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Adhesion of *E. coli* and *E. coli* O157: H7 Isolates from a Typical Tropical Abattoir on Wood, Steel and Glass Surfaces

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ABSTRACT

Adhesion of bacteria to meat processing surfaces may act as potential sources of transmission of pathogens in meat industry. Several studies have shown that adhesion of bacteria to surfaces partly depends upon the nature of the inert surfaces and partly upon the bacterial surface properties. The aim of this study was to compare the adhesion of *E. coli* and *E. coli* O157: H7 on wood, glass and steel surfaces. Twenty abattoir isolates of *E. coli* (8) and *E. coli* O157:H7 (12) from meat tables in Ibadan municipal abattoir, Nigeria were assayed for bacterial adhesion. Bacterial adhesion was assessed on wood, glass and steel surfaces at ambient temperature for 24 and 72 h. Attachment to surfaces was quantified using a crystal violet binding assay. In this study, *E. coli* isolates adhered on all the three surfaces studied, viz. wood, glass and steel. The isolates varied in their ability to adhere to the surfaces. The adhesion by isolates at 24 and 72 h were significantly different ($p<0.05$). The mean absorbance values (nm) at 24 h showed that *E. coli* O157:H7 (SEH1) adhere more bacterial cells on wood (0.51±0.02), steel (0.15±0.00) and glass (0.12±0.01) surfaces than *E. coli* (SE) which was wood (0.38±0.02), steel (0.13±0.01) and glass (0.07±0.01). At 24 and 72 h, a significant difference ($p<0.05$) was observed between the isolates for wood and glass. However, at 24 h, a significant difference ($p<0.01$) ensued between *E. coli* O157:H7 and *E. coli* for steel. *E. coli* and *E. coli* O157:H7 adhere more bacterial cells on wood than on steel and glass. Bacterial adhesion is influenced by strains with high virulence factors and an extension of incubating period. Proper surface selection can reduce the ability of bacteria adhesion on meat contact surfaces.

Key words: Adhesion, *E. coli* O157:H7, abattoir, virulence, incubation period, contact surfaces

INTRODUCTION

Adhesion of microorganisms to food processing equipment surfaces is of great concern to the food industry. Adhered microorganisms to solid surfaces can have the potential to act as a chronic source of microbial contamination, which may compromise food quality and represent a significant health hazard (Barners *et al*., 1999). The ability of bacteria to adhere to food contact surfaces compromises the hygiene of those surfaces. Surface physicochemical properties of the bacterial cell as well as of the materials such as hydrophobicity and roughness, are determinant during the initial attachment phase (Teixeira and Oliveira, 1999; Alves *et al*., 1999; Fonseca *et al*., 2001). Bacterial adhesion is affected by nutrient availability and the growth stage of the bacterial cells (Kumar and Anand, 1998). The pH, temperature of the medium and cell structures (Extracellular Polymeric Substances; flagella; ionic concentration) affect bacterial adhesion (Gengeorgis, 1995).

Bacterial adhesion is the first step in complex process of biofilms formation (Donlan, 2002). Biofilms are formed by adhesion of bacteria cells to surfaces through an exopolymeric matrix. This matrix is important in formation and structure of the biofilm and also on the protection of the bacterial cells. The bacteria in biofilms acquire new physiological properties and confer protection against environmental stresses such as UV radiation, pH shifts, osmotic shock and desiccations (Davey and O'Toole, 2000). It has been determined that in all natural habitats, bacteria prefer to reproduce on the surfaces rather than in the liquid phase (Carpentier and Cerf, 1993). In addition various isolates have been made from biofilms in food processing environment (Batool and Shahida, 2005; Salamitou *et al*., 2009; Puyate and Rim-Rukeh, 2008; Manijeh *et al*., 2008a). The effect of various media on biofilm formation has also been studied (El-Abed *et al*., 2011).

The presence of biofilm on foods and food contact surfaces often adversely impacts food safety especially in minimally processed foods and raw foods (Frank, 2001). Biofilm has ability to readily form in the food industry environment because of the availability of water nutrients and surfaces for attachment (Gibson *et al*., 1999). Midelet and Carpentier (2004) also reported that the contaminating potential of a surface depends not only on the level of contamination but also on the nature, structure and history of the contamination. This is a problem in the food industry because the hygiene of the surface affects the overall quality and safety of the product (Hyde *et al*., 1997).

There are hundreds of strains of *Escherichia coli* most of which are harmless. *E. coli* had earlier being detected in tap water and raw milk (Soomro *et al*., 2002; Azad *et al*., 2003; Kanwal *et al*., 2004) *E. coli* O157: H7 produces a powerful toxin that can cause several illnesses. *Escherichia coli* O157:H7 was first recognized as an enteric pathogen in 1982 (Riley *et al*., 1983). *E. coli* serotype O157:H7 is a gram negative rod shaped bacterium and differs markedly from other pathogenic *E. coli*. In particular, the O157:H7 serotype is negative for invasiveness, adheres through the *E. coli* common pilus (ECP) (Rendon *et al*., 2007) doesn't produce heat stable or heat labile toxins and is non-hemolytic. In addition, *E. coli* O157:H7 is usually sorbitol negative whereas 93% of the *E. coli* ferments sorbitol (Wells *et al*., 1983). *E. coli* serotypes (*E. coli* 055:H7 and *E. coli* O157:H7) are most closely related and diverged from a common pathogenic ancestor that possessed the ability to form attaching and effacing lesions. *E. coli* O157:H7 serotypes apparently arose as a result of horizontal gene transfer of virulence factors (Whittam *et al*., 1988). *E. coli* O157:H7 is diagnosed when the sample is cultured on sorbitol agar, 0157 colonies appear clear due to their ability (unlike other *E. coli* serotypes) to ferment sorbitol.

E. coli O157:H7 is known to produce Exopolysaccharides (EPS) (Mao *et al*., 2001) which can provide a physical barrier to protect cells against environmental stresses. Some studies have shown that EPS produced by *E. coli* O157:H7 acts as an anti adhesive, affecting the attachment of cells on the surface of stainless steel (Ryu *et al*., 2004b). *E. coli* O157: H7 has also been shown to produce curli; a thin coiled fimbriae-like extracellular structure. Unlike non pathogenic *E. coli*, curli production by *E. coli* O157:H7 is uncommon but can occur in association with csgD promoter point mutations (Uhlich *et al*., 2001). Curli produced by non-pathogenic *E. coli* enhanced the attachment of cells on the surface of polystyrenes (Prigent-Combaret *et al*., 2000).

E. coli O157:H7 has been found to form biofilm on stainless steel (Dewanti and Wong, 1994; Ryu *et al*., 2004b; Ryu *et al*., 2004a). *E. coli* 0111 has also been shown to produce biofilms on food contact glass surfaces (Movassagh and Karami, 2010).

Therefore, the aim of this study was to assess the adhesion ability of *E. coli* and *E. coli* O157:H7 strains on wood, steel and glass surfaces.

MATERIALS AND METHODS

This study was conducted in the month of January and August, 2010.

Bacterial strains and culture conditions: This study was conducted in the month of January and August, 2010. Twenty strains of abattoir isolates of *E. coli* (8) and *E. coli* O157:H7 (12) from meat tables at Ibadan municipal abattoir, Nigeria were used in this study. Preliminary identification of the isolate was based on morphological characteristics, fermentation of sorbitol MacConkey (*E. coli* O157:H7) and procedures described by Barrow and Feltham (1993). Definitive identification of the isolate as *E. coli* O157:H7 was done using *E. coli* O157:H7 antiserum (Difco laboratories). The strains were grown in nutrient agar (Fluka 7014, Germany) slope and stored at 4°C. All strains were then transferred from the nutrient agar into the MacConkey medium and incubated for 24 h at 37°C. All the strains were subsequently purified under the same conditions. The grown pure cultures were used for subsequent quantification of biofilms assessment.

Growth of bacteria on surfaces

Preparation of surfaces: Two hundred and sixty-four chips (88 each (4x2x1 cm) for glass (Easy way medical, England) steel (type 304, #4, Ajaokuta steel co., Nigeria) and wood (softwood, Oak) were used. The chips were washed with detergent (Unilever, Lagos, Nigeria), rinsed with sterile distilled water and air-dried. The chips were then wrapped in aluminum foil and placed in hot air oven (Elektro-Helios, Sweden) at 75°C for 30 min for sterilization before use.

Assessment of *E. coli* and *E. coli* O157:H7 strains adhesion: A discrete colony of a 24 h test cultures on nutrient agar (Fluka 7014, Germany) was used for adherence assessment. One colony each for *E. coli* strains (SEH1-12 and SE1-8) on nutrient agar slope was transferred into each of 20 sterile glass jars with lids containing 150 mL of nutrient broth (Fluka 7014, Germany). An uninoculated nutrient broth was used as control. The inoculated broth culture was incubated at 37°C for 24 h. Two hundred and sixty-four sterile chips (88 chips each for glass, steel and wood) 4 cm by 2 cm were used. The chips were washed with detergent, rinsed with sterile distilled water and air-dried before being placed into hot air oven (Elektro-Helios, Sweden) at 75°C for 30 min. After 24 h of incubation of inoculated broth culture, four of steel, glass and wood chips were put into each glass jars with lid. All the glass jars were incubated at ambient temperature of (26-28°C) for 24 and 72 h. At the end of each incubation period, a set of chips (2 of each wood, steel and glass) were removed from the broth culture for bacteria adherence quantification.

Quantification method

Crystal violent binding assay: Crystal violet binding assay was performed to assess bacterial adhesion as described by Stepanovic *et al.* (2004) at wavelength of 520 nm for *E. coli* strains using a spectrophotometer (Springfield, UK). The absorbance of negative control was subtracted from the absorbance values to determine the actual values (Pawar *et al.*, 2005).

Statistical analysis: All tests were carried out in duplicate and results presented in Mean±Standard Deviation (Mean±SD). A one-way Analysis Of Variance (ANOVA) was used to determine significant differences between means for each surface and strains. Student-test was

used to assess significant differences between strains. All data were analyzed using SPSS 15 (2006), Chicago, IL., USA. Statistical significance was evaluated at $p<0.05$ and $p<0.01$. Charts were plotted using Microsoft Excel 2009.

RESULTS AND DISCUSSION

Evaluation of bacterial adhesion by *E. coli and E. coli* O157:H7 in this study revealed that these bacteria possess a high capacity to adhere on 3 surfaces used (wood, glass and steel) (Table 1). Other researchers have observed similar results which showed that *E. coli* and *E. coli* O157:H7 are able to produce and adhere on various surfaces including glass, stainless steel, rubber and polypropylene (Dewanti and Wong, 1994; Kusumaningrum *et al.*, 2003; Stopforth *et al.*, 2003; Ryu *et al.*, 2004a; Rodriguez and Mclandsborough, 2007). Reports on biofilm formation in other bacteria pathogens in meat process environment had also been made(Manijeh *et al.*, 2008b).

At 24 h, Strain *E. coli* O157:H7 (SEH1) adhered more bacteria on wood, glass and steel surfaces than *E. coli* which was significant at ($p<0.05$) for wood and glass and significant at ($p<0.01$) for steel. At 72 h, Strain *E. coli* O157:H7 (SEH1) also adhered more bacteria on wood, glass and steel surfaces than *E. coli*. These values were significant at ($p<0.05$) for wood and glass but not significant for steel (Table 1). This is in agreement with previous studies by Hood and Zottola (1995) and Gulsun *et al.* (2005) in which the level of biofilm formation of the isolates increases with the virulence characteristics: i.e., strains with higher virulence produced more biofilms than strains with lower virulence. Higher producers of this factor have higher virulence than lower producers. Several workers have reported cellulose, biofilm and fimbria production to have influenced virulence (Hood and Zottola, 1995). Numerous studies have examined factors and growth conditions that affect bacteria adherence on various surfaces. However, most of these previous studies assessed only a few strains. The mean absorbance values (nm) on wood, steel and glass surfaces by strains of *E. coli* O157:H7 (SEH1) (0.59±0.02; 0.19±0.01; 0.15±0.01) and *E. coli* (SE) (0.48±0.03; 0.18±0.01; 0.10±0.00) showed significant differences ($p<0.05$) in bacterial adherence on the 3 surfaces (Table 1). *E. coli* O157:H7 and non *E. coli* O157:H7 strains showed greater adherence on wood followed by steel and glass. The findings obtained is in agreement with the report by Donlan (2002) and Sinde and Carballo (2000) in which they reported that glass and stainless steel are hydrophilic materials while wood and plastic are hydrophobic materials thereby encouraging adhesion. Donlan (2002) also reported that adhesion is the first step in complex process of biofilms formation. Hydrophobic materials are reported as surfaces that provide a greater bacterial adherence (Djordjevic *et al.*, 2002). Fletcher and Loeb (1979) noted that large numbers of bacteria attached to hydrophobic surfaces with little or no surface charge and moderate numbers

Table 1: Comparison of bacterial adhesion produced by *E. coli O*157:H7 and *E. coli* on the 3 surfaces

		Surfaces (Mean±SD)		
Strains	Period (h)	Wood	Steel	Glass
E. coli O157:H7	24	0.51 ± 0.02^{a1}	0.15 ± 0.00^{b1}	0.12 ± 0.01^{c1}
E. coli	24	0.38 ± 0.02^{a2}	0.13 ± 0.01^{b1}	0.07 ± 0.01^{c2}
E. coli O157:H7	72	0.59 ± 0.02^{a1}	0.19 ± 0.01^{b1}	0.15 ± 0.01^{c1}
E. coli	72	0.48 ± 0.03^{a2}	0.18 ± 0.01^{b1}	0.10 ± 0.00^{c2}

Mean with the same superscript (alphabet) in the column is not significantly different at 0.05 levels. Mean t-value significant at 0.05 level with the same superscript (numbers) in the row is not significantly different at 0.05 levels for 24 and 72 h

attached to hydrophobic metals with positive charge or neutral charge and very few attached to hydrophilic negatively charged substrates. The importance of hydrophobicity in bacteria attachment has been highlighted by other investigators also (Characklis, 1990). Variation in biofilm density depending on surface was also reported by Karunasagar *et al.* (1996) with *Vibrio harveyi*. This could be one possible explanation for the ability of these bacteria to adhere in high numbers on the wood surface as observed in our results. Abrishami *et al.* (1994) reported that conditioning of the wood surface could also be a factor that aids adherence of microbes. Snyder (1997) also reported that bacteria are shown to be less adherent to steel and glass and are more easily removed as well. Although, high level of bacterial adhesion was seen on wood during this study but Abrishami *et al.* (1994) reported that conditioning of the wood was shown to decrease bacteria penetration . A significant difference ($p<0.05$) was observed between 24 and 72 h incubation periods for *E. coli* and *E. coli* O157:H7 strains (Table 1). The assessment of *E. coli* O157:H7 and *E. coli* strains showed an increase in bacteria adherence with an extension of incubation time. variations in bacteria adherence were also observed among the strains on all the three surfaces at 24 and 72 h of incubation (Fig. 1, 2). This variations were significant ($p<0.05$) in four of the strains. Individual variation among strains even within the same specie is an important factor to be penetration .A significant difference ($p<0.05$) was observed between 24 and 72 h incubation periods for *E. coli* and *E. coli* O157:H7 strains (Table 1). Other researchers have observed similar results of variation in strains within the same specie. Adetunji (2010) and Moltz and Martin (2005) reported quantitative recovery of biofilm and purified cellulose from culture. Their report reflected some variations among strains and an increase with extension in incubation period for biofilms on glass and stainless steel, respectively.

The ability of abattoir isolates of *E. coli* O157:H7 to adhere more bacteria than *E. coli* on the surfaces is of public health importance. A greater number of *E. coli* O157:H7 and *E. coli* adherence was observed on wood surfaces which are common surfaces used in the display and processing of meat in most developing countries. Use of wood as a food contact surface has reduced in the

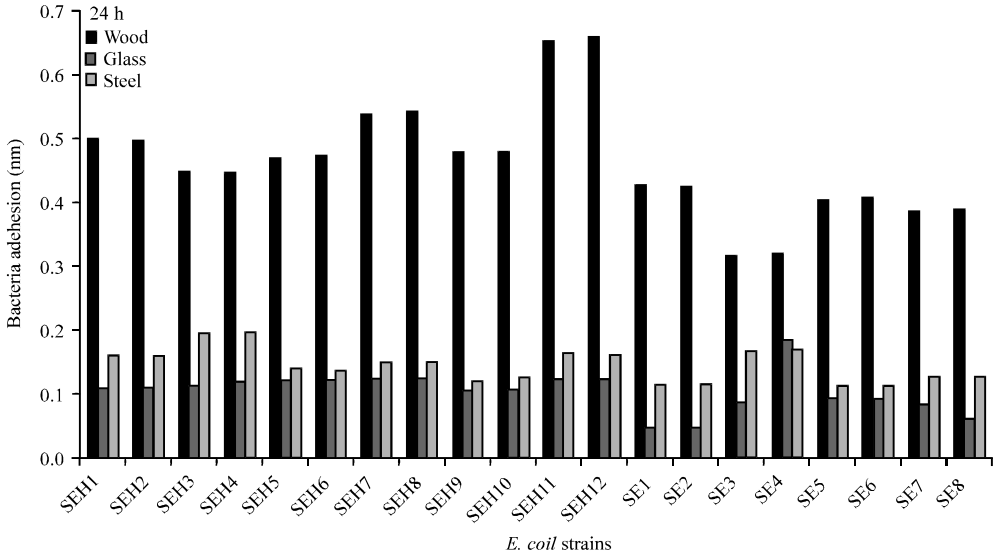

Fig. 1: Variations in bacteria adhesion by *E. coli* O157:H7 (SEH) and *E. coli* (SE) on wood, glass and steel surfaces at 24 h

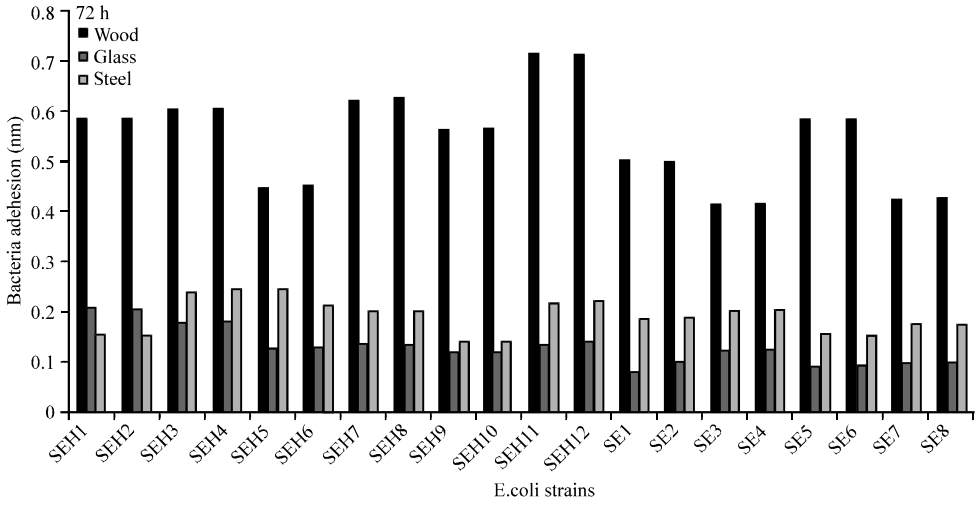

Fig. 2: Variations in bacteria adhesion by *E. coli* O157:H7 (SEH) and *E. coli* (SE) on wood, glass and steel surfaces at 72 h

international community because it is a porous and absorbent material where organic matter along with bacteria can become entrapped; cross-contamination is a main concern (Lauzon, 1998). Glass is sometimes used for food contact surfaces because of its smooth and corrosion-resistant surface (Dunsmore *et al*., 1981). Stainless steel resists impact damage better than glass but is vulnerable to corrosion, while rubber surfaces are prone to deterioration and may develop surface cracks where bacteria can accumulate (Leclercq-Perlat and Lalande, 1994). Equally important in meat contact surface is the cleanability of the surface once bacteria forming biofilms have attached. In an earlier study biofilm forming bacteria isolates were detected after cleaning processes and the use of sanitizer (Manijeh *et al*., 2008a). Proper surface is essential for avoiding cracks and dead areas where organic material could accumulate thereby forming biofilms. This is a major concern in meat processing surfaces. Thus many factors should be considered in designing meat contact surfaces to resist biofilm forming bacterial contamination.

CONCLUSION

Arising from this study, the best surface for meat display or processing are glass surfaces. Bacterial adhesion is also aided by virulence factors and extension of incubating period. Proper surface selection can reduce the ability of bacteria adhesion on meat contact surfaces. In developing world, there is need to change the use of wood as medium for meat display because it allows high level of bacteria attachment. Knife cuts on edges allows meat residues, thus creating suitable medium for biofilms adherence and making cleaning difficult.

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