Assessment of bacteriological quality ready to eat salads selected retail outle (work in progress)

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- Ready meals markets -major growth sectors in recent decades
  - changes in consumer attitudes
- In particular, consumption of ready to eat (RTE) salads on the rise.
  - Salads: mixtures of minimally processed ready-to-eat vegetables/fruits (that is raw vegetables/fruits which have been washed, peeled, sliced, chopped or
    'shredded) with or without dressing

- Ready for immediate consumption at pt of sale
- Can be obtained from fresh products through selection, washing, peeling, cutting, sanitization, rinsing, drying & packaging

Can be raw /cooked, hot / chilled & can be consumed without further heat treatment

- In Europe, RTE salads sector now accounts for an annual consumption in excess of 480,000 tonnes.
- The market has expanded by 20% in the UK over the past five years
- Similar scenario in developing countries
- Minimally processed leafy vegetables/salads: very attractive RTE products to consumers looking for healthy and convenient meals

- Demographic trends
  - Smaller household sizes,
  - Ageing population,
  - Increased mobility, large numbers of itinerary workers
  - less family or home centered activities
  - higher incomes & rising numbers of households where all adults work
    - less time available for food preparation

- Consequence: proper food handling have been transferred from individuals/families' to food vendors
- Minimally processed cut and packaged salads exposed to a range of conditions during harvest, preparation, & distribution
- Increase potential for microbial contamination.

- Many raw fruits & vegetables contaminated with human pathogens pathogenic *E.coli* and *Salmonella* spp
- Outbreaks of gastrointestinal illness due to bacteria, viruses & parasites reported for different intact/processed vegetables



- Microbiological safety of these foods of special concern due to absence of lethal treatments during processing
- Increased consumption of RTE salads together with associated risk of disease to which consumers may be exposed: matter of great concern



- *E. coli,* member of the genus *Escherichia* within family *Enterobacteriaceae*
- Normal flora in human & animals
- Presence in foods indicates excessive human handling
- Members widely distributed in envt
- Contaminated food & water -major sources by which bacteria are spread.



 Selected strains can cause a wide variety of infections

- A subgroup called Enterohaemorrhagic *E. coli* (EHEC) can cause food borne illness
- *E.coli*, particularly serotype O157:H7 has become an important food borne
  pathogen

- Responsible for gastroenteritis epidemics in North America, Europe, Asia & Africa
- Most frequently implicated foods have been undercooked, contaminated ground beef, raw milk, unpasteurised cider & apple juice, bean sprouts or fresh leafy vegetables such as lettuce & spinach

- A number of studies carried out in relation to microbiological status of salads & vegetables
- A study in UK (2001)indicated that vast majority of retail bagged prepared RTE salad vegetables samples analysed of acceptable microbiological conditions (PHLS standards)

- Another study conducted in United States (US) in 2010 on 208 packages revealed that *E.coli* found in only 2.5% of samples
- More recently, a study conducted in Brazil revealed that out of 162 minimally processed samples of leafy vegetables analysed, 53.1% contained *E.coli*

- Most contaminated vegetables were mixtures of spring onion/ parsley and kale, while lettuce samples were the least contaminated.
- E. coli is more suitable than thermotolerant coliforms as indicator of faecal contamination because it better correlates with potential contamination by enteric pathogens

- The microbial load & presence of bacterial pathogens in foods: good indication of food quality & of potential health risk they pose to consumers
- E. coli: commonly used as surrogate indicator, its presence in food generally indicates direct & indirect faecal contamination & poor hygienic conditions

- Levels of these organisms: an indirect measure of potential for dangerous faecal pathogens to be present
- Ideally, E.coli should not be detected
  - Level of less than 3 per gram is considered as satisfactory
  - 3-100: marginal
  - greater than 100 as unsatisfactory (FSANZA, 2001).

- Highlights necessity for implementation of GHP from farm to fork to prevent contamination &/or bacterial growth in these salad products
- Such baseline studies are very important
- Risk assessment of RTE salads is thus highly relevant.



- Past research carried out in the Division (1998) in view of threats associated with consumption of salad vegetables (watercress, lettuce and carrots) has indicated that micro-organisms such as *Escherichia coli*, *Aeromonas* spp. as well as yeast & moulds were present.
- *E.coli*, in particular, was isolated in large numbers from samples analysed.

 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, 2011). However the report does not contain information pertaining to causative agents.



**Figure 1:** Reported number of food poisoning cases (1988-2010) **Source:** Annual Vital Statistics on Health, Ministry of Health and Quality of Life, 2011

# Objectives of the study

- Lack of information concerning the microbiological quality of RTE salads sold in local supermarkets despite their increased consumption
- Given recent outbreaks of *E.coli* in RTE salad products globally, we pursue this line of investigation



# Objectives of the study

- The purpose of this study is focused on assessing occurrence & level of *E. coli* in RTE readily available on local market.
- We target selected retail outlets selling RTE salads for sample collection.
- Some 250 samples will be analysed.



# Materials and methods

- Convenience sampling strategy was adopted.
- 5 samples of minimally processed leafy vegetables/salads (minimum 100 g in market packages), acquired from selected retail outlets around the island as from April 2012.



#### Materials and methods

- Samples analysed using ISO 16649, part 2 (2001)
  - horizontal method used for the enumeration of ß-glucuronidase-positive *E.coli* in products intended for human consumption
  - colony count technique at 44°C on a solid medium containing chromogenic ingredient for the detection of enzyme ß glucuronidase.

# Materials and methods

10 g of test sample weighed into a sterile stomacher bag.

After incubation, typical blue colonies in each dish counted 90 ml of sterile peptone salt solution added as diluent.

Inoculated dishes inverted and incubated at 44°C for 18 to 24 hours.

> 1 ml of initial suspension aseptically transferred to each of two dishes. 1 ml of the subsequent decimal dilution aseptically transferred to each of 2 other Petri dishes



Mixture homogenised.

Large particles allowed to

settle for up to 15 min.











Mixed salad, maize, pepper, onion, watercress, beetroot, cabbage (white and purple), carrot, lettuce, dressing Beetroot, pepper, maize, white and purple cabbage, cucumber (green) Beetroot, lettuce, carrot, cheese Beetroot, egg, potato, onion Beetroot, potato, parsley, pepper

Cabbage, dressing, carrot, onion; Cabbage (purple and white), carrot; Cabbage (purple and white)

Raisin, carrot, white cabbage, dressing; Carrot, onion, pine-apple; Carrot julienne with dressing; Baby carrot, onion, potato, pepper, spring onion; Mushroom, pepper (red, green, yellow),onion; Mixed grilled vegetables salad: squash, pepper, aubergine, onion, chilli, parsley, pepper Lettuce (washed and ready to use); Cucumber, lettuce, tomato, cheese, maize; Egg ham salad, maize, dressing, onion; Red, yellow and green pepper, cucumber (green), tomato; Cucumber (white and green), carrot



Potato, mayonnaise, spring onion; Potato parsley, onion, egg; Broccoli, cauliflower, carrot, onion; Alfalfa sprouts; Mixed fruit salad, apple, strawberry, orange, grape, pommelo, pear

• *E.coli* was detected in 10 of 40 samples.

 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.





 But, based on the PHLS Microbiological Guidelines for some RTE foods sampled at point of sale, 1 sample was found in unacceptable range

| Number of samples with <i>E.coli</i> |      |     |            |         |                          |
|--------------------------------------|------|-----|------------|---------|--------------------------|
| Satisfactory                         | (<20 | CFU | Acceptable | (20-100 | Unsatisfactory (≥100 per |
| per g)                               |      |     | CFU per g) |         | g)                       |
| 6                                    |      |     | 3          |         | 1                        |



- Based on Australian guidelines, 1 sample in unsatisfactory range (>100) and 10 out of 40 samples in marginal range (3 to 100 CFU).
- Although range of *E.coli* present not alarming, it is clear that contamination of faecal origin is found in 10 out of 40 samples analysed.

The rate of contamination by *E. coli* (25%) found in present study higher than those reported by Abadias et al. (2008) for samples of fresh chopped vegetables in Spain (11.4%) and by Sagoo, et al. (2003) for vegetable salads from United Kingdom (1.3%).



- However, Prado et al. (2008) reported high contamination by *E. coli* in Brazilian samples of minimally processed vegetables (30%).
- Difference in results could be attributed to level of contamination & food safety assurance systems found in different countries.

# Conclusion

- Results obtained in this study indicate need for implementing food safety assurance programmes in the production chain of RTE salads to improve their microbiological quality.
- In particular, need for food processors and consumers to adopt hygienic practices to minimize risks of transmission of foodborne pathogens through this kind of foods.