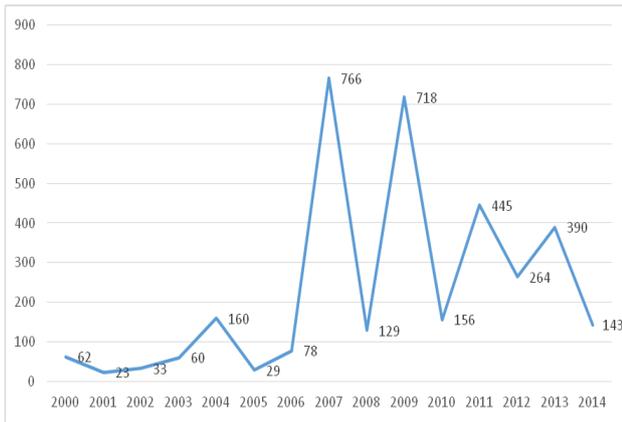


Figure 1. Reported number of food poisoning cases (2000-2014)
(Source: MOH, 2014)

RTE salads in Mauritius. Food poisoning statistics compiled by the Ministry of Health and Quality of Life indicate that there has been an increase in the number of food poisoning cases over the last few years with peaks in 2007 as is depicted in Figure 1 (MOH, 2014). However the report does not contain detailed information pertaining to causative agents or to cases associated with consumption of ready-to-eat salads.

As a result of the lack of information concerning the microbiological quality of RTE salads sold in supermarkets despite their increased consumption and given the recent outbreaks of *E. coli* 0157 in RTE salad products in several countries, we pursued this line of investigation with focus on *E. coli*, an indicator organism of faecal contamination. The purpose of this study is focused on assessing the occurrence and level of *E. coli* in RTE salads available on the local market.

Materials and methods

Sample collection

In the present exploratory study, a convenience sampling strategy was adopted. Five samples of ready-to-eat vegetable or fruit salad (minimum 100 g in market packages), were randomly purchased as from April 2012 to December 2013 on a regular basis from selected retail outlets around Mauritius. Retail outlets were chosen in seven out of the nine districts in Mauritius. Emphasis was laid on hypermarkets; in areas where there were no hypermarkets, supermarkets were selected. The samples were transferred immediately to the laboratory in cooler boxes packed with ice packs, and analysed during the day.

Microbiological analysis

Some 110 samples at 22 retail outlets [5 samples

per outlet as recommended by the ICMSF (2002)] were tested using ISO 16649, part 2 (ISO, 2001), a horizontal method used for the enumeration of β -glucuronidase-positive *E. coli* in products intended for human consumption or animal feeding. This method uses a colony count technique at 44°C on a solid medium containing a chromogenic ingredient for the detection of enzyme β -glucuronidase. 10 g of the test sample was weighed into a sterile Stomacher® (Seward, England) bag. 90 ml of sterile peptone water (Oxoid, UK) was added as diluent. The mixture was homogenised in a stomacher at normal speed for 60 seconds. Large particles were allowed to settle for up to 15 minutes. 1 ml of this initial suspension was aseptically transferred to each of two dishes, by means of a sterile pipette. 1 ml of the subsequent decimal dilution was aseptically transferred to each of two other Petri dishes. 15 ml of Tryptone Bile Agar with X-Glucuronide (TBX) medium (Oxoid, UK) was carefully mixed with the inoculum by rotating the dishes and the mixture was allowed to solidify. Inoculated dishes were inverted and incubated at 44°C for 18 to 24 hours. After incubation, typical blue colonies in each dish containing less than 150 typical CFU and less than 300 total (typical and any other colonies) CFU were counted using a colony counter (Colony Counter 570, Suntext, Taiwan).

Statistical analysis

The count for each plate was expressed as colony forming unit () per gram or ml as per ISO 16649 (2) using both duplicate results per sample. Average number of microorganisms (as well as log CFU) obtained in each outlet was calculated. Data was analyzed using Microsoft Excel.

Results and Discussion

All but one sample purchased were locally manufactured. Samples were available in prepacked form or sold in take-away containers (self-service or served by a stores attendant) and were being displayed in clean conditions (as visually assessed). It is to be noted that the RTE salads available in the surveyed outlets consist of both RTE minimally processed vegetables and fruits (for example, cabbage, lettuce, carrot, pepper, beetroot, cucumber, apple, soyabean sprouts, tomato, maize, pine-apple), as well as minimally processed vegetables mixed with cooked ingredients (for instance, octopus and tomato salad). There is thus a wide variety of salads on sale on the local market, indicating that there is interest for such products.

The average results of analysis per outlet are

Table 1. Average results per outlet

Average number of β -glucuronidase positive <i>E. coli</i> (CFU per g)	Outlet No
Not detected	2, 3, 4, 6, 10, 15, 16, 17, 20, 21
<20	1, 5, 8, 14, 18
20-100	9, 12, 13, 19
=100	7, 11, 22

provided in Table 1. *E. coli* was not detected in samples purchased at 10 out of 22 outlets surveyed, but was detected in 33 of 110 samples analysed. There was a variation in the average results per outlet, ranging from “not detected” to 220 CFU per gram. This large variation could be explained by various factors such as the type of vegetables or fruit, the level of hygiene during the processing stages and the storage conditions at the retail outlet. Beuchat (2004) considers that differences between hydrophobic cuticle, diverse surface morphology, and abrasions in the epidermal tissues of fruits and vegetables can also account for differences in contamination. During harvest, the superficial microbiota of vegetables comprises mainly Gram negative saprophytes (Kokkinakis *et al.*, 2007) but pathogenic organisms (plant and human pathogens) can also be found. Contamination of vegetables can occur when animal manures are used as fertilizer in vegetable production and through contaminated irrigation water (Ingham *et al.*, 2004). Moreover, bird and mammal activity may result in contamination of vegetable production soils (Ingham *et al.*, 2004) and ultimately, vegetables.

Moreover, RTE vegetables and fruit can be obtained through various steps, which may not be efficient to eliminate contamination of such products with micro-organisms; besides this, the storage under refrigerated conditions may favour the growth of psychrotrophic pathogenic and spoilage microorganisms (Aguado *et al.*, 2004; Gleeson and O’Beirne, 2005).

Based on the local regulations which stipulate that for RTE foods, up to 1000 *E. coli* is allowed per gram (Schedule 8 under the Food Act of 1998), the results are not alarming. However, it is clear that contamination of faecal origin is found in 30 % of the samples analysed. Since food borne illness is a major international health problem with heavy economic consequences (Duff *et al.*, 2003), it is important to adopt good hygiene practices during the preparation of such products.

The rate of contamination by *E. coli* (30%) found in the present study was higher than those reported

Table 2. Number of samples with β -glucuronidase positive *E. coli* in the unsatisfactory range

Outlet no	Ingredients	Results (CFU per gram)
7	carrot, onion, pine-apple	810
9	carrot, cucumber (green and white), lettuce, onion, lemon	140
	carrot, cucumber (green and white), lettuce, onion, lemon	270
11	tomato salad	680
12	carrot, cucumber (green and white), lettuce, onion, lemon, cabbage, beetroot	240
	carrot, cucumber (green and white), lettuce, onion, sweet corn, lemon, cabbage, beetroot, pepper	109
13	Cabbage, carrot, cucumber (green and white), sweet corn, lettuce, onion, lemon	145
19	cucumber (green), carrot, pepper	255
22	carrot, cucumber (green and white), lettuce, onion, lemon	723
	carrot, beetroot	100
	carrot, beetroot, cabbage	245

by Sagoo, *et al.* (2003) for vegetable salads from United Kingdom (1.3%) and by Abadias *et al.* (2008) for samples of fresh chopped vegetables in Spain (11.4%). However, Prado *et al.* (2008) reported similar contamination by *E. coli* in Brazilian samples of minimally processed vegetables (30%). This difference in results could be attributed to the food safety assurance systems found in different countries. It could also be the difference in the method used for determination of *E. coli*. However, if a more in-depth analysis is carried out based on the PHLS Microbiological Guidelines for some RTE foods sampled at point of sale (PHLS, 2000), 11 samples were found in the unacceptable range, that is greater than 100 colony forming units of *E. coli* per gram of samples analysed.

Interestingly out of the 11 samples containing above 100 CFU per gram, 10 included carrots as ingredient (Table 2). Thus the level of contamination may depend on the type of vegetable. The contamination rates for different vegetables vary under identical growing conditions and the emerging root is a key area of bacterial attachment (Ingham *et al.*, 2004); the ability to colonize vegetable roots and shoots varies for different strains of a bacterial species (Ingham *et al.*, 2004).

Conclusion

Changes in demographic patterns has caused an increase in the demand of minimally processed ready to eat foods with important health, economic and social impacts. The quality and safety of ready-to-eat salads depend on various intrinsic and extrinsic factors. Populations of *E. coli* are often

used for monitoring the sanitary conditions of foods, but selected serotypes of this species may also be involved in cases of foodborne diseases. EHEC (enterohemorrhagic *E. coli*) and EPEC (classical enteropathogenic *E. coli*) strains have emerged as important pathogens and have been responsible for foodborne outbreaks in many countries. In this study, we used *E. coli* as an indicator of hygiene. The results obtained indicate the need to further investigate into the type of *E. coli* present and other pathogens such as *Listeria monocytogenes* and *Salmonella* spp.

There is need for food processors and consumers to adopt good hygiene practices to minimize the risks of transmission of foodborne pathogens through these ready-to-eat foods. Preventative food safety assurance programme such as the Hazard Analysis Critical Control Point could also be built into such processing lines. In certain cases, RTE salads are available in self-service system which increases the likelihood of contamination. The quality of the raw materials used in the preparation of salads is critical and should therefore be monitored. Training on food hygiene practices in handling of ready to-eat foods should be regularly provided to the operators as the product is quite sensitive. Such training should be evaluated for its efficiency. Guidance documents could be issued to food operators processing or handling such products.

Acknowledgements

The authors are grateful to the Officer in Charge of the Food Technology Laboratory for her support, to the Ministry of Agro-Industry and Food Security, for financing the project and to anonymous reviewers for their comments.

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