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Clinical Paper

Public Health Bacteriology of Commercially Composted Domestic Food and Garden Waste by the 11 Councils in Northern Ireland – Persistence Of *Clostridium perfringens* and Implications for Local Food Safety

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ABSTRACT

Background: New legislation has been introduced in Northern Ireland from April 2017, requiring domestic households to recycle all domestic food waste items. The purpose of this study was to examine the bacteriology of compost derived from this source which is supplied free-of-charge to the public for horticultural use.

Methods: Municipal compost samples were compared microbiologically with commercial garden compost samples, examining total viable count and diversity of bacteria cultivated.

Results: The municipal compost had a mean Total Viable Count (TVC) of 1.53 x 10^8 colony forming units (cfu)/ g compost, whereas the commercial garden compost had a mean TVC of 4.5 x 10^7 cfu/g compost. *Campylobacter, Salmonella* and *Listeria* were not isolated from any compost. Twenty-three bacterial genera were represented amongst the 46 organisms recovered. Of these, *Pseudomonas aeruginosa* and *Clostridium perfringens* were the most clinically significant.

Discussion: The application of municipal compost to cultivate non-food plants, such as roses and flowers presents minimal risk, provided safe handling practices are adopted and hands are thoroughly washed and dried afterwards. However, if applied to soil growing food produce, it is important that municipal compost does not enter the food chain without an effective critical control point which would prevent germination of spores of *Clostridium perfringens*. Risk may be increased in products such as marinades, home canning, home bottling and associated products. These methods of food preparation are common in ethnic Eastern European populations in Northern Ireland, where awareness of risk needs to be increased.

INTRODUCTION

New legislation has been introduced in Northern Ireland (NI) from April 2017, requiring households in each of the 11 local council regions to recycle all domestic food waste items. As a result, NI households collect all uneaten food, food that has passed its "*Best Before*" or "*Use By*" date and raw food, including raw meat. Items are usually deposited into brown bins, used solely for collecting domestic organic waste, although some councils, for example, Ards and North Down Borough Council, use green bins. Food waste is collected along with other sources of domestic organic waste from each household, including garden waste materials. The waste is usually collected on a bi-weekly basis by the local council authority and sent for commercial composting. At certain times of the year, residents can freely collect the composted material, termed "*Rosey-lea*" from their local council, especially during International Compost Awareness Week, usually in April-May each year. Given that waste food

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entering the composting cycle may be heavily contaminated with foodborne pathogens, we examined the public health bacteriology of the composted material which the public may use for the cultivation of fruit and vegetables.

METHODS

Source of composts and sampling protocol

Two types of compost were examined in this study, municipal compost from domestic food and green waste material (n=10) and commercially available garden compost (n=2). Municipal compost was obtained from Mid & East Antrim Council Recycling Yard for the period 01 April – 01 June 2017. Commercially available garden composts (75L) were purchased from a local hardware shop.

Microbiological examination of composts

(i). Total viable count (TVC): The total number of culturable bacteria was enumerated quantitatively as follows:- Serial dilutions of each compost were prepared in peptone saline solution (Oxoid CM0733) and 100μl of each dilution was spread onto the surface of Standard Plate Count (SPC) agar (Oxoid CM0463; Oxoid Ltd., Basingstoke, UK) and were incubated both aerobically and anaerobically at 20°C for 72h. After this period, all colonies on the plate were counted and the count expressed as colony forming units (cfu) per g compost.

(ii). Identification of organisms from composts: SPC plates from (i) above were examined for different colonial types. Resulting colonies were purified and subcultured on SPC agar, as detailed above prior to identification using the MALDI-TOF system (Biomerieux, France), in accordance with the manufacturer's instructions.

(iii). Detection of the presence of *Salmonella*, *Campylobacter* & *Listeria* in compost:

(a). *Salmonella*

25g of compost was weighed into a sterile stomacher bag and a 1 in 10 dilution made using buffered peptone water. Samples were then stomached for 30 seconds prior to transfer to glass honey jars and incubated at 37°C for 18 hours. Following incubation, 0.1mL aliquot of the pre-enriched culture was transferred to 10mL of Rappaport Vassiladis Soya Peptone (RVS) Broth and Muller Kauffmann Tetrathionate Novobiocin Broth (MKTTn), ensuring particulate matter was not collected and incubated in a water bath at 37°C for 24 hours. RVS and MKTTn broths (10μl) were subcultured to Xylose Lysine Desoxycholate agar and Brilliant Green Agar and incubated at 37°C for 24 hours.

(b). *Campylobacter*

25g of compost was weighed into a sterile plastic closure bag and homogenised in Bolton broth at a 1 in 10 dilution. Three to four bags were placed in 2.5L plastic rectangular boxes (Thermofisher) and incubated in a microaerophilic atmosphere (Campygen, Oxoid) at 37°C for four to five hours before being transferred to 41.5°C for a further 44 hours. Broths were then sub-cultured onto *Campylobacter* selective agar (mCCDA) and again using the plastic boxes and microaerophilic atmosphere, incubated at 41.5°C for 48 hours.

(c) ***Listeria***

25g of compost was weighed into a sterile stomacher bag and homogenised with approximately half of 225ml of Half-Fraser broth. Contents of the stomacher bag were transferred to the honey jar with the remaining Half-Fraser broth and incubated at 30°C for 44 hours. Half-Fraser broths were sub-cultured (0.1mL) to Full Fraser broth (10mL) and incubated at 37°C for 44hrs as well as being sub-cultured to *Listeria* chromogenic agar and Oxford agar and incubated at 37°C for 48 hours. Plates were examined for growth at 24 and 48 hours.

RESULTS

In this study, 10 municipal compost samples were compared with two commercial garden compost samples. The municipal compost samples were taken from a single site over a 12-week period. The municipal compost had a mean Total Viable Count (TVC) of 1.53 x 10^8 colony forming units (cfu)/ g compost, [Range: 9.1 x 10^5 – 5.1 x 10^8 cfu/g], whereas the commercial garden compost had a TVC of 4.5 x 10^7 cfu/g compost. [Range: 1.2 x 10^7 – 7.8 x 10^7 cfu/g]

Overall, this study collected 46 bacterial isolates (43 from municipal compost and 3 from commercial garden compost). Twenty-three bacterial genera were represented amongst the 46 organisms isolated from both compost types, comprising 14 (14/23; 61%) Gram-negative genera, including *Achromobacter, Acinetobacter, Brevundimonas, Chryseobacterium, Comomonas, Fusobacterium, Morganella, Neisseria, Parabacteroides, Proteus, Providencia, Pseudomonas, Serratia* and *Shewanella*. Nine (9/23; 39%) Gram-positive genera were identified, including *Aerococcus, Bacillus, Clostridium, Corynebacterium, Paenibacillus, Rhodococcus, Staphylococcus, Turicella* and *Vagococcus*.

Clostridium perfringens was the only organism which was isolated from both municipal and commercial composts and all other organisms isolated were unique to that compost type. In addition, this species was the most frequently isolated (5/46; 10.8%) from compost.

Campylobacter, Salmonella and *Listeria* were not isolated from any compost sample examined.

DISCUSSION

The recent requirement to recycle all food waste material in Northern Ireland and the potential for NI householders to utilise the resulting compost provides challenges for public health. Local council guidance now requires all domestic food waste to be collected for commercial composting.

Significantly, this includes raw meat (red meats, chicken, pork and fish) which may contain foodborne bacterial pathogens, such as *Salmonella, Campylobacter, Listeria* and pathogenic *E. coli* organisms. A failure to eliminate these pathogens during the composting process will allow their potential re-entry into the food chain, when used for growing fruits and vegetables.

Recycling waste is a prudent idea for a sustainable green environment but there must be checks and balances to ensure minimum public health risk. EU legislation for recycling food/animal waste ingredients recommends stringent composting pit temperatures of 70°C or separate exposure to this temperature for at least 1 hr before the batch is released back to human and/or animal habitats.

This study of the microbiology of municipal and commercial garden composts demonstrated the presence of a diverse variety of bacterial genera and species, with 23 bacterial genera being identified from 46 bacterial isolates obtained. This represents a huge diversity reflecting heterogenous composition of the samples. With the exception of *Bacillus clausii*, all bacteria identified have been reported in the literature as potential human pathogens.

Whilst these organisms may have the potential to infect humans, they generally remain infrequent causes of infection. However, there are two notable exceptions; the Gram-negative bacterium *Pseudomonas aeruginosa* (*P. aeruginosa*), and the Gram-positive spore-forming bacterium, *Clostridium perfringens* (*C. perfringens*). *C. perfringens* was isolated in 4/10 municipal composts as well as in both commercial garden composts.

Three species of *Pseudomonas* were isolated from the compost material; *P. aeruginosa, P. mendocina* and *P. stutzeri*. Of these, *P. aeruginosa* is the most clinically significant, particularly in patients with cystic fibrosis (CF), where this organism is responsible for increased morbidity and mortality.[1] CF patients and the parents of CF children often alter their lifestyle in order to reduce the risk of contact with *P. aeruginosa* by avoidance of reservoirs of this organism, especially water sources (hot tubs, jacuzzis). This report has identified municipal compost as a source of *P. aeruginosa*, so CF families should be aware of the risks of handling compost or home-grown produce.

Although *C. perfringens* may be commensal in the human intestine, illness is caused by eating food contaminated with large numbers of *C. perfringens* bacteria that produce sufficient toxin to cause illness. The incubation period is usually 6-24 hours post ingestion, followed by diarrhoea and abdominal cramps, but generally not with fever or vomiting. Symptoms usually last up to 24h, although in severe cases, symptoms can last for one to two weeks.[2] *C. perfringens* can survive high temperatures. During cooling and holding of food at temperatures from 12°C–60°C, the bacteria may grow and they can grow very rapidly between 43°C–47°C.[2]

C. perfringens is an important aetiological agent of food-poisoning in Northern Ireland, where 24 cases were recorded in 2016.[3] Of these, the majority (17; 70.8%) were in patients aged 65 years or older, with the remainder (7; 29.1%) in patients 45-64 years, representing an age specific incidence of 5.7 and 1.5 per 100,000 population. It is unknown if any of these cases were related to produce grown in compost.

Nevertheless, it is important that we examine the implications of this data. Foodborne outbreaks of *C. perfringens* involving leafy and vine-stalk vegetables have been described.[4] The growing social trends of sustainability and consuming organic foodstuffs, as well as an ageing susceptible population are factors which could exacerbate *C. perfringens* food-poisoning. Growing and cooking home produce has become more popular in recent years, especially amongst older people.

In Eastern European cultures, there is a tradition of preserving food through home canning, as well as preparing food marinades, which have a relatively high pH and are left at room temperature to mature. Such practices have led to outbreaks of *Clostridium* food-poisoning.[5,6] Therefore, whilst it may be relatively safe for the NI indigenous population to immediately prepare dishes from home-grown vegetables, where *Clostridium*-contaminated compost has been applied, Marinating or home canning may increase *Clostridium* growth and create a food safety vulnerability amongst some ethnic groups.

In conclusion, the composts examined in this study contained a diverse range of bacteria, which may cause human infection. In particular, *Clostridium perfringens* is an important foodborne pathogen. The application of compost to cultivate non-food plants, such as roses and flowers presents minimal risk, provided safe handling practices are adopted and hands are thoroughly washed and dried after completion of gardening activities. However, if compost is applied to soil growing edible produce, it is important that the produce does not enter the food chain domestically or commercially without the inclusion of an effective critical control point, which would eliminate *Clostridium perfringens* and prevent germination of its spores. The latter is particularly important if home canning and marinating are employed – such practices are common in ethnic Eastern European populations in NI, where educational awareness needs to be increased to avoid potential food-poisoning. Anyone handling compost should ensure that their hands are washed with warm water and soap and are thoroughly dried after exposure.

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