E. coli from dishcloths as an indicator of hygienic status in households

V. Keshav, C. A. Kruger, A. Mathee, N. Naicker, A. Swart and T. G. Barnard

ABSTRACT

E. coli is routinely used as an indicator of fecal pollution, although some strains are capable of causing diarrhea. E. coli was used as a model organism for this study to assess the possibility that dishcloths used in households could contribute to the occurrence of diarrhea. Dishcloths (n = 424) were collected from five suburbs in Johannesburg (South Africa) as part of a larger Health, Environment & Development (HEAD) study. Results for the total coliforms indicated that on average 81% of the samples analyzed had total coliform counts of more than 1,000 cfu/100 ml per 25 cm² cloth. The E. coli results indicated that 40% of the samples had culturable E. coli present with 17% of the samples showing the presence of >1,000 cfu/100 ml per 25 cm² cloth. Except for the samples from Bertrams, all the pathogenic E. coli genes could be detected in various combinations in the different samples. Since all the diarrheagenic E. coli strains detected can be accepted as culturable due to the enrichment step, there is a clear danger of contamination of food and surfaces exposed to the contaminated dishcloths. The results indicated that there is a need for public education regarding hygiene in the households, especially if the same dishcloth is used for various tasks.

Key words | contaminated dishcloths, fecal contamination, home-based hygiene

INTRODUCTION

According to the United Nations (2013) the Millennium Development Goal (MDG) Goal 7, focusing on sanitation, hygiene and water quality, has achieved a 13% increase (1990–2010) in providing access to improved drinking water sources globally. However, over 40% of people in sub-Saharan Africa still do not have access to improved water sources and 2.5 billion (10⁹) people lack access to adequate sanitary facilities. Despite the progressive efforts as outlined in the MDGs to improve the lives of 100 million people living in slum dwellings by 2015, the hindrance often encountered is due to negligence of hygienic practices within the society. Infectious diseases are frequently reported, due to deviations from hygiene practices executed in households which differ amongst individuals, to communities, states and countries. Factors such as age, health and immunity of individuals may also affect public health. Moreover, many domestic households contribute to a decrease in water quality, sanitation and hygiene practices leading to disease outbreaks (Curtis et al. 2003; Bloomfield et al. 2007; Aiello et al. 2008; Pickering et al. 2011; Stauber et al. 2013).

Soares et al. (2012) stated that up to 87% of reported outbreaks originated either from improperly prepared or consumed food in homes. Furthermore bacterial cells variably adhere to different materials such as wood, plastic, glass or stainless steel equipment or surfaces used in domestic kitchens and thus, further contribute in the spread of microorganisms (Ojima et al. 2002; Moore & Griffith 2006; Rossi et al. 2015). In addition it was stated that bacterial strains were more prevalent on wet substances such as drain taps, dish-washing sponges, counter towels, sinks and bathroom sponges. An essential study carried out by Rayner et al. (2004) reported the prevalence of biofilms on various surfaces in households. Biofilms are habitually resistant to general cleaning protocols used at homes and hence these protocols may be insufficient to remove such contamination. Such findings are building evidence of the
survival of microorganisms in domestic kitchens and their ability to act as fomites to cross contaminate other areas. Table 1 summarizes the frequently reported sources considered hazardous in spreading infections.

Of the many bases of potential carriers identified, kitchen dishcloths and sponges were reported to have the highest bacterial count as they mostly remain wet and serve both as a reservoir and disseminator of pathogens (Kagan et al. 2002; Aiello et al. 2008). In many African homes, dishcloths are used for multiple purposes such as cleaning dishes, wiping various surfaces, walls and floors, and consequently potentially transferring pathogens throughout the household. Sharma et al. (2009) reported that dishcloths transferred Escherichia coli O157:H7 (E. coli) to surfaces more frequently than Salmonella species. Additionally Kagan et al. (2002) stated that E. coli cells may survive in temperatures higher than 50 °C. This is of concern as water used to wash food products, kitchen surfaces and utensils is usually below 50 °C and hence contributes in the spread of pathogenic bacteria. Pathogenic E. coli, namely enterotoxigenic E. coli (ETEC), enterohemorrhagic E. coli (EHEC), enteroaggregative E. coli (EAEC), enteroinvasive E. coli (EIEC) and enteropathogenic E. coli (EPEC), targets the gastrointestinal tract of humans and is responsible for causing infections leading to diarrhea and dysentery (Donnenberg 2002).

In an attempt to understand the occurrence of microorganisms in households, E. coli was chosen as an indicator organism to evaluate the hygienic status of dishcloths and sponges. The occurrence of E. coli is an indication of fecal contamination and can also indicate the presence of other pathogens such as Shigella, Salmonella, Aeromonas, Campylobacter jejuni, Yersinia and Campylobacter coli associated with fecal pollution (Liu et al. 2011), further

<table>
<thead>
<tr>
<th>Source</th>
<th>Contact</th>
<th>Identified transmission pathway to humans</th>
<th>Risk Involved</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dishcloths</td>
<td>Indirect</td>
<td>Raw food, water, hands, airborne, skin scales</td>
<td>Wet or moist conditions are ideal for survival of bacterial cells up to several hours</td>
<td>Kagan et al. (2002), Mattick et al. (2003a, 2003b), Bloomfield et al. (2007), Rossi et al. (2013)</td>
</tr>
<tr>
<td>Food</td>
<td>Direct/indirect</td>
<td>Uncooked food, water and hands</td>
<td>Serves as a nutritional source for bacteria</td>
<td>Jong et al. (2008), Pickering et al. (2011), Beltran et al. (2013)</td>
</tr>
<tr>
<td>Floors, furniture and walls, surfaces</td>
<td>Indirect</td>
<td>Wash-cloths, hands, airborne, skin scales</td>
<td>Bacterial cells are known to survive in dust</td>
<td>Rayner et al. (2004), Bloomfield et al. (2007), Sinclair &amp; Gerba (2010), Chang et al. (2013)</td>
</tr>
<tr>
<td>Fomite</td>
<td>Indirect</td>
<td>Hands, airborne</td>
<td>Carrier for bacterial cells</td>
<td>Sinclair &amp; Gerba (2010)</td>
</tr>
<tr>
<td>Personal hygiene</td>
<td>Direct</td>
<td>Unwashed hands</td>
<td>Lack of knowledge/practice</td>
<td>Curtis et al. (2005)</td>
</tr>
<tr>
<td>Toys</td>
<td>Indirect</td>
<td>Hands, mouth</td>
<td>Carrier for bacterial cells and direct mouth contact with kids</td>
<td>Stauber et al. (2015)</td>
</tr>
<tr>
<td>Toilets, baths, sinks</td>
<td>Indirect</td>
<td>Skin scales, airborne, hands</td>
<td>Bacterial cells adhere to surfaces and can be viable up to several hours</td>
<td>Kagan et al. (2002), Ojima et al. (2002), Sinclair &amp; Gerba (2010)</td>
</tr>
<tr>
<td>Utensils and equipment</td>
<td>Indirect</td>
<td>Hands, food, wash-cloths</td>
<td>Carrier for bacterial cells</td>
<td>Soares et al. (2012), Jensen et al. (2013)</td>
</tr>
<tr>
<td>Water</td>
<td>Direct/indirect</td>
<td>Consumption, uncooked food, cross-contaminated dishcloths, utensils and surfaces</td>
<td>Temperature of water used for various household chores is generally below 50 °C</td>
<td>Jong et al. (2008), Mattick et al. (2003a, 2003b)</td>
</tr>
<tr>
<td>Waste disposal</td>
<td>Indirect</td>
<td>Hands, airborne</td>
<td>Serves as a nutritional source for bacteria</td>
<td>Scott (1999)</td>
</tr>
</tbody>
</table>
increasing the risk for diarrhea and other related infections. Thus dishcloth testing could be the link to measure unsanitary hygienic practices at homes traced with *E. coli* pathogens isolated from other various contaminated water sources.

**METHODOLOGY**

**Growth and maintenance of bacterial strains**

A commensal *E. coli* strain was isolated and confirmed using the API 20E strip. This strain was cultured using the Plate Count Agar (PCA) (Oxoid) method and incubated at 37°C for 16 hours under aerobic conditions. This was used as a positive control for the Colilert® Quanti-Tray®/2000 method. *Klebsiella pneumoniae* [KLEPN 01, National Health Laboratory Service (NHLS)], and *Pseudomonas aeruginosa* (PSEAE 01, NHLS) were cultured using the Plate Count Agar (PCA) (Oxoid) method and incubated at 37°C for 16 hours under aerobic conditions. This was used as a negative control for the Colilert® Quanti-Tray®/2000 method.

**Sample collection**

To test for the presence of *E. coli*, dishcloths (*n* = 424) were collected from participating households in Hillbrow (*n* = 50), Bertrams (*n* = 25), Riverlea Extension 1 (*n* = 101), Braamfischerville (*n* = 105) and Hospital Hill (*n* = 143) that formed part of a larger Health, Education & Development (HEAD) study in Johannesburg, South Africa. Participants were asked to place the dishcloths in sterile bags in exchange for new dishcloths. Before sample analysis, a brief description on the quality of dishcloths based on dirty/clean, wet/dry was recorded and the actual measurements were noted. Working under sterile conditions each dishcloth was folded in half and cut into a 5 cm × 5 cm (length × breadth) sample and placed into a sterile (Colilert® antifoam) container, for further microbial analysis.

**Microbial analysis**

The Colilert® Quanti-Tray®/2000 system (Iddex) was used to culture and detect the presence of coliforms and *E. coli* cells from the collected dishcloth samples. This was done by mixing each (5 × 5 cm) piece of dishcloth with 100 ml sterile distilled water and the Colilert®-18 media. The mixture (without the cloth) was then poured into the Quanti-Tray®/2000 and sealed with the Quanti-Tray® sealer followed by an incubation period of 18 hours at 37°C. After incubation the Quanti-Trays® were placed under a 366 nm ultraviolet light for the detection of fluorescent wells, which would indicate viable *E. coli* cells. Two milliliters of the Colilert®-18 media was extracted from the positive *E. coli* Quanti-Tray® wells using a 1 ml Neomedic disposable syringe (Kendon Medical Supplies) and transferred into a 2 ml 96 well plate (Uniplate®, Whatman®). The plates were centrifuged at 13,000 × g for 15 minutes at 4°C to pellet out the cells. The supernatant was discarded and the pelleted cells were held at 4°C for DNA extractions.

**DNA extraction**

Deoxyribonucleic acid (DNA) extractions were performed using the DX Universal Liquid Sample DNA extraction kit (Corbett Life Sciences). The pellets were resuspended with 100 μl of DX liquid digest buffer [100 μl DXL with 10% (v/v) DX digest enzyme] per well and incubated for 10 minutes at room temperature. A volume of 400 μl of DX Binding buffer with DX binding additive (w/v) was added to each well and the lysate was mixed and incubated for 5 minutes at room temperature. All the lysate was transferred to a filter plate (Unifilter®, Whatman®) placed on a manifold (Whatman®, Merk). A vacuum of 25 kPa was applied to the manifold to filter out the cell debris within the wells of each plate. A volume of 200 μl of DX Binding buffer with DX Binding additive was added to each well and a vacuum of 25 kPa was applied. Each well was washed twice with 600 μl of DX Wash buffer. A further volume of 600 μl of DX Final wash buffer was added and vacuum filtered for 5 minutes at 25 kPa. A sterile 250 μl plate (Uniplate®, Whatman®) was placed inside the manifold to collect the eluted DNA. Elution buffer (150 μl) was added to each well and further incubated for 5 minutes at room temperature. The DNA was eluted by applying a 30 kPa vacuum for 1 minute. The eluted DNA was used as a template for polymerase chain reactions.
Polymerase chain reaction

The polymerase chain reaction (PCR) method was performed using the 8-gene multiplex PCR protocol that was developed by Omar et al. (2010). The multiplex PCR targeted six E. coli groups; namely (1) ETEC containing the \(l_t\) and \(st\) gene, (2) EHEC containing the \(stx1\) and \(stx2\) gene (3) EIEC containing the \(ial\) gene, (4) EAEC containing the \(eagg\) gene, (5) EPEC containing the \(eagg\) gene and (6) E. coli commensal containing the \(mdh\) gene. The primers used for the amplification of these genes and the predicted sizes are indicated in Table 2.

The E. coli genes were amplified using a 20 \(\mu\)l reaction mixture that contained 10 \(\mu\)l of the 2 \(\times\) Qiagen® m-PCR master mix (HotstartTaq DNA polymerase, 10 \(\times\) buffer, 2 mM MgCl\(_2\) and dNTP mix), 1 \(\mu\)l 5 \(\times\) Q-solution, 5 \(\mu\)l of PCR grade water, 2 \(\mu\)l of the primer mix \([0.1 \mu M\) of \(Md\)h and \(l_t\) primers (F and R), 0.2 \(\mu M\) of \(ial\) and \(eagg\) primers (F and R), 0.3 \(\mu M\) of \(eaeA\) and \(st\) primers (F and R), 0.5 \(\mu M\) of \(stx1\) and \(st\) primers (F and R)] (Table 2). PCR reactions were performed in a Biorad Mycycler™ thermal cycler under the following conditions: an initial denaturation at 95°C for 15 minutes, denaturation at 94°C for 45 seconds, annealing at 55°C for 45 seconds, extension at 68°C for 2 minutes (35 cycles) and final elongation at 72°C of 5 minutes (Omar et al. 2010).

Gel electrophoresis

Amplified DNA were electrophoresed on a 2.5% (w/v) agarose gel (1.25 g agarose, 50 ml of 50 \(\times\) TAE buffer \([0.04 M\) Tris-acetate, 0.001 M EDTA, pH 8.0]), pre-stained with ethidium bromide (0.5 \(\mu g/ml\)) and visualized under UV illumination using the Gene Genius Bio Imaging System.

Statistical analysis

Statistical analysis was performed using Sigma Plot software Version 12.0. Data were analyzed according to the one sample \(t\)-test. This statistical analysis was used to determine the significance between total number of samples and experimental groups where values in the 95% confidence interval \((P < 0.05^{*}, P < 0.01^{**}\) or \(P < 0.001^{***}\)) were accepted as statistically different.

RESULTS

The following characteristics were noted prior to sampling of the dishcloths. Of the 424 samples collected 37% were identified as drying cloths, 63% as wash cloths and 0.7% as sponges. The sanitary status of the cloths was recorded

### Table 2 | Primer sequences used in the multiplex PCR reaction and expected PCR product sizes for each gene (Omar et al. 2010)

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>Primer</th>
<th>Primer sequence (5'-3')</th>
<th>Size (bp)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. coli</td>
<td>(mdh(F))</td>
<td>GGT ATG GAT CGT TCC GAC CT GAG AGA ATG GTA ACA CCA GAG T</td>
<td>300</td>
<td>Turr et al. (2002)</td>
</tr>
<tr>
<td></td>
<td>(mdh(R))</td>
<td>GGC AGA ATG GTA ACA CCA GAG T</td>
<td></td>
<td></td>
</tr>
<tr>
<td>EIEC</td>
<td>(ial(F))</td>
<td>GGAT ATG ATG ATG ATG AGT GGC GGA GGC CAA CAA TTA TTT CC</td>
<td>630</td>
<td>Paton &amp; Paton (1998)</td>
</tr>
<tr>
<td></td>
<td>(ial(R))</td>
<td>GGA GGC CAA CAA TTA TTT CC</td>
<td></td>
<td></td>
</tr>
<tr>
<td>EHEC/EPEC</td>
<td>(eaeA(F))</td>
<td>CTG AAC GGC GAT TAC GCG AA CAC GAT ACG ATC CAG</td>
<td>917</td>
<td>Aranda et al. (2004)</td>
</tr>
<tr>
<td></td>
<td>(eaeA(R))</td>
<td>CAG GAT ACG ATC CAG</td>
<td></td>
<td></td>
</tr>
<tr>
<td>EAEC</td>
<td>(eagg(F))</td>
<td>AGA CTC TGG CGA AAG ACT GTA TC ATG GCT GTC TGT AAT AGA TGA CAA GAA C</td>
<td>194</td>
<td>Kong et al. (2002)</td>
</tr>
<tr>
<td></td>
<td>(eagg(R))</td>
<td>GAC CTA GGC GAT TAC GCG AA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>EHEC</td>
<td>(stx1(F))</td>
<td>ACA CTG GAT CTC AGT GG CTC AAT CCT CCA TTA TG CCT TGC AAC TGA GCA CTA CTG TGT AAT AGA TGA CAA GAA C</td>
<td>614</td>
<td>Moses et al. (2006)</td>
</tr>
<tr>
<td></td>
<td>(stx1(R))</td>
<td>ATA GAT CTC AGT GCG ACG GTT CCA TGA CAA CGG ACA TCA GTT</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(stx2(F))</td>
<td>CCA TGA CAA CGG ACA GCA GTT CCA TGA CAA CGG ACA TCA GTT</td>
<td>779</td>
<td>Moses et al. (2006)</td>
</tr>
<tr>
<td></td>
<td>(stx2(R))</td>
<td>CCT TGC AAC TGA GCA CTA CTG TGT AAT AGA TGA CAA GAA C</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ETEC</td>
<td>(lt(F))</td>
<td>GCC GAC AGA TTA TAC CGT GC TTT CCC CTC TTT TAG TCA GTC AAG TG TGT AGC AGA TTA TAC CGT GC</td>
<td>330</td>
<td>Pass et al. (2000)</td>
</tr>
<tr>
<td></td>
<td>(lt(R))</td>
<td>CCG TCT CTA TAT GCC TGC TTT CCC CTC TTT TAG TCA GTC AAG TG</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(st(F))</td>
<td>GCC AGG ATT ACA ACA AAG TTC ACA GCC AGG ATT ACA ACA AAG TTC ACA</td>
<td>160</td>
<td>Pass et al. (2000)</td>
</tr>
<tr>
<td></td>
<td>(st(R))</td>
<td>GCC AGG ATT ACA ACA AAG TTC ACA GCC AGG ATT ACA ACA AAG TTC ACA</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
as 56% dirty cloths and 44% clean cloths based on visual observations. Table 3 distinguishes the cloth type and sanitary status of dishcloths collected from different study areas.

The analyzed cloths detected total coliforms in 98 of the samples, of which 81% had bacterial counts of higher than 1,000 cfu/25 cm² (Table 4). In addition 42% of the cloths analyzed tested positive for the presence of E. coli (Table 5).

Positive E. coli samples were further tested for the presence of pathogenic E. coli strains. The results indicated that ETEC was detected in 15% of the samples, EHEC was detected in 9% of the samples, EAEC was detected in 8% of the samples, EIEC was detected in 4% of the samples and EPEC was detected in 4% of the samples. Figure 1 shows the pathogens detected from each sampling area.

### DISCUSSION

From the results presented, Braamfischervelles showed the highest occurrence of contamination with E. coli. This observation was unexpected as Braamfischervelle is a relatively developed area containing recently built Reconstruction and Development Programme (RDP) housing. This could be attributed to the need for education regarding personal hygiene practices for people moving from informal settlements to the RDP housing. A study reported by Pickering et al. (2011) compared the bacterial count from Tanzanian houses using pit latrines containing concrete slabs to those without the concrete slabs. Their findings reported no difference of fecal contamination within the house. However, contaminants were observed in the soil. Thus although RDP houses in Braamfischervelle contain latrines, education on personal hygiene is
particularly needed to prevent unsanitary practices within homes. Furthermore, Sinclair & Gerba (2013) conclude that containing an improved latrine lessens the potential contamination burden but is not effective on its own. It additionally requires people to practice sanitary conditions, have improved water systems, control the household climate and perform cleaning activities regularly.

Although such activities are generally practiced within households, it was interesting to note that bacteria on contaminated dishcloths survived 24–48 hours after drying. Kagan et al. (2002) stated that drying the cloths alone is not an indication of hygienic practices. Furthermore, Mattick et al. (2003a, 2003b) state that the temperature of water used to wash dishcloths and tea towels is generally below 40°C which is not efficient to destroy bacterial...
contaminants and hence plays an important role in the spread of microorganisms around the household.

Moreover, Ojima et al. (2002) along with Beltran et al. (2013) stated that cleaning dishcloths with regular detergent or soaps is not sufficient to destroy pathogenic strains and suggested soaking the dishcloths in sodium hypochlorite followed by a wash in water or a dishwasher with a temperature above 40 °C. If such a protocol is applied in cleaning dishcloths within households, the bacterial load would be significantly reduced.

Areas such as Bertrams and Hillbrow had a variation of bacterial counts for total coliforms as well as pathogenic E. coli. However no pathogenic E. coli was detected from samples collected from Bertrams; this was unexpected as these regions lack access to improved water sources as well as latrines. However, the occurrence of total coliforms may indicate the occurrence of other pathogenic bacteria and requires further analysis.

Understanding the movement of bacteria in low income households in Africa is important for the development of hygienic programs and education. The source tracking of bacterial contamination within households can be done using several protocols and would assist in getting a better idea of how the bacteria are spread. However, to discontinue the spread of contaminants within the household and surrounding environment Curtis et al. (2003) suggest that a multidisciplinary method is required if hygienic programs are to be designed.

**CONCLUSION**

The results in this study indicate that there is a need for public education regarding hygiene in the households. Bacterial contamination is inevitable in such conditions and hence requires urgent attention to achieve the MDGs.

It has been demonstrated that dishcloths are important indicators of hygienic status in the households especially if the same cloth is used for various tasks. The presence of both total coliforms and E. coli in the samples can also indicate the presence of other pathogens further increasing the risk for diarrhea and other related infections.

Since all the diarrheagenic E. coli strains detected from the dishcloths can be accepted as culturable, due to the enrichment step, there is a danger of contamination of food and surfaces exposed to the dishcloths. Such occurrences may indicate the source of pathogens traveling into water and food sources contributing to diarrheal outbreaks.

**ACKNOWLEDGEMENTS**

The authors would like to acknowledge the publication workshop arranged by the South African Young Water Professionals during which this manuscript was prepared. We would also like to convey our appreciation to the Department of Science and Technology, Water Institute of Southern Africa and the University of Johannesburg for covering the costs of the workshop. Additionally we would like to acknowledge the Department of Water and Environmental Health, University of Johannesburg.

**REFERENCES**


Chang, H., Ko, E. & Ju, S. 2013 Effectiveness of a dry kitchen system on controlling the microbiological safety of food and contact surfaces. *Food Science and Biotechnology* 22, 1137–1144.


Donnenberg, M. S. 2002 Infections Caused by *Escherichia coli* and Other Enteric Gram-Negative Bacilli. Section 7, Chapter VIII. (D.C. Dale, ed.) Scientific American Medicine.

Jensen, D. A., Friedrich, L. M., Harris, L. J., Danyluk, M. D. & Schaffner, D. W. 2015 Quantifying transfer rates of


First received 31 July 2014; accepted in revised form 31 March 2015. Available online 21 May 2015.