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The survival of *Escherichia coli* O157 on a range of metal surfaces

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Abstract

Escherichia coli O157:H7 is a serious pathogen causing haemorrhagic colitis. It has been responsible for several large-scale outbreaks in recent years. *E. coli* O157:H7 is able to survive in a range of environments, under various conditions. The risk of infection from contaminated surfaces is recognised, especially due to the low infectious dose required. In this study, a high concentration (10^7 cells) of *E. coli* O157 was placed onto different metals and survival time measured. Results showed *E. coli* O157 to survive for over 28 days at both refrigeration and room temperatures on stainless steel. Copper, in contrast, has strong antibacterial properties (no bacteria can be recovered after only 90 min exposure at 20 °C, increasing to 270 min at 4 °C) but its poor corrosion resistance and durability make it unsuitable for use as a surface material. Other copper-containing alloys, such as copper nickels and copper silvers, have improved durability and anticorrosion properties and greatly reduce bacterial survival times at these two temperatures (after 120 min at 20 °C and 360 min at 4 °C, no *E. coli* could be detected on a copper nickel with a 73% copper content). Use of a surface material with antibacterial properties could aid in preventing cross-contamination events in food processing and domestic environments, if standard hygiene measures fail.

Keywords: *E. coli*; Metal alloys; Survival; Surfaces; Cross-contamination

1. Introduction

Foodborne bacterial infections can affect high numbers of people with large-scale outbreaks occurring. There have been a number of outbreaks due to *Escherichia coli* O157:H7. This is one of the verocytotoxigenic *E. coli* (VTEC) (also called Shiga toxin-producing *E. coli* [STEC]) strains and was first isolated in the US in 1975 (Riley et al., 1983). *E. coli* O157:H7 has since been found to be responsible for a number of large outbreaks of haemorrhagic colitis in several countries including the USA, Japan and the UK (Neill, 1994). An estimated 73,500 cases of illness, 2000 hospitalisations and 60 deaths occur in the

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United States each year as a result of *E. coli* O157 infection (Mead et al., 1999). Together with an estimated 31,200 cases of non-O157 VTEC infection, these cost the United States almost $1 billion a year in medical costs and lost productivity (Buzby, 2002). The September 2003 issue of the *World Health Organisation Surveillance Programme for Control of Foodborne Infections and Intoxications in Europe* newsletter focussed entirely on *E. coli* O157 outbreaks, illustrating the continued importance of this pathogen.

E. coli O157:H7 is common in the gastro-intestinal (GI) tract of many farm animals, and therefore, these animals can act as a reservoir for this and other VTEC bacteria (Faith et al., 1996; Chapman et al., 1997; Hancock et al., 1997; Rice et al., 1997; Bouvet et al., 2001). Infection can be spread by contamination of carcasses and work surfaces in abattoirs with faeces (Neill, 1994; Gill et al., 1999; Siragusa et al., 1999; McEvoy et al., 2003). The bacterium can often be recovered from faeces as demonstrated by Wells et al. (1991), where viable bacteria were detected in the faeces of 8.4% of healthy dairy cows and 19% of heffers and calves. A study by Bouvet et al. (2001) in three French abattoirs found 50% of pig carcasses containing VTEC strains. Outbreaks have also been linked to other food produce including milk (unpasteurised and contamination post-pasteurisation), cheese, yoghurt, cooked meats, meat pies, dry cured salami, manuring of raw vegetables, unpasteurised apple juice and potable water (Armstrong et al., 1996; Beuchat and Ryu, 1997; Gonzalez-Garcia, 2002).

One of the biggest problems with controlling VTEC outbreaks is the low infectious dose required for infection to occur (Tilden et al., 1996; Tuttle et al., 1999; Strachan et al., 2001; Teunis et al., 2004). This means that even slight contamination of surfaces or work areas may cause serious infection (Beuchat and Ryu, 1997). In this way, *E. coli* O157:H7 presents a serious public health risk from cross-contamination from surfaces to food produce. This has implications for a range of food handling and production industries including abattoirs, dairies, butchers, chilled food counters in supermarkets, salad and chilled food preparation factories. The public health risk is not restricted to food production/handling industries but also has implications in domestic premises and potable water supplies (Keevil et al., 1999; Keevil, 2000).

Bacteria are able to survive on a variety of materials, but survival rates differ on different types of materials. Copper is known to have inhibitory effects on various microorganisms including *Legionella pneumophila*, compared to stainless steel and plastics (Domek et al., 1984; Schoenen and Schlomer, 1989; De Veer et al., 1994; Rogers et al., 1994a,b; Faundez et al., 2004). Preliminary studies in the UK indicated similar results for *E. coli* O157 (Keevil et al., 1999). It has been found that *E. coli* O157 is robust in being able to survive in a range of environments and under differing potential stresses for long periods of time (Maule, 1997; Keevil et al., 1999). A recent (2001) outbreak of *E. coli* O157 was investigated by Varma et al. (2003) who found contamination of a building to be the source of infection, with *E. coli* surviving 42 weeks in a stressful environment. These all raise the question of persistence on work surfaces.

The aim of the current study is to investigate the survival of *E. coli* O157 (NCTC 12900) on a wide range of copper-containing alloy materials that might be suitable for use as work surfaces in industrial and domestic environments. The work involves applying a high concentration of bacterial cells (to represent a "worst case" scenario) onto each alloy and monitoring the bacterial levels over time at two temperatures: 20 °C and 4 °C, representing room and refrigeration temperature environments.

2. Materials and methods

2.1. Culturing

An original stock culture of *E. coli* O157 NCTC 12900 was grown from Selectrol discs (TCS Microbiology, UK). To set up the stock culture, a single disc was placed into a sterile 100 ml Schott bottle containing 40–50 ml tryptone soya broth (TSB) (Oxoid, UK). The culture was then put onto microbeads for long-term storage at −80 °C (Protect System, Fisher Scientific, UK).

For each new experiment, cultures which were between 15 and 20 h old were used, in an attempt to ensure continuity throughout the study. To set up a culture, a vial of beads was removed from the freezer

and allowed to thaw at room temperature. A single bead was aseptically transferred to a sterile 25 ml universal containing 5–10 ml TSB. The cultures were incubated at 37 °C until required.

2.2. Experimental method

A 20 μl aliquot of culture was placed on each coupon to be tested. Prior to use, the metal coupons were wiped with ethanol and allowed to air dry. The coupons were housed within a plastic container to minimise contamination from the laboratory environment. After the required incubation time, the coupon was transferred into a sterile 50 ml centrifuge tube containing 10 ml autoclaved phosphate-buffered saline (PBS) (Oxoid, UK) and 10–20 autoclaved, 2 mm-diameter glass beads (VWR, UK). This was then mixed thoroughly using a vortex mixer for 1 min. Serial dilutions were made in PBS. The efficiency of this removal method was tested using the viability testing technique described below.

A 50 μl aliquot of each dilution was pipetted onto a nutrient agar plate (Oxoid, UK) which had been pre-warmed (agar plates were pre-warmed to room temperature (20 ± 5 °C) to prevent any temperature shock to the bacterial cells). This was then spread over the surface of the plate using a sterile, disposable, plastic L-shaped spreader. Agar plates were incubated, lid down, at 37 °C for 18–24 h before colonies were counted. All experiments were replicated at least three times, by repeating on different days with different bacterial cell suspensions. Within each experiment several dilutions were made. The mean was calculated from a minimum of six data points. The detection limit for this experimental method was 100 bacteria based on counts of colony-forming units. However, additional experiments were carried out where the total 10 ml volume of PBS was filtered onto a 0.2 μm-pore-diameter membrane. The membrane was then transferred onto a nutrient agar plate and incubated as described previously. These experiments were carried out at the time points where no viable bacteria could be detected.

A total of 22 wrought metal alloys were tested; Table 1 details the composition of each alloy. The alloys tested were separated into six groups: coppers, brasses, bronzes, copper nickels, copper nickel zinc (copper silvers) alloys, and stainless steel. Historically, bronze has been considered to be a copper tin alloy, but now the name is given to a wide range of alloys with a varied composition. The copper nickel zinc alloys are also commonly referred to as copper silvers due to their colour. All alloys were manufactured in the same way, with no additional surface treatments.

Table 1
Alloy composition (wt.%) (data supplied by the Copper Development Association Inc., US)

Alloy UNS number	Cu	Zn	Sn	Ni	Al	Mn	Fe	Cr	P	Si	Ti	Mg
Coppers												
C10200	100											
C11000	100											
C18080	99						0.1	0.5			0.1	
C19700	99						0.7		0.3			
Brasses												
C21000	95	5										
C23000	85	15										
Y90*	78	12		3		7						
Bronzes												
C51000	95		5						0.2			
C61500	90			2	8							
C63800	95				3					2		
C65500	97					1				2		
C68800	74	23			3							
Cu–Ni												
C70250	96			3						0.7		0.2
C70600	89			10			1					
C71000	79			21								
C71300	75			25								
C71500	70			30								
C72900	77		8	15								
Cu–Ni–Zn												
C73500	72	10		18								
C75200	65	17		18								
C77000	55	27		18								
Stainless steel												
S30400	0			8			74	18				

Cu=copper, Zn=zinc, Sn=tin, Ni=nickel, Al=aluminium, Mn=manganese, Fe=iron, Cr=chromium, P=phosphorus, Si=silicon, Ti=titanium, Mg=magnesium.

2.3. Bacterial staining procedures

The fluorochrome, 4′,6′-diamidino-2-phenylindole (DAPI) (Sigma Chemical Co., UK) was used to assess

the efficiency of bacterial removal from coupon surfaces using the glass beads/vortex method described. DAPI stains the nuclear material of bacterial cells blue, allowing visualisation under epifluorescence microscopy. Coupons were tested by placing in sterile Petri dishes, following bacterial removal (as a positive control, coupons which had not gone through the bacterial removal procedure were also tested), and 1 ml DAPI (final concentration 5 μg ml^{-1}) added. Petri dishes were incubated at room temperature in the dark for 15 min. Excess DAPI was gently removed and coupons examined under episcopic differential interference contrast (EDIC) and epifluorescence (EF) illumination on a Nikon Eclipse ME600 microscope (Best Scientific, UK), using long working distance objectives (Keevil, 2003).

E. coli O157 were also observed directly on the various coupons, throughout the study, using the bacterial viability stain, 5-cyano-2,3-ditolyl tetrazolium chloride (CTC) (Polysciences Inc., US). The methods used followed the procedure provided by the company. CTC is a monotetrazolium redox dye which, when it is biologically reduced, produces a fluorescent insoluble formazan. This means that actively respiring bacterial cells fluoresce bright red after the application of this dye. *E. coli* O157 contaminated coupons were placed in sterile Petri dishes and 1 ml CTC (final concentration 4.0 mM) added. The Petri dishes were incubated in the dark at 37 °C for 2 h. Following incubation, excess stain was removed and the coupon gently flooded with filter-sterilised deionised water. The coupons were examined under EDIC and EF illumination.

3. Results

The data for all alloys are summarised in Table 2, where the results are given for the two test temperatures (20 °C and 4 °C). The table gives the mean data from the experiments and indicates the exposure time after which an initial reduction (of at least 1 log) in bacterial numbers is observed and the time required for no viable bacteria to be detected (all replicates for each alloy required the same period for the bacterial number to fall to zero, i.e., there was no standard deviation for time). The detection limit for this direct plating procedure was 100 bacteria allowing for all dilution steps. At each time point where no viable bacteria could be detected, additional experiments filtering the total diluent volume onto membranes were carried out and in all cases no colonies were seen after appropriate incubation. This indicates that no viable bacteria were present on the coupon after that exposure time.

Using the two bacterial staining procedures described, it was found that the glass beads/vortex

Table 2
Elapsed time of initial bacteria count reduction (of at least 1 log) and for zero bacteria count at the two temperatures tested: 20 °C and 4 °C

Alloy UNS number	% Cu	Elapsed time (min) 20 °C		Elapsed time (min) 4 °C	
		Initial reduction	Zero count	Initial reduction	Zero count
Coppers					
C10200	100	45	75	90	180
C11000	100	75	90	180	270
C18080	99	45	75	180	270
C19700	99	45	75	90	180
Brasses					
C21000	95	60	90	90	180
C23000	85	30	60		
Y90	78	90	120	180	270
Bronzes					
C51000	95	60	105	180	270
C61500	90	105	180	No reduction	Not reached
C63800	95	60	90	90	180
C65500	97	45	65	90	270
C68800	74	120	270	No reduction	Not reached
Cu–Ni					
C70250	96	90	105	90	270
C70600	89	90	105	180	360
C71000	79	90	120	No reduction	Not reached
C71300	75	75	120	270	360
C71500	70	105	Not reached	No reduction	Not reached
C72900	77	120	360	No reduction	Not reached
Cu–Ni–Zn					
C73500	72	60	90	No reduction	Not reached
C75200	65	90	105	No reduction	Not reached
C77000	55	90	Not reached	No reduction	Not reached
Stainless steel					
S30400	0	180	Not reached	No reduction	Not reached

method was efficient at removing bacteria from the coupons. Prior to removal, bacterial cells could clearly be seen on the coupons under both EDIC and EF illumination; however, in no case could any cells be seen following removal. The viability stain was also used throughout the study to check the data obtained from direct plating, in each case where no colony grew on the agar plates no bacteria could be observed using the stains and direct microscopical analysis of the coupons agreeing with results obtained when the total diluent volume was filtered onto a membrane.

3.1. Coppers

Four different coppers were tested (Table 2, Fig. 1a and b), all with either 99% or 100% copper content. In general, at both temperatures, all members of this group, exhibited similar behaviours. At room temperature (20 °C), the numbers of viable bacteria decreased rapidly (Fig. 1a). No viable bacteria were found on any of the coupons after only 75–90 min exposure to the alloys, equating to a 7 log kill. With the exception of alloy UNS C11000, there had been a 4 log reduction in bacterial numbers within 60 min exposure. At the lower temperature (4 °C), the bacteria remained viable for longer (Fig. 1b). At this temperature, no viable bacteria could be detected on alloys UNS C10200 and C19700 after 180 min exposure, for alloys UNS C11000 and C18080 this was observed after 270 min.

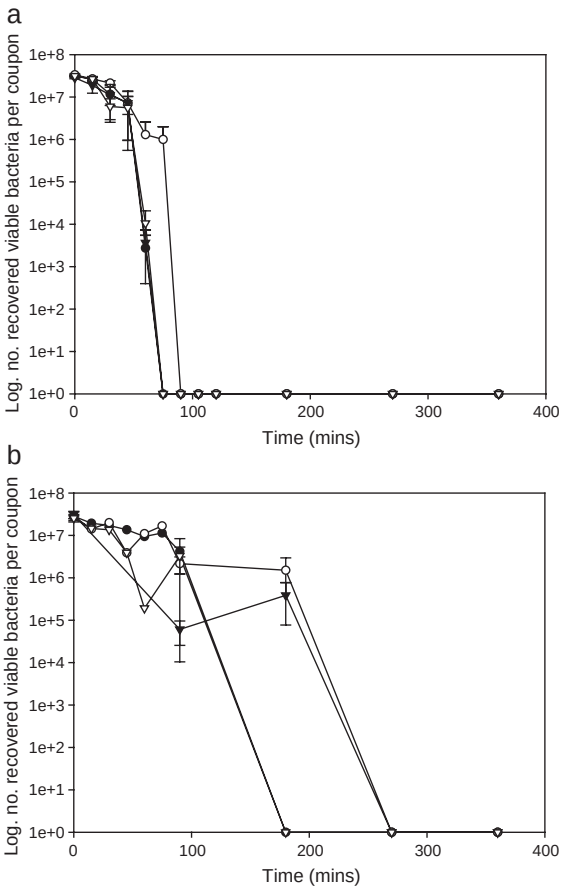

Fig. 1. (a and b) Decrease in bacterial numbers with exposure time on copper family alloys. (a) Room temperature (20 °C). (b) Refrigeration temperature (4 °C). ● UNS C10200, ○ UNS C11000, ▼ UNS C18080, ▽ UNS C19700. Mean values are plotted with error bars calculated from the standard deviation.

3.2. Brasses

A total of three different brasses were tested (Table 2). The copper content of these alloys varied between 78% and 95%, and the observed results were more varied than seen with the high copper alloys. The inhibitory effect of the brasses was similar within the group, but less pronounced when compared with the coppers. All the brass alloys also contain zinc but this did not seem to correlate with bacterial survival times.

3.3. Bronzes

A total of five bronzes were tested (Table 2). In the bronzes tested, the copper content ranged from 74% to 97%. As expected from such a diverse range of alloys, the results obtained from the bronzes do not all follow the same general patterns. At room temperature, there was some correlation between the inhibition effect and the inverse of the copper content. For example, the bacteria survived longest (no viable cells being detected after 270 min exposure) on alloy UNS C68800, which has the lowest copper content of 74%. In contrast, no bacteria could be detected on alloy UNS C65500 (copper content of 97%) after only 65 min exposure. A similar pattern occurred when the experiments were carried out at the lower temperature (4 °C), with no reduction in bacterial numbers being observed on two of the bronzes after the maximum exposure period of 360 min. These two alloys were

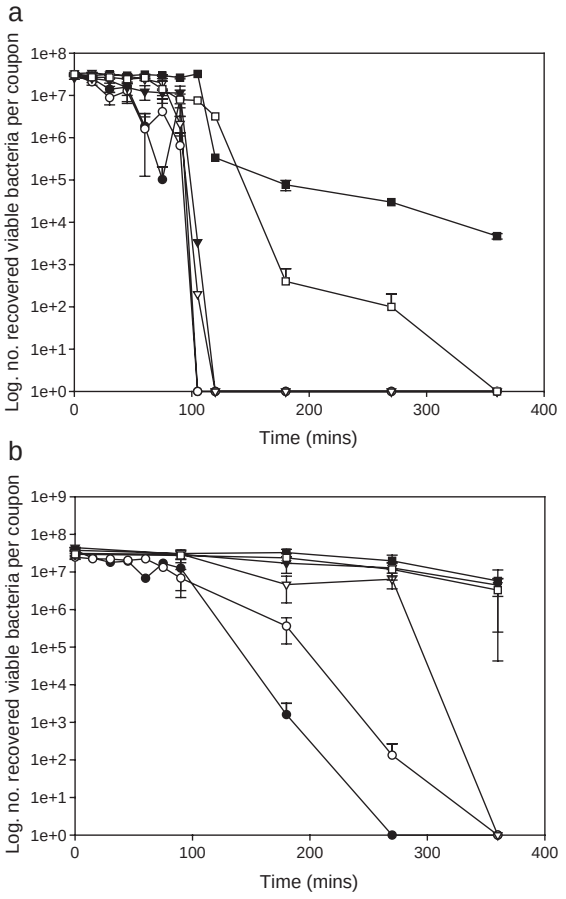

Fig. 2. (a and b) Decrease in bacterial numbers with exposure time on copper nickel family alloys. (a) Room temperature (20 °C). (b). Refrigeration temperature (4 °C). ● UNS C70250, ○ UNS C70600, UNS ▼ C71000, ▽ UNS C71300, UNS C71500, □ UNS C72900. Mean values are plotted with error bars calculated from the standard deviation.

UNS C68800, which has the lowest copper content of this group (74%) and contains a high zinc content (23%), and UNS C61500, which has a comparatively high copper content (90%) but also has the highest aluminium content (8%) of any of the alloys tested.

3.4. Copper nickels

The copper content of the six copper nickels tested ranged from 70% to 96% and the nickel content from 3% to 30%. The only alloy containing another metallic element is UNS C72900, which contains 8% tin. Excluding this alloy, there was an increase in bacterial survival times with decreasing copper and increasing nickel content at both temperatures (Table 2, Fig. 2a and b). Bacterial cells survived on alloy UNS C72900 for the entire duration of the experiment at both temperatures, even though its copper content was higher and its nickel content lower than some of the other alloys within this group.

3.5. Copper–nickel–zinc alloys

The copper–nickel–zinc alloys are commonly referred to as copper silvers due to their colour. Only three members of this family of copper alloys were tested (Table 2). They all contain comparatively

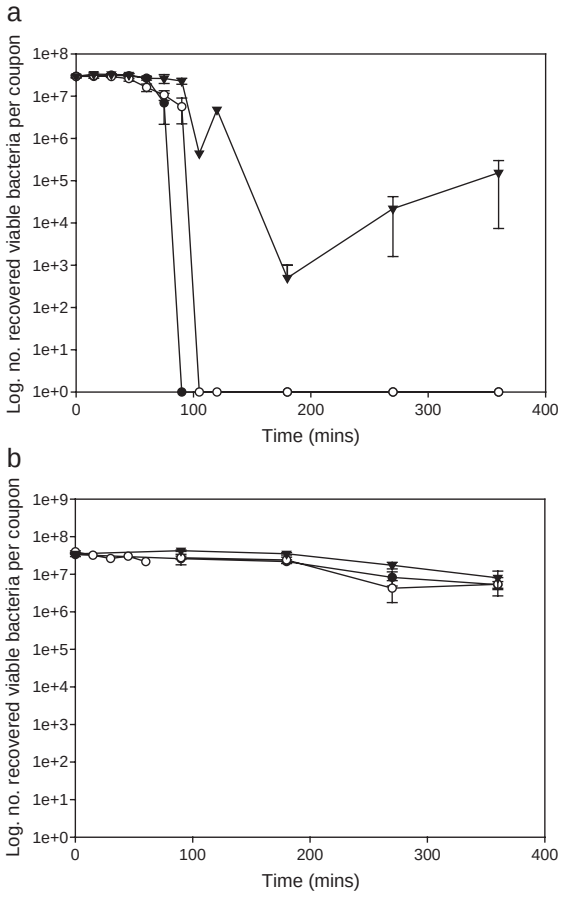

Fig. 3. (a and b) Decrease in bacterial numbers with exposure time on copper nickel zinc (copper silver) family alloys. (a) Room temperature (20 °C). (b) Refrigeration temperature (4 °C). ● UNS C73500, ○ UNS C75200, ▼ UNS C77000. Mean values are plotted with error bars calculated from the standard deviation.

low amounts of copper, ranging from 55% to 72%, all contain 18% nickel and the content of zinc ranged from 10% to 27%. At room temperature, a 2 log reduction in the number of viable bacteria was seen after exposure to alloy UNS C77000 which has the lowest copper content of 55% and the highest content of zinc (27%) (Fig. 3a). No bacteria could be detected on the other two coupons after only 90–105 min exposure, even though the copper content was comparatively low. At the lower temperature, there was no significant reduction in bacterial numbers for any of the copper–nickel–zinc alloys (Fig. 3b).

3.6. Stainless steel

For comparison, coupons of stainless steel were tested under the same conditions. The stainless steel alloy used, UNS S30400, is commonly used in many applications. It contains 74% iron, 18% chromium and 8% nickel. At both temperatures, no significant reduction in bacterial numbers was observed over the course of the standard experiments (360 min exposure). Longer term experiments were carried out (Fig. 4), with exposure over 28 days. At room temperature, the numbers of viable bacteria fell by 1 log during the first 180 min and then by 5 logs within the first two days before remaining constant for the remainder of the test period. The initial 1 log reduction corresponded to the time required for the aliquot of bacterial culture to dry onto the metal coupon. At the lower temperature, the decrease was more gradual, and the numbers of bacteria remained slightly higher over the course of the experiment. After 28 days incubation, at both temperatures, viable bacterial cells could be observed clearly using the CTC viability stain.

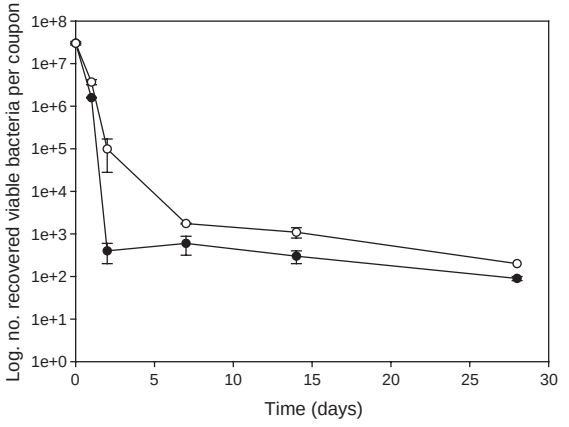

Fig. 4. Decrease in bacterial numbers with exposure time on stainless steel (UNS S30400). ● Room temperature (20 °C), ○ refrigeration temperature (4 °C). Mean values are plotted with error bars calculated from the standard deviation.

4. Discussion

Stainless steel is a commonly used material for surfaces in a variety of environments where bacterial contamination could be an important health risk, including food processing and handling industries, abattoirs, hospital environments, public transit systems, drinking water systems and in domestic premises. We have found that *E. coli* O157 was able to survive, in a desiccated state, for more than 28 days at both refrigeration and room temperatures on stainless steel (Fig. 4). There was a 5 log reduction in viable bacteria within the first 2 days exposure, but then the population density remained constant at 1×10^4 cfu ml^{-1}. This clearly constitutes a potentially serious public health risk as the infectious dose is thought to be very low (Tilden et al., 1996; Tuttle et al., 1999; Strachan et al., 2001; Teunis et al., 2004).

In this study, the inhibitory effects of a total of 21 copper-containing alloys were tested on cultures of *E. coli* O157. Some general trends are evident from the results. It is clear that *E. coli* O157 does survive for different periods of time on different surfaces. Also, bacterial survival was extended at the lower, refrigeration temperature (4 °C) compared to room temperature (20 °C) in all cases. Extended survival times at the lower temperature were expected based on knowledge of bacterial physiology in environmental conditions (Crane and Moore, 1984). *E. coli* O157 were grown in a broth medium (tryptone soya broth) which provides similar nutrients to those found in meat products. Bacteria were then left on the coupons for varying amounts of time before being removed by vortexing with glass beads. Tests on coupons, using bacterial stains (including the viability stain, CTC), before and after vortexing showed the removal procedure to be efficient. A high concentration of *E. coli* O157 was used to simulate a "worst case" scenario based on the premise that survival of a lower concentration of bacteria would be inhibited in the same or less time. Other studies have used similar or lower concentra-

tions of bacteria (Rogers et al., 1994a,b; Keevil et al., 1999; Faundez et al., 2004) and have obtained similar results.

The main aim of this work was to assess the antibacterial properties of other metal alloys containing copper and compare their performance to pure coppers and stainless steel. Compared to stainless steel, all the copper-containing alloys exhibited increased antibacterial activity. *E. coli* O157 only survived for very short periods of time on the pure coppers. This is to be expected as copper is well documented as being inhibitory to the survival of several microorganisms (Domek et al., 1984; Schoenen and Schlomer, 1989; De Veer et al., 1994; Rogers et al., 1994a,b; Keevil et al., 1999). However, copper itself would not be a suitable alternative material for surfaces, even though it has such strong antibacterial properties. It is soft, not durable and it tarnishes easily. There are many other copper-containing alloys which have characteristics which make them more suitable.

Three brasses were tested; these all showed a decrease in survival time with increasing copper content. Brass was commonly used in the past for door knobs and push plates. An early study by Kuhn (1983) compared the bactericidal properties of copper, brass and stainless steel door knobs. Copper and brass were found to have strong bactericidal properties compared to the stainless steel and the continued use of brass (as it is harder and more durable than copper) was recommended in hospital settings to aid in the prevention of nosocomial infections.

The copper nickels and copper silvers (copper nickel zinc alloys) have much greater corrosion resistance and so are more suitable for general use in industrial and domestic environments. They are widely used in marine environments due to their anticorrosion properties. All of these alloys showed greater antibacterial activity when compared to stainless steel.

In this study, we have demonstrated how different materials can reduce the survival of *E. coli* O157. Other work has mainly concerned with the relative adherence of bacteria to various surface types (Merritt et al., 2000; Cookson et al., 2002). Stainless steel has often been selected as the most appropriate surface material because it has poor adhesion characteristics (Merritt et al., 2000; Cookson et al., 2002) and can easily be cleaned. However, as shown in the current study, *E. coli* O157 can survive for extended periods of time when dried onto the surface indicating that a potential contamination risk could occur if a surface was not adequately cleaned.

Cross-contamination events in abattoirs (Neill, 1994; Gill et al., 1999; Siragusa et al., 1999; Bouvet et al., 2001; Warriner et al., 2002; McEvoy et al., 2003) and food industries (Armstrong et al., 1996; Beuchat and Ryu, 1997; Gonzalez-Garcia, 2002; Warriner et al., 2002) have been shown to be responsible for many of the recent produce-related outbreaks. The potential also exists for widespread cross-contamination within domestic environments and is reviewed by Mattick et al. (2003) and illustrates the ease with which this could occur. Surface contamination was also found to be a serious risk area in a study of Japanese households by Ojima et al. (2002). In addition, biofilm development has been shown to be widespread on domestic surfaces (Rayner et al., 2004). Both in industrial and domestic settings the additional antibacterial benefit of certain materials could be useful in reducing the number of contamination incidents. The data from this preliminary investigation indicate that there are suitable alternative materials to the use of stainless steel, especially at times when hygiene measures fail.

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