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FOOD SAFETY IN THE DOMESTIC ENVIRONMENT

Thesis presented to *Escola Superior de Biotecnologia* of the *Universidade Católica Portuguesa* to fulfil the requirements of Master of Science degree in Food Innovation

By **Inês Gonçalves de Azevedo Moreira**

(June, 2012)

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Under the supervision of Prof. Doutora Paula Cristina Maia Teixeira
and
Doutora Joana Gabriela Laranjeira da Silva

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Resumo

O principal objetivo deste estudo foi avaliar a importância da segurança alimentar em ambientes domésticos.

A prevalência e identificação de bactérias de origem alimentar foi levada a cabo através da recolha de amostras em várias localizações de 15 casas, tais como maçanetas de portas, puxadores do frigorífico e máquina de lavar louça, botões de fogão, superfícies de preparação de alimentos, torneiras e toalhas de cozinha, bem como das patas de animais domésticos que usualmente têm acesso à área da cozinha, e ainda puxadores e torneiras de WC.

Um questionário foi também preparado e efetuado ao responsável pelas tarefas domésticas de modo a avaliar a experiência em práticas de higiene alimentar.

A deteção e quantificação de microrganismos de origem alimentar foram realizadas de acordo com os métodos descritos na International Standards Organization (ISO), resultando num total de 125 isolados de *Enterobacteriaceae* spp. (19 isolados de *Salmonella* spp., 46 de *Escherichia coli* e 60 de outras *Enterobacteriaceae*), 86 de *Staphylococcus* coagulase-positive, 5 de *Listeria* spp. e 13 de *Escherichia coli*. No entanto, nas 175 amostras analisadas não foi detetado *Campylobacter* spp..

A resistência aos antibióticos ampicilina, cloranfenicol, ciprofloxacina, gentamicina, ácido nalidíxico, tetraciclina, trimetropin e nitrofurantoína foi avaliada nos 3 grandes grupos dos 125 isolados de *Enterobacteriaceae* spp. (19 isolados de *Salmonella* spp., 46 de *Escherichia coli* e 60 de outras *Enterobacteriaceae*).

Escherichia coli e *Salmonella* spp. demonstraram resistência à ampicilina, cloranfenicol, tetraciclina, ácido nalidíxico e nitrofurantoína, enquanto outras *Enterobacteriaceae* apresentaram resistência apenas à ampicilina, trimetropin e nitrofurantoína. Resistência múltipla aos antibióticos descritos ocorreu maioritariamente nos isolados de *Escherichia coli* mas também em isolados de *Salmonella* spp. e de outras *Enterobacteriaceae*; no entanto, todos os isolados mostraram sensibilidade a antibióticos de grande importância clínica, como as fluoroquinolonas e os aminoglicosídeos.

Abstract

The main purpose of the work was to evaluate the significance of food safety in domestic environments.
The prevalence and identification of food-borne pathogens were assessed by taking swabs from several points in 15 houses, such as knobs of doors, refrigerators and dishwashers, stove buttons, surfaces of preparation of foods, taps and kitchen towels, as well as from domestic animals' feet that usually have access to the kitchen area, and WC knobs and taps.
A questionnaire was also prepared and administered to the person responsible for domestic tasks in order to evaluate their experience of hygienic practices.
Detection and quantification of food-borne microorganisms was made according to the methods described in the International Standards Organization (ISO), resulting in a total of 125 *Enterobacteriaceae* spp. isolates (19 *Salmonella* spp. isolates, 46 of *Escherichia coli* and 60 of other *Enterobacteriaceae*), 86 *Staphylococcus* coagulase-positive isolates, 5 *Listeria* spp. isolates and 13 *Escherichia coli* isolates. No *Campylobacter* spp. was found in the 175 analyzed samples.
Antibiotic resistance to ampicillin, chloramphenicol, ciprofloxacin, gentamicin, tetracycline, nalidixic acid and trimethoprim was evaluated in the 3 major groups of the 125 isolates of *Enterobacteriaceae* spp. (19 *Salmonella* spp. isolates, 46 of *Escherichia coli* and 60 of other *Enterobacteriaceae*). *Escherichia coli* and *Salmonella* spp. showed resistance to ampicillin, chloramphenicol, tetracycline, nalidixic acid and nitrofurantoin, while other *Enterobacteriaceae* presented resistance only to ampicillin, trimethoprim and nitrofurantoin. Multiple antibiotic resistance occurred mainly in *Escherichia coli* isolates but also in *Salmonella* spp. and other *Enterobacteriaceae*; nevertheless all the isolates showed sensitivity to antibiotics of clinical importance, such as fluoroquinolones and aminoglycosides.

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Table of contents

1. Introduction

Every year, millions of people worldwide experience foodborne diseases and illnesses resulting from the consumption of contaminated food, which has become one of the most common public health problems in the contemporary world (Notermans *et al.*, 1995; WHO, 2004).

Foodborne diseases impose a big burden on health and millions of people fall ill and many die as a result of eating unsafe food, so a resolution was adopted by WHO and its Member States to recognize food safety as an essential public health function, and to develop a Global Strategy for reducing the weight of foodborne diseases (WHO, 2002). In May 2010 the World Health Assembly approved a new resolution on food safety - Advancing Food Safety Initiatives (WHA, 2010) – of which the main goal is to update the current WHO Global Strategy for Food Safety (WHO, 2002).

The main purpose of this Master's thesis was to evaluate the significance of food safety in the domestic environment.

1.1. Food Safety in the domestic environment

Food safety is an important issue for consumers; they need to know how to safely prepare and handle food. Knowledge on safe food practices reduces consumer health risks from foodborne diseases that commonly result from poor food-handling and hygiene practices. These are thought to be the cause of a significant amount of foodborne illness, in the domestic environment (Scott, 1996; Fischer *et al.*, 2006).

As consumers, we expect food to be harmless, tasty and nutritious. Yet, every year millions of people become ill as a result of eating contaminated food. In fact, the World Health Organization (WHO) estimates that approximately 10 to 30% of the population in developed countries experience food poisoning annually (WHO, 2007). From farm to fork, microorganisms are transferred to our food through contact with contaminated water, insects, animals, humans, other contaminated foods and air. Microorganisms are able to multiply in our food and sometimes to produce toxins during processing and storage. When foods are eaten, e.g. raw, undercooked or simply cross-contaminated after cooking, we can consume these bacteria and/or their toxins. They can progress into our intestines and invade the cells lining the gut and/or the blood stream and potentially every organ in our bodies (Bolton and Maunsell, 2006).

The expression "diseases of alimentary origin" is vulgar and traditionally used to designate a group of symptoms which include gastric disturbances, usually involving vomiting, diarrhoea, fevers and abdominal pains, that can occur individually or in combination (Pinto, 2007).

Many indicators show that foodborne diseases are increasing in the domestic environment, mostly due to inappropriate food handling preparation and storage by consumers in their own kitchens.The main problem is that home-based outbreaks are not often identified nor reported which understates the real situation (Scott, 1996; Fisher *et al.*, 2006).

It is difficult to estimate the global incidence of foodborne disease, but according to WHO (2007) around 1.8 million people died from diarrheal diseases, mostly due to contamination of food and drinking water. In the United States up to 76 million cases of foodborne diseases, resulting in 325,000 hospitalizations and 5,000 deaths, are estimated to occur every year. In developing countries, a wide range of foodborne illnesses is usually the biggest problem and the high prevalence of diarrheal diseases suggests major primary food safety problems. Although most foodborne diseases are sporadic and often not reported, foodborne disease outbreaks may take on massive proportions.

Foodborne diseases are commonly considered as one of the biggest problems of public health in most countries and the reduction of these diseases is one of the main goals in national and international food safety programmes. Poor food handling and hygiene practices in our homes seem to be a key element in the prevention of foodborne diseases (Noronha *et al.*, 2006).

Because of its own nature, the domestic environment is a multifunctional place and this has a direct impact on the need for food safety improvement. First of all, the domestic environment contains occupants of assorted ages and diverse health status. Particularly, the emergent elderly and immunocompromised populations living at home are often at a higher risk for the acquisition of foodborne diseases as well as for a more severe disease outcome (Scott, 2003).

Consumers must know and be aware of the need for good hygiene practices at home to prevent the occurrence of infectious diseases. The biggest problems in achieving these improvements are educating the public and promoting behavioural changes. Inappropriate hand washing, food handling and preparation, short cooking times and long storage in non-appropriate conditions at home, can all permit proliferation of microorganisms. Pathogenic microorganisms are being carried to our homes through people, food, domestic animals, contaminated water and by air. These microorganisms are being disseminated to various surfaces throughout the home by cross-contamination, indicating the need for behavioural changes in our daily life (Gorman *et al.*, 2002). Many consumers don't know that raw food is one of the sources of bacterial contamination in our kitchen. Even more, consumers are not aware that the human body carries lots of pathogenic microorganisms being the main source of cross-contamination during food handling and preparation (Scott, 1996). Additionally, to its human occupants, the home is often a shelter for pets. Domestic cats and dogs frequently serve as reservoirs for microorganisms and, thus, are potential sources of infection. These animals can transfer their intrinsic microflora to the kitchen food handling surfaces, increasing the risk of cross-contamination to food (Scott, 2003) .

Foods and microorganisms have long and healthy associations such as the nutritional significance and as an ideal culture media for microbial development. Microbial growth in foods can result in preservation or spoilage, depending on the microorganisms involved and food storage conditions. Microorganisms can be used to convert raw foods into gastronomic delights, including cheeses, pickles, sausages, wines, beers and other alcoholic beverages. On the other hand, foods also can act as a vehicle for disease transmission. During the entire sequence of food handling, from the producer to final consumer, microorganisms can affect food quality and human health. Contamination

by disease causing microorganisms can occur at any point in the food handling sequence (Prescott *et al.*, 1999).

Around the world in the near future, foodborne diseases will continue to be an issue of major concern. Public instruction can be seen as a key factor in the improvement of food safety practices at home and the benefits of food hygiene education would include a decrease in the occurrence of foodborne illness as well as a population better prepared to meet the needs for safer food (Scott, 2003).

1.2. Antibiotic resistance in *Enterobacteriaceae* species

The *Enterobacteriaceae* family is frequently used as an indicator of faecal contamination during food microbiological analyses, and contains important zoonotic bacteria such as *Salmonella* spp. and *Escherichia coli*. *Enterobacteriaceae* may originate severe infections, and unfortunately several of the most important members of this family are becoming progressively more resistant to currently available antimicrobials such as tetracyclines and fluoroquinolones (Fritsche *et al.*, 2005; Paterson, 2006; Denton, 2007).

Nowadays, the antimicrobial agents used to treat or prevent bacterial infections in animals are basically the same classes of compounds that are used in human medicine. In both cases the use of antibiotics not only causes an increase of resistance in pathogenic bacteria, but also in the endogenous flora of these animals. These animals' resistant bacteria can infect or reach the human population not only by direct contact, but also via food products of animal origin (van den Bogaard and Stobberingh, 2000).

The choice of antibiotics becomes more limited, since the bacteria are also resistant to other drugs. For example, when established more than two decades ago, the fluoroquinolones, particularly ciprofloxacin, were considered the "new penicillins" because they were secure, bactericidal and exhibited a relatively broad spectrum of activity. Even though resistance to fluoroquinolones was not observed in this present study, over the past decade the emergence of high-level, fluoroquinolone resistance among *Escherichia coli* and other clinically important pathogens such as *Staphylococcus aureus* and *Pseudomonas aeruginosa*, has been witnessed (Piddock, 1999). Nevertheless, *E. coli*, the leading cause of urinary tract infection and Gram-negative bacteraemia, which was naturally susceptible to ampicillin, nowadays 50-60% of isolates present resistance worldwide (Wu *et al.*, 1992).

This resistance phenomenon requires continual vigilance and measures have to be found in order to control the further spread of resistance by pathogens included in the *Enterobacteriaceae* family. Another aspect of concern is related to the increase in multi-resistance now common in both community and hospital isolates (Shannon and French, 2004).

However, little information relative to enteric bacteria isolated from domestic settings is currently available. Consequently, a second main goal of the present study was to investigate the prevalence of antimicrobial susceptibility found in *Enterobacteriaceae* isolates found in the domestic environment and try to make a comparison with some other studies. The potential repercussion of these results in microbiological safety terms, especially concerning the development and spread of antimicrobial resistance to the food chain, will be discussed.

1.3. Food Safety survey: knowledge levels of consumers

Increasingly, food safety awareness levels are essential for food poisoning prevention. The main sources of infection in the domestic environment are people, pests, pets and contaminated food and water. Therefore home hygiene isn't just daily cleaning the house but also knowing how to prevent contamination. Microbes are constantly transmitted by direct contact with people or animals, through contaminated food, water, surfaces and air. When preparing contaminated food, pathogens easily spread onto cooking utensils, such as cutting boards and knives, or onto surfaces when using kitchen cloths (Beumer and Kusumaningrum, 2003).

Consumers need to know which behaviours are more likely to result in illness in order to make decisions about food handling and consumption behaviours, making education the main focus to reduce foodborne diseases (Jevsnik *et al.*, 2008).

In this study it seemed important to design a questionnaire with some questions related with food safety and cleaning habits which was administered to the responsible persons in each house.

1.4. Aims of the study

In order to evaluate the significance of food safety in domestic environments, the prevalence and identification of food-borne pathogens were assessed by analysing several points in 15 houses and then several objectives were established:

- Estimate potential risks of cross contamination in the domestic environment;
- Evaluate the prevalence of some foodborne pathogens, namely *Enterobacteriaceae* spp*., E. coli*, *S. aureus*, *L. monocytogenes* and *Campylobacter* spp., at various defined points in different houses;
- Characterization of the presumptive *Enterobacteriaceae* spp. isolates in order to obtain representative groups according to their metabolic characteristics;
- Determine antibiotic susceptibility of *Enterobacteriaceae* isolates;
- Correlate the microbiological results obtained for each domestic environment with the results of the questionnaire applied at each house.

2. Material and Methods

2.1. Sampling

During the period January 2008 to July 2008, the detection and/or enumeration of *Enterobacteriaceae*, coagulase-positive *Staphylococcus*, *E. coli*, *L. monocytogenes* and *Campylobacter* spp. in 15 different private homes was assessed by taking several cotton swabs from various defined points i.e. knobs of doors, refrigerators and dishwashers, stove buttons, surfaces used for preparation of foods, taps and kitchen towels, WC knobs and taps and from domestic animals' feet that usually have access to the kitchen area. Samples were taken after the normal daily cleaning of the house, then collected, stored in thermo bags and further analysed as soon as they arrived in the laboratory.

2.2. *Campylobacter* spp. detection

The detection of *Campylobacter* spp. *was* performed according to International Standard Organization (ISO) 10272-1 methodology. After sampling, cotton swabs were immediately inoculated in Bolton Broth (Biokar) and incubated at 37 ºC for 4 to 6 hours and subsequently at 41.5 ºC for 44 hours in a microaerobic environment. Using the spread plate technique, 0.1 mL samples were inoculated onto modified Cefoperazone Charcoal Deoxycholate Agar (mCCDA, Oxoid) and incubated for 48 hours at 41.5 ºC under microaerobic conditions, using a specific incubator.

Characteristic colonies (gray, flat, with metallic shine and with swarming tendency) were selected and sub-cultured on Columbia agar with 5% sheep blood (BioMérieux) and incubated between 24 to 48 hours at 41.5 ºC under microaerobic conditions. After this period characteristic colonies were confirmed through direct microbiologic examination, oxidase test and growth on blood agar under aerobic and microaerobic conditions during 44 hours at 41.5 ºC. Small curved bacilli, with rapid motility, corkscrew shape, oxidase positive and that do not grow under aerobic conditions at 41.5 ºC were incubated on Tryptic soy agar (TSA, Biokar), for 24 hours at 37 ºC and then stored, in triplicate, at - 80 ºC in Trypticase Soy Broth (TSB, Pronadisa-Conda Lab) containing 30% (v/v) of glycerol (Sigma).

2.3. Coagulase-positive *Staphylococcus* enumeration

The enumeration of coagulase-positive *Staphylococcus* was performed according to the ISO 6888-1 methodology.

After sampling, cotton swabs were immediately inoculated in 10 mL of Buffered Peptone Water (BPW, Oxoid). Decimal dilutions were prepared with sterile Ringer's solution (Lab M) and enumeration was performed by the spread plate technique on Baird Parker Agar (BPA, Biokar Diagnostic) with egg yolk (Bio-Rad) (0.5 mL from the initial suspension, in duplicate, and 0.1 mL of each dilution) and further incubated at 37 ºC for 48 hours.

Characteristic (with an opaque halo surrounded by a zone of clearing) and non-characteristic black colonies were counted and from each plate, five characteristic and five non-characteristic colonies were selected and then sub-cultured in Brain Heart Infusion broth (BHI, Merck), for 24 hours at 37 ºC.

Coagulase test was performed by adding 150 µL of the BHI suspension to 250 µL of rabbit plasma (Biokar Diagnostic) and incubating for approximately 12 hours at 37 ºC. *S. aureus* and *S. epidermidis* were used as positive and negative controls, respectively. All coagulase positive colonies (gelling of the plasma) were isolated on TSA, incubated for 24 hours at 37 ºC and then stored, in triplicate, at - 80 ºC in TSB containing 30% (v/v) of glycerol.

2.4. *Listeria monocytogenes* detection

The detection of *L. monocytogenes* was performed according to the ISO 11290-1 methodology.
After sampling, cotton swabs were transferred to 10 mL of half-Fraser broth (Biokar Diagnostics) and incubated at 30 ºC for 48 h. Aliquots (1 mL) of these primary enrichments were transferred to 10 mL of secondary enrichment Fraser broth (Biokar Diagnostics) and incubated at 30 ºC for 48 h. A loopful of each primary enrichment culture and of the secondary enrichments after 24 and 48 hours of incubation, were streaked separately onto PALCAM (Merck) and ALOA (BioMérieux) agar plates. Characteristic colonies (blue/green with an opaque halo in ALOA and green/gray with black precipitate in PALCAM) were selected after incubation at 37 ºC for 48 hours, five typical colonies per plate (when possible) were transferred onto PALCAM Agar, incubated at 37 ºC for 48 hours.
Pure cultures were tested for sugars fermentation, mannitol (0.5% w/v), rhamnose (1% w/v) and xylose (0.5% w/v) and CAMP with *S. aureus* NCTC 1621 and *Rhodococcus equi* NCTC 25923. *L. monocytogenes* positive colonies were then stored, in triplicate, at - 80 ºC in TSB containing 30% (v/v) of glycerol.

2.5. *Escherichia coli* enumeration

The enumeration of *E. coli* was performed according to the ISO 16649-2 methodology. After sampling, cotton swabs were immediately inoculated in 10 mL of BPW. Decimal dilutions were prepared with sterile Ringer's solution and enumeration was performed by the pour plate technique (1 mL of each dilution) in Tryptone Bile X-glucuronide Agar (TBX, Bio-Rad). The plates were further incubated at 44 ºC for 48 hours.
Characteristic blue/green colonies were counted and from each plate, five different colonies were selected, sub-cultured in TSA, for 24 hours at 37 ºC and then stored, in triplicate, at - 80 ºC in TSB containing 30% (v/v) of glycerol.

2.6. *Enterobacteriaceae* spp.

2.6.1. Enumeration and detection

The enumeration and detection of *Enterobacteriaceae* were performed according to the ISO 21528-2 methodology.
After sampling, cotton swabs were immediately inoculated into 10 mL of BPW. Decimal dilutions were prepared with sterile Ringer's solution and enumeration was performed by the pour plate technique in Violet Red Bile Glucose Agar (VRBGA, Biokar Diagnostic) (1 mL of each dilution plus

overlay). Plates were then incubated at 37 ºC for 24 hours. Simultaneously, the detection of *Enterobacteriaceae* as described for the enumeration but with the inclusion of an enrichment step in BPW for 24 hours at 37 ºC before the enumeration.

In both cases, characteristic red colonies were counted. From each plate, five individual colonies were randomly selected and then sub-cultured in TSA, for 24 hours at 37 ºC. Confirmation of isolates was performed according to the results obtained for the glucose fermentation and for the oxidase positive test. Presumptive *Enterobacteriaceae,* glucose fermenting and oxidase negative isolates, were then stored, in triplicate, at - 80 ºC in TSB with 30% (v/v) of glycerol.

2.6.2. Identification Tests

Different tests were performed in order to confirm the identification of the isolates to the family level and to group them on the basis of specific biochemical characteristics. Controls and working cultures were recovered from frozen storage in TSB for 24 hours at 37 ºC and then inoculated onto TSA (incubated at 37 ºC for 24 hours). All controls used in identification tests are part of ESB culture collection.

2.6.2.1. Growth on MacConkey agar plates

MacConkey agar medium (Merck) is selective for Gram negative bacteria and can differentiate those bacteria that are able to ferment lactose. Isolated colonies of presumptive *Enterobacteriaceae* grown on TSA were streaked on the surface of MacConkey agar plates and further incubated for 18 to 24 hours at 37 ºC. *Salmonella* spp. were used as a negative control, colonies of non-lactose fermenting organisms are colourless. *E. coli* was used as a positive control - colonies of lactose fermenting organisms are red and surrounded by a turbid zone due to the precipitation of bile acids as a result of acid pH.

2.6.2.2. Triple Sugar Iron test

A colony of presumptive *Enterobacteriaceae* grown in TSA was inoculated onto Triple Sugar Iron (TSI) slants and incubated for 24 h at 37 °C. This medium was used to observe the degree of acid produced and to differentiate between non-fermenters, glucose-fermenters (which produce a relatively small amount of acid) and those which ferment lactose and/or sucrose in addition to glucose (producing a relatively large amount of acid which diffuses throughout the medium). Organisms which produce hydrogen sulfide from the reduction of thiosulfate are easily detected because the H_2S reacts with the iron in the medium to produce ferrous sulfide, a black precipitate. Five controls were used, namely *Klebsiella* spp. *Salmonella* spp., *E. coli*, *Proteus vulgaris*, and a negative without inoculum.

In the case of *Klebsiella* spp. the TSI tube became yellow with some cracks because it ferments all three sugars producing gas. For *Salmonella* spp. the butt of the tube presented

a black cracked precipitate indicating glucose fermentation with gas and H_2S production but the slant colour is red because only glucose is fermented and the bacterium is capable of utilizing and fermenting glucose, but not lactose or sucrose. *E. coli* fermented all sugars with gas formation and that's why the tube presented a yellow colour with big cracks. *Proteus vulgaris* fermented all three sugars with gas and H_2S formation (yellow with a black precipitate colour with cracks). The non-inoculated tube remained red, the characteristic colour of the original medium.

2.2.2.3. Indole production from tryptophan

The indole test determines the ability of an organism to produce indole from the degradation of the amino acid tryptophan, which is hydrolyzed by tryptophanase to produce three possible end products, one of which is indole. BPW was inoculated with one isolated colony grown in TSA and further incubated at 37 °C for 24 to 28 hours. After this period 0.5 mL of Kovac's reagent (Merck) was gently added. The presence of a red or red-violet colour in the surface alcohol layer of the broth was considered a positive result. A negative result appeared yellow. *E. coli* and *Salmonella* spp were used as positive and negative controls, respectively.

2.7. Antibiotic susceptibility of *Enterobacteriaceae* spp.

2.7.1. Minimal Inhibitory Concentration (MIC) estimation

For each isolate, the minimum inhibitory concentration MIC (µg/mL) of eight antibiotics was determined by the agar microdilution method, according to the Clinical and Laboratory Standards Institute (CLSI, 2007). Antibiotics were chosen on the basis of their ability to provide a diverse representation of different classes of antimicrobial agents.

Each test was carried out on Muller-Hinton Agar (MHA) (BioMérieux) with cation adjusted for ampicillin (AMP) (Fluka) and on MHA for the seven other tested antibiotics – ciprofloxacin (CIP), chloramphenicol (CHL), gentamicin (GEN), nalidixic acid (NAL), nitrofurantoin (NIT), tetracycline (TET) and trimethoprim (TMP) (kindly supplied by the company Labesfal, Portugal). With the exception of TMP ranging from 0.0156 to 128 µg/mL; all the other antibiotic concentrations ranged from 0.0156 to 512 µg/mL. Inocula were prepared from overnight cultures on TSA plates, by suspension in sterile Ringer's solution in order to obtain turbidity equivalent to 0.5 McFarland standards. Approximately 1 µL was positioned on each plate containing antibiotic with an automatic plating system (Mast Group, Ltd.). All isolates were grown in plates of MHA and MHA with cation adjusted with no antibiotic. The quality control strains *Enterococcus faecalis* ATCC 29212 and *E. coli* ATCC 25922 were used to monitor the accuracy of MICs (CLSI, 2007). Plates were incubated for 24 hours at 37 ºC. Classification of isolates according to their susceptibility (as sensitive, intermediate or resistant) was based on the values recommended by the CLSI (2007; Table 2A – MIC Interpretative

Standards (µg/mL) for *Enterobacteriaceae*). Isolates exhibiting resistance to at least two of the antimicrobial agents were considered to be multi-resistant strains.

2.8. Domestic survey

A questionnaire was designed and some questions related with food safety and cleaning habits were administered to the responsible persons in each house.

This questionnaire included the following questions:

- Is there any domestic animal in your house?
- Does your pet stay inside, outside your house or both?
- When it's inside your house does it stay in the kitchen area?
- What kind of pet do you possess? A cat, a dog or something else?
- If you own a cat, what sort of sand do you buy?
- When you use WC do you wash your hands always, most of times, rarely or never?
- In the kitchen area do you usually wash your hands when you go to WC, handle raw, cooked or ready to eat food?
- How often do you normally clean door knobs?
- Do you use detergent, disinfectant, water or something else for knobs cleaning?
- How often do you normally clean kitchen taps?
- Do you use detergent, disinfectant, water or something else for taps cleaning?
- How often do you normally clean kitchen counter?
- Do you use detergent, disinfectant, water or something else for kitchen counter cleaning?
- How often do you normally clean kitchen stove buttons?
- Do you use detergent, disinfectant, water or something else for kitchen stove buttons cleaning?
- How often do you normally clean the dishwasher knob?
- Do you use detergent, disinfectant, water or something else for dishwasher knob cleaning?
- How often do you normally clean the refrigerator knob?
- Do you use detergent, disinfectant, water or something else for refrigerator knob cleaning?
- Do you use kitchen cloths?
- Do you use wood or plastic cutting board?
- Do you have different cutting boards for vegetables, meat, fish, cooked and raw food?
- Do you have any kind of doubt about proper food safety behaviours?

All the data was evaluated and combined, using Excel, in order to obtain comparative results between different houses and surfaces.

3. Results and Discussion

Increasingly, food safety awareness levels are essential for preventing food poisoning. Domestic environment is frequently contaminated by people, pests, pets, food and water and daily cleaning the home hygiene may not be enough therefore it is rather important to know how to prevent contamination. Taking this into consideration, in this section, all the results obtained from the domestic setting will be presented and discussed.

3.1. Campylobacter spp. detection

Although *Campylobacter* is often present in domestic environments when high risk foods, like chicken, are prepared in domestic kitchens, resulting in cross contamination (Humphrey *et al.*, 2001), this bacteria was not detected in any of the 15 houses sampled in this study. This agrees with the results of Speirs *et al.* (1995), who also failed to identify this pathogen in a large variety of sites examined in 46 domestic kitchens.

These bacteria are generally sensitive to the extra-intestinal environment and conditions common in kitchens, such as high or low temperature and drying environment, that may not only cause a reduction in the viable population, but also injure surviving cells. There is much to be learned about *Campylobacter* behaviour, and particularly about the best methods for their isolation from non-clinical samples. Usually *Campylobacter* can take a very long time to repair cellular damage and begin to grow, which can result in some false negative results being obtained (Humphrey *et al.*, 2001).

3.2. Coagulase-positive *Staphylococcus* count

A total of eighty six samples of coagulase-positive *Staphylococcus* were collected from all places in all 15 houses analysed. As can be seen in **Figure 1**, the greatest numbers were detected from the feet of domestic animals, WC tap and knob, kitchen counter, cooking stove buttons, refrigerator and dishwasher handles. According to some authors it is quite common to find *S. aureus* in domestic environments since it is a common inhabitant of the human nose, throat and skin (Arbuthnott *et al.*, 1990) and therefore more likely to contaminate foods by direct or indirect human contact during domestic food handling (Kusumaningrum *et al.*, 2003). This microorganism can survive for between 2 and 4 days on surfaces, and is easily transferred from such sites to food by a range of mechanisms (Kusumaningrum *et al.*, 2003). *S. aureus* is also commonly found in a wide range of food products such as meat, cheese and milk and from environmental sources such as soil, air and water (Kloos and Schleifer,1986).

Domestic animals analysed in this study, both canine and feline species, demonstrated high carrier rates of coagulase-positive *Staphylococcus* on their feet, which is considered normal since they are usually found inside and outside of the house. These animals may possibly serve as a source of

pathogenic staphylococci, since the rates isolated are indicative of a potential reservoir of pathogenic organisms (Morrison *et al.*, 1961).

Up to 39% of domestic food poisoning outbreaks are due to food preparers' hands (Ryan *et al.*, 1996) and therefore all sites which are directly contacted by fingers, are potentially contaminated. Places like taps, knobs, stove buttons, kitchen counter and handles (refrigerator and dishwasher) were shown to carry high counts since cross-contamination to other surfaces by hands, occurs easily. Many studies have noted the ability of pre-inoculated foods to cause cross-contamination of other surfaces and sites in the domestic kitchen (de Wit *et al.*, 1979; de Boer and Hahné, 1990; Scott and Bloomfield, 1990; Bradford *et al.*, 1997; Zhao *et al.*, 1998), thereby identifying the ability of foodborne disease microorganisms to become disseminated from naturally contaminated foods to various hand and food contact surfaces in the domestic kitchen.

3.3. *Listeria monocytogenes* detection

Since *Listeria* spp. are commonly found in the general environment (Beumer *et al.*, 1996; Azevedo *et al.*, 2005), the presence of these organisms in the domestic environment is not surprising. Several food products have been associated with *Listeria* contamination, such as milk and dairy products, various meats and meat products such as beef, pork, fermented sausages, fresh produce such as radishes, cabbage, seafood and fish products (Gadhi and Chikindas, 2007). Through cross contamination this pathogen can spread, adapt to survive and grow in a wide range of environmental conditions.

In this study *Listeria* spp. were present in low numbers and only on five of the 11 sites analysed (**Figure 2**). *L. monocytogenes* was not isolated from any of the samples, however *L. seeligeri*, *L. innocua* and *L. grayi* were found in the WC (tap and knob), kitchen tap and kitchen counter, respectively (**Table 1**).

Azevedo *et al.* (2005) found that *L. monocytogenes* was present in three domestic refrigerators out of the 86 investigated and *L. grayi* and *L. innocua* were also isolated from four and one refrigerators, respectively. This may indicate that these pathogens may normally exist in our kitchen, although apparently at low numbers and frequency.

Table 1 - Occurrence of *Listeria* spp. in the domestic environment.

Type of sample (Number)	Listeria number present (%)	Isolated *Listeria* spp.
Domestic Animal (30)	1 (**3.3**)	1 (*Listeria* spp.)
WC Tap (15)	1 (**6.7**)	1 (*Listeria seeligeri*)
WC Knob (15)	1 (**6.7**)	1 (*Listeria seeligeri*)
Kitchen Tap (15)	1 (**6.7**)	1 (*Listeria innocua*)
Kitchen Counter (15)	1 (**6.7**)	1 (*Listeria grayi*)

A study carried out by Beumer *et al.* (1996) demonstrated that *L. monocytogenes* and *L. innocua* are the usual *Listeria* spp. found in domestic environments. According to Beumer's (1996) study, *Listeria* spp. is frequently found in wet places and up to 37% was found in dishcloths. Taps are by their nature

places were water is normally present and that can explain *Listeria* presence in our samples. Generally dishcloths are used to clean other surfaces in the kitchen and in the course of cleaning cross contamination can occur which may explain *Listeria* presence on the kitchen counter. Other workers have recognized the potential for spread of microbial contamination via cleaning utensils and the potential for some microorganisms to persist in the environment (Davis *et al.*, 1968; Westwood and Mitchell, 1971).

Humans are exposed to this pathogen on a regular basis, because of its ubiquity in food products and the wider environment (Farber and Losos, 1988; Farber and Peterkin, 1991), therefore it is likely that domestic animals' paws will also be contaminated with this kind of pathogen.

L. monocytogenes has also been shown to adhere to various surface materials normally in contact with foods, such as stainless steel, rubber, glass and polypropylene (Blackman and Frank, 1996; Mafu *et al.*, 1990) which can explain the WC knob contamination.

3.4. *Escherichia coli* count

Beumer and Kusumaningrum (2003) stated that places or objects with high numbers of microorganisms, which can simply be transmitted to other surfaces, are considered as reservoirs/disseminators and that even though raw material is most likely the major cause of contamination in the kitchen, the adjacent areas could also act as sources of free-living bacterial populations.

In a study carried out by Gorman *et al.* (2002), *E. coli* was isolated from chicken samples which cross-contaminated one or more surfaces in the domestic kitchen, namely dishcloths, person's hands, refrigerator handles, oven door handles and counter-tops. This was not surprising since *E. coli* is a normal inhabitant of the chicken intestine and contamination may occur during evisceration. *E. coli* are frequently isolated from human or animal faeces or from food products, like poultry (Saénz *et al.*, 2001).

In this study several surfaces and utensils were contaminated with *E. coli* (**Figure 3**), resulting in collection of 13 samples, which is in agreement with several other studies where domestic kitchens were investigated for the presence of food pathogens (de Wit *et al.*, 1979; de Boer and Hahné, 1990; Beumer and Giffel, 1999). High numbers of *E. coli* ($> 10^3$ CFU/swab) were detected on domestic animals, WC and kitchen taps, kitchen knob and counter, stove buttons, cutting board, refrigerator and dishwasher handle. Surprisingly the kitchen cloth, which is generally recognized as a potential source for spreading microorganisms, since bacteria tend to persist in these vehicles (Josephson *et al.*, 1997; Rusin *et al.*, 1998), presented low contamination level ($< 10^2$ CFU/swab).

According to Adiga *et al.* (2012) among the different places in the kitchen, water taps were found to be most contaminated followed by stove knob, towel and refrigerator handle. The high incidence of pathogens on water taps and stove knobs/buttons, which are usually touched with unwashed hands

during cleaning of raw food, was not a surprise since the moisture creates an ideal environment for bacterial growth.

Previous studies acknowledged that the kitchen generally shows more bacterial contamination than the bathroom (Finch *et al.*, 1978; Scott *et al.*, 1982; Speirs *et al.*, 1995; Rusin *et al.*, 1998) and there is evidence that the survival and transfer of potentially pathogenic bacteria via environmental surfaces is important (Sanborn, 1963; Humphrey *et al.*, 1994). de Wit *et al.* (1979) showed that following the domestic preparation of chickens contaminated with *E. coli*, the bacteria were isolated from the cutting board, door handles and faucet handles where hand transfer must have occurred.

The presence of enteric bacteria such as *E. coli*, a widely accepted indicator of faecal contamination, might be introduced into the kitchen through raw foods, mainly of animal origin, people, pets and insects and may be a sign of a low level of hygiene among the kitchen users (Scott *et al.*, 1982; William E. Oswald *et al.*, 2007), i.e. poor hand washing.

3.5. *Enterobacteriaceae* spp. count and detection

Enterobacteriaceae isolates were collected and **Figure 4** summarizes *Enterobacteriaceae* distribution in the domestic setting. As can be seen these kinds of microorganisms are distributed all around the house with high isolation rates (> 10^2 CFU/swab). Localities as domestic animal paws, kitchen tap, kitchen counter, stove buttons, refrigerator and dishwasher handle or kitchen cloth show levels of contamination higher than 10^5 CFU/swab.

In a study carried out by Scott *et al.* (1982), more than 80% of the 201 homes examined contained one or more species of enterobacteria and wet places like taps and dishcloths were highly contaminated. The normal contamination of dishcloths and other wet items with large numbers of organisms including enterobacteria, suggests that these objects may act not only as reservoirs but also as disseminators of contamination in the kitchen and although enteropathogenic organisms probably originate from the toilet and toilet usage, hands and cleaning cloths harbour and may disseminate these organisms.

Experimental studies with *Salmonella* spp. and *E. coli* show the likelihood for spread from toilets to bathroom surfaces and hands (Gerba *et al.*, 1975; Barker and Bloomfield, 2000) and from hands to other surfaces (Rheinbaben *et al.*, 2000).

In a study by Curtis *et al.* (2003), microbiological samples proved that faecal contamination of the domestic environment does occur, since faecal coliforms were found at a number of sites, not only in toilets and bathrooms but also in kitchens and on a variety of objects. The fact that a number of bathroom and toilet sites including door handles, were found to confirm signs of faecal contamination, suggests that hand-washing after using the toilet is not always regularly practised.

Several studies show that the intestinal tracts of animals generally harbour *Enterobacteriaceae* (Beutin, 1999; Guardabassi *et al.*, 2004; Cobeljic *et al.*, 2005; Jimenez *et al.*, 2011); therefore it is normal that domestic animals may introduce these types of pathogens into the domestic setting.

Adiga *et al.* (2012) studies demonstrated that bacterial contamination in the kitchen is common and among 10 kitchens analysed several sites, like refrigerator handle, kitchen stove and water taps, were infected with faecal microorganisms.

Enterobacteriaceae presence in all 11 places of the house, analysed in this study, is in accordance with several studies which showed that various species of bacteria can live on kitchen surfaces and cross-contamination can easily occur contaminating the food preparation counter, cloths, utensils and hands (de Wit *et al.*, 1979; Ak *et al.*, 1994; Scott, 1996; Gorman *et al.*, 2002; Beumer and Kusumaningrum, 2003).

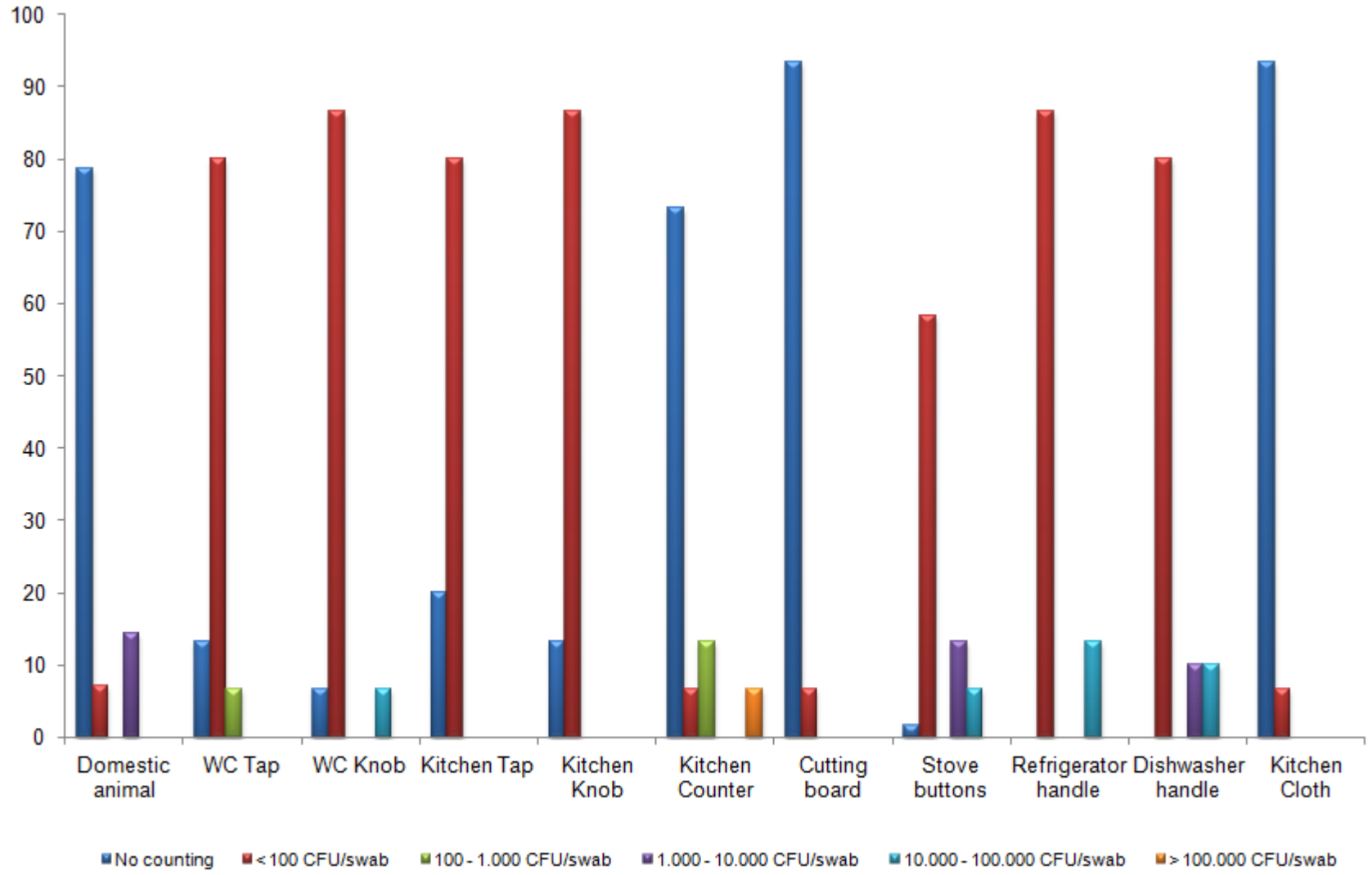


Figure 1 - Coagulase-positive *Staphylococcus* count (%) by locality in domestic environments.

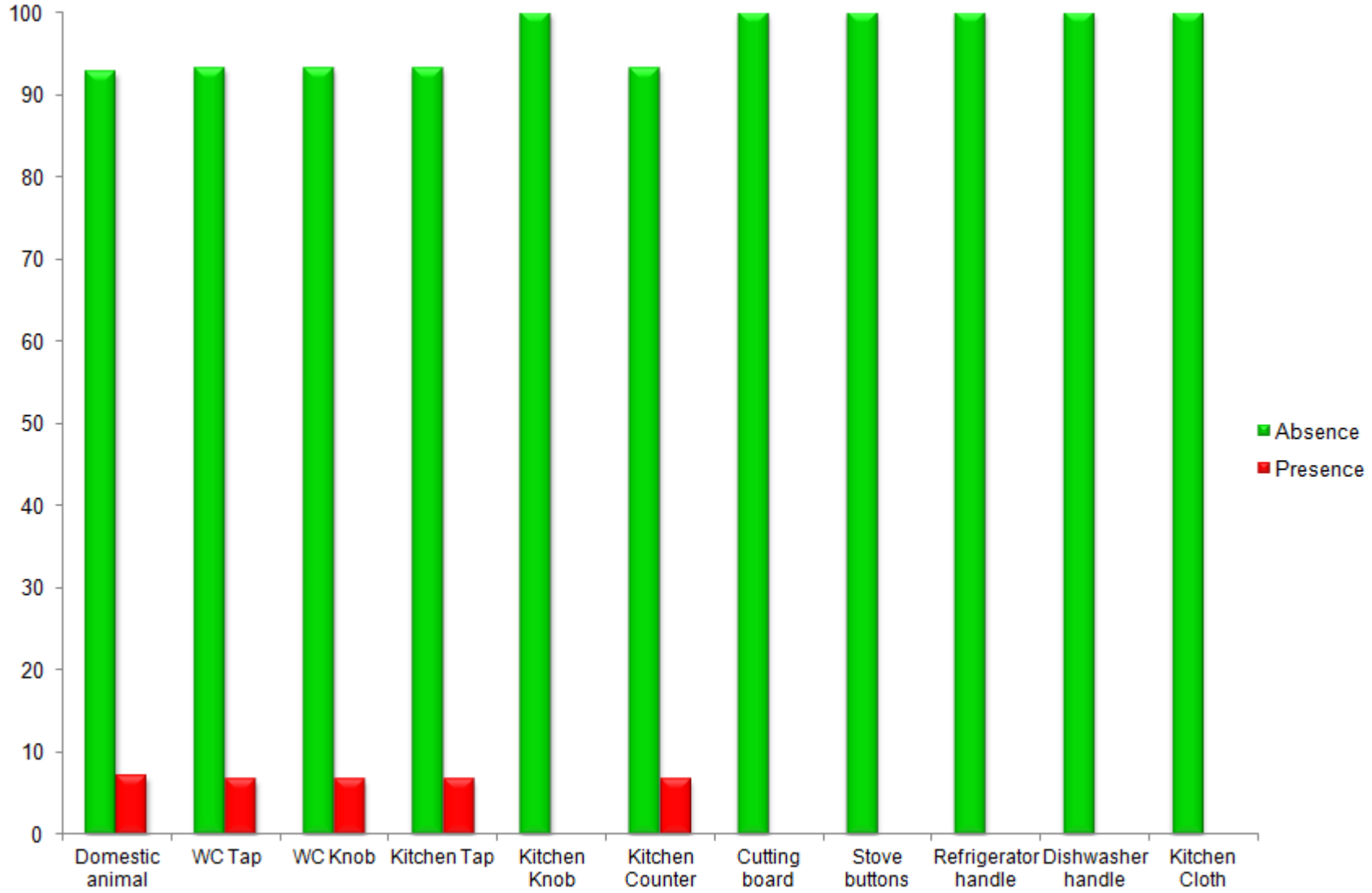


Figure 2 - *Listeria* spp. detection (%) by locality in the domestic environment.

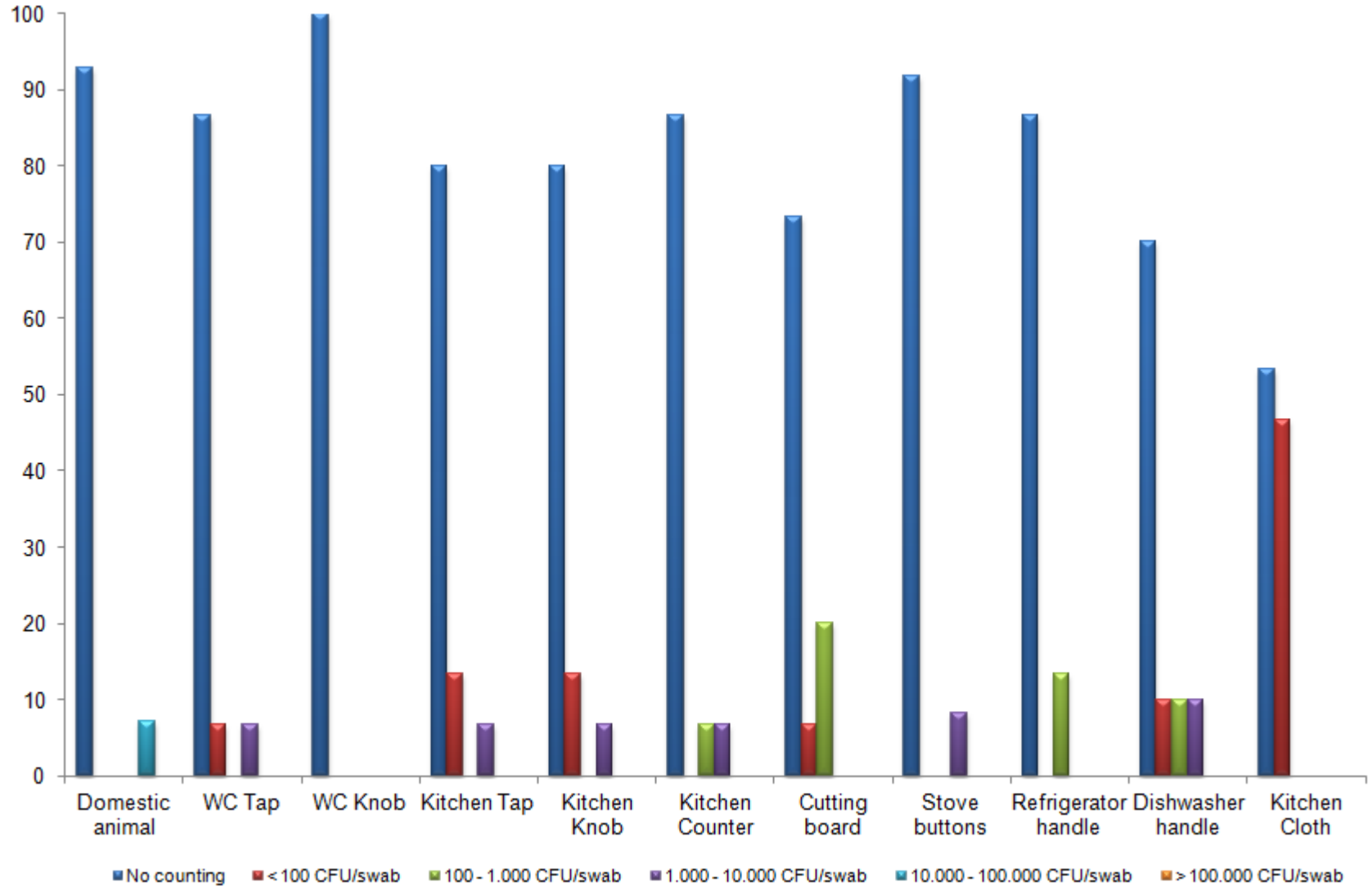


Figure 3 - *Escherichia coli* counts (%) by locality in the domestic environment.

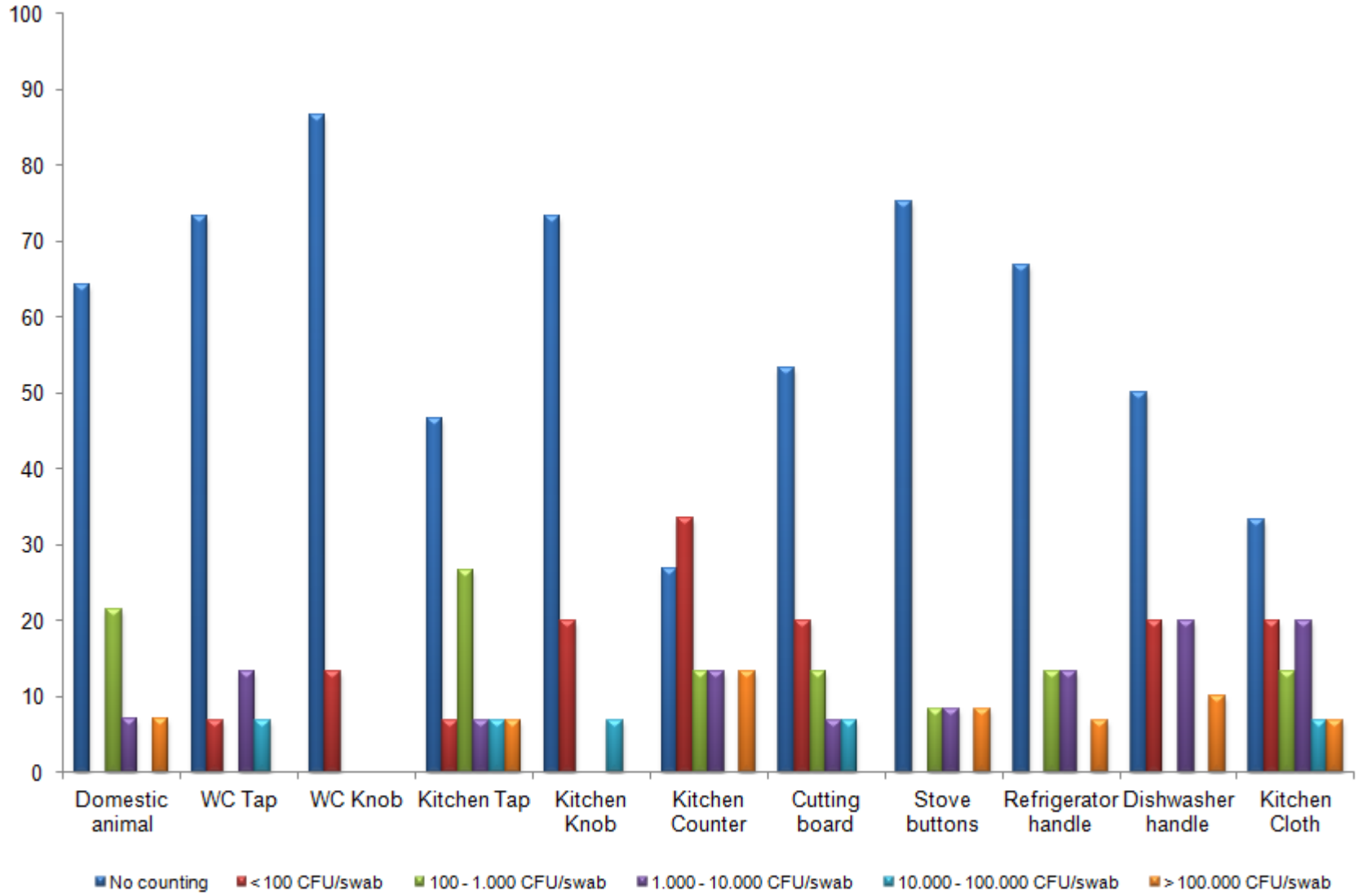


Figure 4 - *Enterobacteriaceae* counts (%) by locality in the domestic environment.

3.6. *Enterobacteriaceae* antibiotic resistance

For antibiotic resistance, all 351 isolates suspected to be *Enterobacteriaceae* were Gram-stained and tested for oxidase, catalase activity and fermentation of glucose. A total of 125 isolates glucose-fermenting, Gram-negative, oxidase-negative, catalase-positive were considered to belong to the family *Enterobacteriaceae*, and only these were included in further testing. After primary identification, *E. coli* and *Salmonella* spp. were differentiated from all the other *Enterobacteriaceae* as shown in **Table 2**.

Table 2 - *Escherichia coli* and *Salmonella* spp. differentiation by biochemical tests

Microorganism	**Biochemical Tests**					
	Indole	Triple Sugar Iron Agar (**TSI**)				
		Glucose	Lactose	Sucrose	H_2S	Gas
Escherichia coli	+	+	+	+	-	+
Salmonella spp.	-	+	-	-	+	+

From this differentiation all 125 samples were separated in 3 major groups, for antibiotic resistance tests, namely 46 of *E. coli*, 19 of *Salmonella* spp. and all the remaining 60 isolates of other *Enterobacteriaceae* (**Table 3** - see pag. 25).

High frequencies of antimicrobial resistance have been previously found in *Enterobacteriaceae*, in faecal flora as well as in clinical isolates (Kelch and Lee, 1978; Levy *et al.*, 1988; Lester *et al.*, 1990; Bonten *et al.*, 1992; Leistevuo *et al.*, 1996). Yet little or nothing is reported about antibiotic resistance in *Enterobacteriaceae* isolates found in the domestic setting, but there is plenty of evidence for enteric organisms found in this study with their origins in animals, humans and/or food.

Both animals and humans can introduce enteric pathogens into the dosmestic environment by cross-contamination when hands are poorly washed and paws have a direct contact to domestic surfaces. Food related problems arise when antimicrobials are used to treat infections and resistance is developed. In animals as in humans misuse of antibiotics may not only cause an increase of resistance in pathogenic bateria, but also in the endogenous flora of these animals. Resistant bacteria from these animals may be transferred to the human population, not only by direct contact, but also through food products of animal origin. These resistant bacteria may then either colonise humans and/or transfer their resistance genes to other bacteria in the human intestinal flora (van den Bogaard and Stobberingh, 2000).

At this time, it is well acknowledged that several antimicrobial resistant bacteria isolated from humans originated mainly from animals raised for human consumption (Aarestrups, 2000) and that such resistant bacteria may contaminate the meat derived from those animals (Sáenz *et al.*, 2001). As a result, development of antimicrobial resistance amongst bacterial isolates from animal supplies can represent potential hazards to consumers through foodborne infections caused by these bacteria. In the past years, several studies have reported the antimicrobial resistance of some *Enterobacteriaceae* genera isolated from poultry, such as *E. coli* and *Salmonella* spp. (Antunes *et al.*, 2003; Cormican *et*

al., 2001; Guerra *et al.*, 2003; Kijima-Tanaka *et al.*, 2003; Sáenz *et al.*, 2001; van den Bogaard *et al.*, 2001).

Enterobacteriaceae family, a group containing some highly pathogenic Gram negative organisms, is universally used as an indicator of faecal contamination during food microbiology analyses and it includes zoonotic bacteria like *Salmonella* and *E. coli*. These microorganisms may cause severe infections and are becoming gradually more resistant to the generally used treatment antibiotics like tetracyclines, aminoglycosides, trimethoprim, fluoroquinolones and chloramphenicol (Paterson 2006), demonstrating multiple resistance and declining activity of several antibiotic groups such as the fluoroquinolones (Rhomberg et al., 2006).

According to **Table 3**, in this study *Enterobacteriacea*e strains were found to be resistant to ampicillin (28.3%), trimethoprim (1.7%) and nitrofurantoin (5.0%). However, all strains were also sensitive to ciprofloxacin, gentamicin and nalidixic acid although resistance to Chloramphenicol and Tetracycline was practically non-existent (95 and 98.3% of sensitive strains, respectively).

Resistance of *E. coli* was found to ampicillin (41.3%), chloramphenicol (4.3%), tetracycline (6.5%), nalidixic acid (6.5%) and nitrofurantoin (4.3%). Nevertheless all strains showed sensitivity to ciprofloxacin, gentamicin and trimethoprim.

Among *Salmonella* spp. isolates, 26.3% were resistant to ampicillin, 5.3% to chloramphenicol, 10.5% to tetracycline, 5.3% to nalidixic acid and 15.8% to nitrofurantoin. Ciprofloxacin, gentamicin and trimethoprim were shown to be very effective with 100% of sensitivity detected.

For all eight antimicrobials tested, overall no resistance was found to ciprofloxacin and gentamicin and for all 3 major groups resistance to ampicillin was common with a top score distinguished for *E. coli* with 41.3% of strains resistant, followed by *Enterobacteriaceae* with 28.3% and *Salmonella* spp. with 26.3%. For nitrofurantoin 15.8% of *Salmonella* spp. strains were resistant, followed by *Enterobacteriaceae* spp. with 5.0% and *E. coli* with 4.3%. This is in accordance with a study carried out by Osterblad *et al.* (1999) where antimicrobial sensitivity was shown in *Enterobacteriaceae* isolated from vegetables.

For example, urinary tract infections is a common illness that afects both community and hospital patients and is often caused by *E. coli* which is naturally susceptible to ampicillin even though about 50 - 60% of isolates are now resistant worlwide (Wu *et al.*, 1992; Chomarat, 2000; Sefton, 2000; Gupta, 2001).

For more than 50 years nitrofurantoin has been an option for the management of urinary tract infection but its use declined with the introduction of alternative antimicrobials, like trimethoprim, although there has been a slow reappearance in its use because of continued low rates of resistance among common urologic pathogens (Hooton and Stamm, 1997). Nowadays, the only indication for nitrofurantoin is the management of bladder infection resulting from susceptible strains of *E. coli*, once it appears to be associated with lower cure rates (approximately 85%) than other first line agents (90% to 95%), like trimethoprim (Warren *et al.*, 1999). In the present study, isolates showed low levels of resistance to nitrofurantoin (15.8%, 5.0% and 4.3% for *Salmonella* spp., *Enterobacteriaceae* and *E. coli* samples,

respectively). A study conducted by Rampling *et al.*, (1990) stated that *Salmonella* Enteritidis, isolated from poultry and from human enteric infection in the United Kingdom, showed high resistance rates which can be explained by the use of nitrofurans in the poultry industry.

Trimethoprim has been the core of therapy for urinary tract infection for the past many years with a 90% success rate as first-line agent indicated for the supervision of acute urinary tract infection and pyelonephritis (Hooton *et al.*, 1995). It is very effective against most *Enterobacteriaceae* like *E. coli*, *Klebsiella* species, *Enterobacter* species, *Morganella morganii*, *Proteus mirabilis* and *Proteus vulgaris* although there has been a significant increase in the prevalence of resistance of *E coli*.

Enterobacteriaceae family has shown worldwide trimethoprim resistance in chicken, pork, fish and even water (Tao *et al.*, 2010; Su *et al.*, 2011; Schwaiger *et al.*, 2012). Generally *E. coli* and *Salmonella* spp. present higher resistance rates although this is not what was found in this study. Domestic environment *Enterobacteriaceae* showed a very low rate of resistance (1.7%) while *E. coli* and *Salmonella* spp. strains were all sensitive to this kind of antimicrobial.

In some countries *Salmonella* and *E. coli* antibiotic resistance rates are reported to be high (Oppegaard *et al.*, 2001; Ronald, 2002; Threlfall, 2002). In a study with 752 *E. coli* isolates from human and animal agriculture sources in several countries, tetracycline high frequency resistance rates were found in isolates from human and turkey samples (56% and 71%, respectively). The resistance profiles for cattle, chicken, and swine were similar with approximately 47% of cattle isolates resistant to tetracycline (Schroeder *et al.*, 2002). This can be explained by the fact that tetracycline is the drug most often used in animal husbandry and is only a drug of second choice in human medicine (Mayrhofer *et al.*, 2004). In another study performed by Schroeder *et al.* (2003) samples taken from retail beef, chicken, pork, and turkey resulted in 472 *E. coli* isolates, 59% of which were resistant to tetracycline, nalidixic acid (8%), and chloramphenicol (6%).

In contrast with these results, domestic isolates showed comparatively low rates of resistance to tetracycline with 6.5% and 10.5% for *E. coli* and *Salmonella* spp., respectively. All other *Enterobacteriaceae* were sensitive to this antimicrobial (98.3%). Although resistance rates related to nalidixic acid (6.5% for *E. coli* and 5.3% for *Salmonella* spp.) and chloramphenicol (4.3% for *E. coli* and 5.3% for *Salmonella* spp.) were much lower, this is in conformity with Schroeder *et al.* (2003) study.

In our study no resistance was determined for gentamicin or ciprofloxacin. This is in agreement with several studies but antimicrobial resistance can be modified depending on the nature of the food production system considered (Osterblad *et al.*, 1999; van den Bogaard *et al.*, 2000; Bywater *et al.*, 2004; Fluckey *et al.*, 2007; Miranda *et al.*, 2008; Knezeviz and Petrovic, 2008).

In an antimicrobial susceptibility study of *Enterobacteriacea*e isolated from vegetables, no resistance was found to nalidixic acid but tetracycline and chloramphenicol showed low resistance rates (5.5 and 12%, respectively) (Osterblad *et al.*, 1999). This is consistent with some other antibiotic resistance studies where *Enterobacteriaceae* were isolated from milk, cheese and other dairy products. Isolates from milk products presented no resistance to nalidixic acid but some resistance was detected for tetracycline (14.28%) and chloramphenicol (9.52%). Among samples of cheese, 24% of isolates were

resistant to tetracycline but no resistance was found to chloramphenicol and nalidixic acid. Dairy products showed high rates of resistance to tetracycline (52.38%), but also no resistance was found to chloramphenicol and nalidixic acid (Hleba *et al.*, 2011).

In our study nalidixic acid, chloramphenicol and tetracycline were shown to be effective against *Enterobacteriaceae* since no resistance was found.

Several studies reported *Salmonella* and *E. coli* food-related isolates showed resistance to trimethoprim and some declare that this resistance profile is due to treatments used in animal medicine (van den Bogaard *et al.*, 2000; Cormican *et al.*, 2001; Sáenz *et al.*, 2001; van den Bogaard *et al.*, 2001; Kijima-Tanaka *et al.*, 2003; Bywater *et al.*, 2004; Fluckey *et al.*, 2007). Although some studies also stated food-related isolates showed sensitivity to trimethoprim (Lundin *et al.*, 2008; Erdington *et al.*, 2009) which is in accordance with this study where all *Salmonella* and *E. coli* strains were sensitive to this antibiotic.

Another interesting way of analysing our results was to organize antibiotic resistance rates by house (**Table 4 to 6** - see pag. 26 to 28) and surface (**Table 7 to 9** - see pag. 29 to 31).

From data in **Table 4** it can be concluded that although other *Enterobacteriaceae* are not present in one house they are widely spread in all the others. Several antibiotic resistant strains were detected, mainly to ampicillin (House 1, 6, 9, 10, 11, 12 and 15) and ranging from 25.0% of isolates in House 9 to 66.7% in House 12, and nitrofurantoin (House 3, 4, 8, 12 and 15), ranging from 16.7% in House 12 to 50.0% in House 3. Only one strain showed trimethoprim resistance in House 1 (9.1%). All isolates were sensitive to ciprofloxacin, gentamicin and nalidixic acid although chloramphenicol and tetracycline present some intermediary isolates.

In conclusion, resistance to more than one antibiotic is verified in *Enterobacteriaceae* isolates from House 1 (ampicillin and trimethoprim), House 12 and House 15 (ampicillin and nitrofurantoin, in both cases). However, isolates from House 5 and House 13 showed no resistance to all 8 antibiotics tested.

In **Table 5** it can be seen that not all houses are contaminated with *E. coli* which is good news since some strains of these bacteria are considered a severe faecal pathogen. Once more strains showed resistance to ampicillin (House 3, 7, 9, 10, 13, 14 and 15) and nitrofurantoin (House 4 and 15) with rates ranging from 20.0% in House 10 to 100% in House 3 and 8.3% in House 15 to 50.0% in House 4, respectively. Moreover in House 13, 50.0% strains were found to be resistant to chloramphenicol, 75.0% to nalidixic acid and another 50.0% to tetracycline. Isolates from House 15 also showed some extra resistance to tetracycline although in low proportion (1 in 11 isolates). All *E. coli* domestic isolates showed no resistance to ciprofloxacin, gentamicin and trimethoprim.

In *E. coli* isolates resistance to more than one antibiotic was confirmed in House 13 (ampicillin, chloramphenicol, nalidixic acid and tetracycline) and House 15 (ampicillin, tetracycline and nitrofurantoin). Nevertheless, strains from House 5, House 8 and House 11 showed no resistance against all 8 antibiotic tested.

Table 6 presents *Salmonella* spp. resistance rates by house and it can can be seen that contamination is lower than for other bacteria presented above, since only 6 in 15 houses show contamination. In this

case isolates exhibit high resistance rates against ampicillin in House 2, 3, 4 and 11 (varying from 25.0% in House 3 to 100% in House 4) while strains were resistant to nitrofurantoin in House 1 (50.0%) and House 3 (25.0%). Some strains were resistant to chloramphenicol (12.5% in House 3), nalidixic acid (50.0% in House 11) and tetracycline (25.0% in House 3). Ciprofloxacin, gentamicin and trimethoprim are once more the most effective antibiotics with 100% sensitive strains.

In this case, *Salmonella* spp. isolates from two houses proved to be resistant to more than one antibiotic namely House 3 (ampicillin, chloramphenicol, tetracycline and nitrofurantoin) and House 11 (ampicillin and nalidixic acid). On the other hand, only House 7 showed no resistance for all 8 antibiotic tested.

Related to **Table 7** it can be seen that *Enterobacteriaceae* show no resistance to chloramphenicol, ciprofloxacin, gentamicin, nalidixic acid and tetracycline even though some strains show intermediary breakpoints for chloramphenicol (domestic animal and kitchen counter) and tetracycline (kitchen tap). Resistance rates were detected against ampicillin (ranging from 9.1% in kitchen cloth to 100% in WC tap) and nitrofurantoin (ranging from 18.2% in kitchen cloth to 50% in dishwasher handle).

In summary, only those *Enterobacteriaceae* isolates found in the kitchen cloth showed resistance to more than one antibiotic (ampicillin and nitrofurantoin) while the refrigerator handle isolates presented no resistance to all 8 antibiotics tested.

From the data in **Table 8**, no *E. coli* was detected in WC knob but in all 9 other surfaces it is widely distributed. High resistance to ampicillin was found 9 localities, not including domestic animals' feet. The kitchen cloth and dishwasher handle isolates presented resistance to chloramphenicol, nalidixic acid and tetracycline while nitrofurantoin resistance was found in the strains from the kitchen counter and dishwasher handle. No resistance was found to ciprofloxacin, gentamicin and trimethoprim.

In conclusion, *E. coli* isolates found in the kitchen cloth and dishwasher handle showed resistance to more than one antibiotic. Resistance to ampicillin (75.0%) and nitrofurantoin (50.0%) was detected in kitchen counter isolates, while those from the kitchen cloth demonstrated resistance to ampicillin (54.5%), chloramphenicol (9.1%), nalidixic acid (18.2%) and tetracycline (9.1%). The dishwasher handle isolates showed low resistance (16.7%) to 5 antibiotics namely ampicillin, chloramphenicol, nalidixic acid, tetracycline and nitrofurantoin. Only strains from domestic animals presented no resistance to all 8 antibiotics tested.

Analysing **Table 9** *Salmonella* spp. shows no isolates from the WC tap, kitchen knob and stove buttons. Resistance was detected for 5 of the 8 antibiotics detected with ampicillin taking the lead with high rates in isolates from kitchen tap (50.0%), cutting board (100%) and kitchen cloth (50.0%). Cutting board also showed evidence for resistance to chloramphenicol (50.0%), tetracycline (100%) and nitrofurantoin (100%) and only one strain (33.3) showed resistance to nalidixic acid (from the kitchen counter). No isolates were resistant to ciprofloxacin, gentamicin or trimethoprim.

Therefore, of the *Salmonella* spp. isolates, only those from the cutting board presented resistance to more than one antibiotic namely, ampicillin, chloramphenicol, tetracycline and nitrofurantoin. In contrast, the isolates from the WC knob, refrigerator and dishwasher handles revealed no resistance.

Overall, multi-resistance, to more than one antibiotic, was found in this study. Analysing **Annex 1**, where all the different antibiotic profiles are shown, in all 15 Houses, we can assume that diverse sources of enteric bacteria were found probably from different origins as animals, humans and/or foodstuff.

Table 3 - *In vitro* susceptibility of *Enterobacteriaceae* isolates to several antibiotics and minimum inhibitory concentration (MIC) breakpoints

		MIC (µg/mL) Breakpoints			Other *Enterobacteriaceae* isolates			*Escherichia coli* isolates			*Salmonella* spp. isolates		
Class	**Antibiotic**	**Sensitive (S)**	**Intermediary (I)**	**Resistant (R)**	**Number of sensitive isolates (%)**	**Number of intermediary isolates (%)**	**Number of resistant isolates (%)**	**Number of sensitive isolates (%)**	**Number of intermediary isolates (%)**	**Number of resistant isolates (%)**	**Number of sensitive isolates (%)**	**Number of intermediary isolates (%)**	**Number of resistant isolates (%)**
Penicillins	Ampicillin	≤ 8	16	≥ 32	37 (61.7)	6 (10.0)	17 (28.3)	21 (45.6)	6 (13.0)	19 (41.3)	10 (52.6)	4 (21.1)	5 (26.3)
Phenicols	Chloramphenicol	≤ 8	16	≥ 32	57 (95.0)	3 (5.0)		44 (95.7)		2 (4.3)	16 (84.2)	2 (10.5)	1 (5.3)
Fluoroquinolones	Ciprofloxacin	≤ 1	2	≥ 4	60 (100)			46 (100)			19 (100)		
Aminoglycosides	Gentamicin	≤ 4	8	≥ 16	60 (100)			46 (100)			19 (100)		
Tetracyclines	Tetracycline	≤ 4	8	≥ 16	59 (98.3)	1 (1.7)		42 (91.3)	1 (2.2)	3 (6.5)	13 (68.4)	4 (21.1)	2 (10.5)
Quinolones	Nalidixic Acid	≤ 16	---	≥ 32	60 (100)			43 (93.5)		3 (6.5)	18 (94.7)		1 (5.3)
Folate pathway inhibitor	Trimethoprim	≤ 8	---	≥ 16	59 (98.3)		1 (1.7)	46 (100)			19 (100)		
Nitrofurantoins	Nitrofurantoin	≤ 32	64	≥ 128	34 (56.7)	23 (38.3)	3 (5.0)	39 (84.8)	5 (10.9)	2 (4.3)	13 (68.4)	3 (15.8)	3 (15.8)

Blank spaces indicate that no MIC value was determined for that concentration.

Table 4 - *In vitro* susceptibility of other *Enterobacteriaceae* isolates to several antibiotics and minimum inhibitory concentration (MIC) breakpoints by house

Antibiotic	MIC (µg/mL) Breakpoints	Number (%) other *Enterobacteriaceae* isolates													
		House 1	House 3	House 4	House 5	House 6	House 7	House 8	House 9	House 10	House 11	House 12	House 13	House 14	House 15
Ampicillin	**Sensitive**	6 (54.5)	2 (100)	1 (33.3)	3 (100)	1 (25.0)	3 (100)	4 (100)	3 (75.0)	6 (60.0)	2 (66.7)	2 (33.3)	1 (100)	1 (50.0)	3 (50.0)
	Intermediary	1(9.1)		2 (66.7)		1 (25.0)								1 (50.0)	1 (16.7)
	Resistant	4 (36.4)				2 (50.0)			1 (25.0)	4 (40.0)	1 (33.3)	4 (66.7)			2 (33.3)
Chloramphenicol	**Sensitive**	11 (100)	2 (100)	3 (100)	3 (100)	3 (75.0)	3 (100)	4 (100)	4 (100)	10 (100)	3 (100)	4 (66.7)	1 (100)	2 (100)	6 (100)
	Intermediary					1 (25.0)						2 (33.3)			
	Resistant														
Ciprofloxacin	**Sensitive**	11 (100)	2 (100)	3 (100)	3 (100)	4 (100)	3 (100)	4 (100)	4 (100)	10 (100)	3 (100)	6 (100)	1 (100)	2 (100)	6 (100)
	Intermediary														
	Resistant														
Gentamicin	**Sensitive**	11 (100)	2 (100)	3 (100)	3 (100)	4 (100)	3 (100)	4 (100)	4 (100)	10 (100)	3 (100)	6 (100)	1 (100)	2 (100)	6 (100)
	Intermediary														
	Resistant														
Nalidixic Acid	**Sensitive**	11 (100)	2 (100)	3 (100)	3 (100)	4 (100)	3 (100)	4 (100)	4 (100)	10 (100)	3 (100)	6 (100)	1 (100)	2 (100)	6 (100)
	Intermediary														
	Resistant														
Tetracycline	**Sensitive**	11 (100)	1 (50.0)	2 (66.7)	3 (100)	4 (100)	3 (100)	4 (100)	4 (100)	8 (80.0)	3 (100)	6 (100)	1 (100)	2 (100)	6 (100)
	Intermediary		1 (50.0)	1 (33.3)						2 (20.0)					
	Resistant														
Trimethoprim	**Sensitive**	10 (90.9)	2 (100)	3 (100)	3 (100)	4 (100)	3 (100)	4 (100)	4 (100)	10 (100)	3 (100)	6 (100)	1 (100)	2 (100)	6 (100)
	Intermediary														
	Resistant	1 (9.1)													
Nitrofurantoin	**Sensitive**	5 (45.5)	1 (50.0)	1 (33.3)	1 (33.3)	2 (50.0)	1 (33.3)	2 (50.0)	1 (25.0)	10 (100)	1 (33.3)	2 (33.3)	1 (100)	2 (100)	5 (83.3)
	Intermediary	6 (54.5)		1 (33.3)	2 (66.7)	2 (50.0)	2 (66.7)	1 (25.0)	3 (75.0)		2 (66.7)	3 (50.0)			
	Resistant		1 (50.0)	1 (33.3)				1 (25.0)				1 (16.7)			1 (16.7)

Blank spaces indicate that no MIC value was determined for that concentration.
Note: No other *Enterobacteriaceae* isolates were detected in House 2 therefore no results were here included.

Table 5 - *In vitro* susceptibility of *Escherichia coli* isolates to several antibiotics and minimum inhibitory concentration (MIC) breakpoints by house

		Number (%) *Escherichia coli* isolates										
Antibiotic	MIC (µg/mL) Breakpoints	House 3	House 4	House 5	House 7	House 8	House 9	House 10	House 11	House 13	House 14	House 15
Ampicillin	**Sensitive**		1 (50.0)	3 (75.0)		4 (100)		3 (60.0)		2 (50.0)		9 (75.0)
	Intermediary		1 (50.0)	1 (25.0)	1 (33.3)			1 (20.0)	3 (100)			
	Resistant	2 (100)			2 (66.7)		2 (100)	1 (20.0)		2 (50.0)	3 (100)	3 (25.0)
Chloramphenicol	**Sensitive**	2 (100)	2 (100)	4 (100)	3 (100)	4 (100)	2 (100)	5 (100)	3 (100)	2 (50.0)	3 (100)	12 (100)
	Intermediary											
	Resistant									2 (50.0)		
Ciprofloxacin	**Sensitive**	2 (100)	2 (100)	4 (100)	3 (100)	4 (100)	2 (100)	5 (100)	3 (100)	4 (100)	3 (100)	12 (100)
	Intermediary											
	Resistant											
Gentamicin	**Sensitive**	2 (100)	2 (100)	4 (100)	3 (100)	4 (100)	2 (100)	5 (100)	3 (100)	4 (100)	3 (100)	12 (100)
	Intermediary											
	Resistant											
Nalidixic Acid	**Sensitive**	2 (100)	2 (100)	4 (100)	3 (100)	4 (100)	2 (100)	5 (100)	3 (100)	1 (25.0)	3 (100)	12 (100)
	Intermediary											
	Resistant									3 (75.0)		
Tetracycline	**Sensitive**	2 (100)	1 (50.0)	4 (100)	3 (100)	4 (100)	2 (100)	5 (100)	3 (100)	2 (50.0)	3 (100)	11 (91.7)
	Intermediary		1 (50.0)									
	Resistant									2 (50.0)		1 (8.3)
Trimethoprim	**Sensitive**	2 (100)	2 (100)	4 (100)	3 (100)	4 (100)	2 (100)	5 (100)	3 (100)	4 (100)	3 (100)	12 (100)
	Intermediary											
	Resistant											
Nitrofurantoin	**Sensitive**	2 (100)		3 (75.0)	3 (100)	3 (75.0)	2 (100)	5 (100)		4 (100)	3 (100)	11 (91.7)
	Intermediary		1 (50.0)	1 (25.0)		1 (25.0)			3 (100)			
	Resistant		1 (50.0)									1 (8.3)

Blank spaces indicate that no MIC value was determined for that concentration.
Note: No *Escherichia coli* isolates were detected in House 1, 2, 6 and 12, therefore no results were here included.

Table 6 - *In vitro* susceptibility of *Salmonella* spp. isolates to several antibiotics and minimum inhibitory concentration (MIC) breakpoints by house

		Number (%) *Salmonella* spp. isolates					
Antibiotic	MIC (µg/mL) Breakpoints	House 1	House 2	House 3	House 4	House 7	House 11
Ampicillin	**Sensitive**	2 (100)	2 (50.0)	4 (50.0)		1 (50.0)	1 (50.0)
	Intermediary		1 (25.0)	2 (25.0)		1 (50.0)	
	Resistant		1 (25.0)	2 (25.0)	1 (100)		1 (50.0)
Chloramphenicol	**Sensitive**	2 (100)	3 (75.0)	6 (75.0)	1 (100)	2 (100)	2 (100)
	Intermediary		1 (25.0)	1 (12.5)			
	Resistant			1(12.5)			
Ciprofloxacin	**Sensitive**	2 (100)	4 (100)	8 (100)	1 (100)	2 (100)	2 (100)
	Intermediary						
	Resistant						
Gentamicin	**Sensitive**	2 (100)	4 (100)	8 (100)	1 (100)	2 (100)	2 (100)
	Intermediary						
	Resistant						
Nalidixic Acid	**Sensitive**	2 (100)	4 (100)	8 (100)	1 (100)	2 (100)	1 (50.0)
	Intermediary						
	Resistant						1 (50.0)
Tetracycline	**Sensitive**		3 (75.0)	6 (75.0)		2 (100)	2 (100)
	Intermediary	2 (100)	1 (25.0)		1 (100)		
	Resistant			2 (25.0)			
Trimethoprim	**Sensitive**	2 (100)	4 (100)	8 (100)	1 (100)	2 (100)	2 (100)
	Intermediary						
	Resistant						
Nitrofurantoin	**Sensitive**		4 (100)	6 (75.0)		1 (50.0)	2 (100)
	Intermediary	1 (50.0)			1 (100)	1 (50.0)	
	Resistant	1 (50.0)		2 (25.0)			

Blank spaces indicate that no MIC value was determined for that concentration.
Note: No *Salmonella* spp. isolates were detected in House 5, 6, 8, 9, 10, 12, 13, 14 and 15, therefore no results were here included.

Table 7 - *In vitro* susceptibility of other *Enterobacteriaceae* isolates to several antibiotics and minimum inhibitory concentration (MIC) breakpoints, by surface

Antibiotic	MIC (µg/mL) Breakpoints	Number (%) *Enterobacteriaceae* isolates										
		Domestic Animal	WC Knob	WC Tap	Kitchen Knob	Kitchen Tap	Stove Buttons	Kitchen Counter	Cutting Board	Kitchen Cloth	Refrigerator Handle	Dishwasher Handle
Ampicillin	**Sensitive**	5 (62.5)	4 (100)		3 (75.0)	4 (50.0)	2 (40.0)	5 (50.0)	1 (25.0)	10 (90.9)	3 (75.0)	1 (50.0)
	Intermediary	3 (37.5)				2 (25.0)	1 (20.0)				1 (25.0)	1 (50.0)
	Resistant			2 (100)	1 (25.0)	2 (25.0)	2 (40.0)	5 (50.0)	3 (75.0)	1 (9.1)		
Chloramphenicol	**Sensitive**	7 (87.5)	4 (100)	2 (100)	4 (100)	8 (100)	6 (100)	8 (80.0)	4 (100)	11 (100)	4 (100)	2 (100)
	Intermediary	1 (12.5)						2 (20.0)				
	Resistant											
Ciprofloxacin	**Sensitive**	8 (100)	4 (100)	2 (100)	4 (100)	8 (100)	6 (100)	10 (100)	4 (100)	11 (100)	4 (100)	2 (100)
	Intermediary											
	Resistant											
Gentamicin	**Sensitive**	8 (100)	4 (100)	2 (100)	4 (100)	8 (100)	6 (100)	10 (100)	4 (100)	11 (100)	4 (100)	2 (100)
	Intermediary											
	Resistant											
Nalidixic Acid	**Sensitive**	8 (100)	4 (100)	2 (100)	4 (100)	8 (100)	6 (100)	10 (100)	4 (100)	11 (100)	4 (100)	2 (100)
	Intermediary											
	Resistant											
Tetracycline	**Sensitive**	8 (100)	4 (100)	2 (100)	4 (100)	7 (87.5)	6 (100)	10 (100)	4 (100)	11 (100)	4 (100)	2 (100)
	Intermediary					1 (12.5)						
	Resistant											
Trimethoprim	**Sensitive**	7 (87.5)	4 (100)	2 (100)	4 (100)	8 (100)	6 (100)	10 (100)	4 (100)	11 (100)	4 (100)	2 (100)
	Intermediary											
	Resistant	1 (12.5)										
Nitrofurantoin	**Sensitive**	3 (37.5)		2 (100)	4 (100)	6 (75.0)	6 (100)	5 (50.0)	2 (50.0)	4 (36.4)	4 (100)	1 (50.0)
	Intermediary	5 (62.5)	3 (75.0)			2 (25.0)		5 (50.0)	2 (50.0)	5 (45.4)		
	Resistant		1 (25.0)							2 (18.2)		1 (50.0)

Blank spaces indicate that no MIC value was determined for that concentration.

Table 8 - *In vitro* susceptibility of *Escherichia coli* isolates to several antibiotics and minimum inhibitory concentration (MIC) breakpoints, by surface

Antibiotic	MIC (µg/mL) Breakpoints	Number (%) *Escherichia coli* isolates									
		Domestic Animal	WC Tap	Kitchen Knob	Kitchen Tap	Stove Buttons	Kitchen Counter	Cutting Board	Kitchen Cloth	Refrigerator Handle	Dishwasher Handle
Ampicillin	**Sensitive**	2 (100)	2 (50.0)	2 (50.0)	2 (50.0)		1 (25.0)	2 (33.3)	3 (27.3)	1 (50.0)	5 (83.3)
	Intermediary		1 (25.0)		1 (25.0)			2 (33.3)	2 (18.2)		
	Resistant		1 (25.0)	2 (50.0)	1 (25.0)	1 (100)	3 (75.0)	2 (33.3)	6 (54.5)	1 (50.0)	1 (16.7)
Chloramphenicol	**Sensitive**	2 (100)	4 (100)	4 (100)	4 (100)	1 (100)	4 (100)	6 (100)	10 (90.9)	2 (100)	5 (83.3)
	Intermediary										
	Resistant								1 (9.1)		1 (16.7)
Ciprofloxacin	**Sensitive**	2 (100)	4 (100)	4 (100)	4 (100)	1 (100)	4 (100)	6 (100)	11 (100)	2 (100)	6 (100)
	Intermediary										
	Resistant										
Gentamicin	**Sensitive**	2 (100)	4 (100)	4 (100)	4 (100)	1 (100)	4 (100)	6 (100)	11 (100)	2 (100)	6 (100)
	Intermediary										
	Resistant										
Nalidixic Acid	**Sensitive**	2 (100)	4 (100)	4 (100)	4 (100)	1 (100)	4 (100)	6 (100)	9 (81.8)	2 (100)	5 (83.3)
	Intermediary										
	Resistant								2 (18.2)		1 (16.7)
Tetracycline	**Sensitive**	2 (100)	4 (100)	4 (100)	4 (100)	1 (100)	4 (100)	6 (100)	10 (90.9)	2 (100)	5 (83.3)
	Intermediary										
	Resistant								1 (9.1)		1 (16.7)
Trimethoprim	**Sensitive**	2 (100)	4 (100)	4 (100)	4 (100)	1 (100)	4 (100)	6 (100)	11 (100)	2 (100)	6 (100)
	Intermediary										
	Resistant										
Nitrofurantoin	**Sensitive**	2 (100)	4 (100)	4 (100)	4 (100)	1 (100)	1 (25.0)	4 (66.7)	9 (81.8)	2 (100)	5 (83.3)
	Intermediary						1 (25.0)	2 (33.3)	2 (18.2)		
	Resistant						2 (50.0)				1 (16.7)

Blank spaces indicate that no MIC value was determined for that concentration.
Note: No *Escherichia coli* isolates were detected in WC knob, therefore no results were here included.

Table 9 - *In vitro* susceptibility of *Salmonella* spp. isolates to several antibiotics and minimum inhibitory concentration (MIC) breakpoints, by surface

Antibiotic	MIC (µg/mL) Breakpoints	Number (%) *Salmonella* spp. isolates							
		Domestic Animal	WC Knob	Kitchen Tap	Kitchen Counter	Cutting Board	Kitchen Cloth	Refrigerator Handle	Dishwasher Handle
Ampicillin	**Sensitive**	3 (75.0)	2 (100)	1 (25.0)	1 (33.3)		1 (50.0)		1 (100)
	Intermediary	1 (25.0)		1 (25.0)	2 (66.7)			1 (100)	
	Resistant			2 (50.0)		2 (100)	1 (50.0)		
Chloramphenicol	**Sensitive**	4 (100)	2 (100)	4 (100)	3 (100)		1 (50.0)	1 (100)	1 (100)
	Intermediary					1 (50.0)	1 (50.0)		
	Resistant					1 (50.0)			
Ciprofloxacin	**Sensitive**	4 (100)	2 (100)	4 (100)	3 (100)	2 (100)	2 (100)	1 (100)	1 (100)
	Intermediary								
	Resistant								
Gentamicin	**Sensitive**	4 (100)	2 (100)	4 (100)	3 (100)	2 (100)	2 (100)	1 (100)	1 (100)
	Intermediary								
	Resistant								
Nalidixic Acid	**Sensitive**	4 (100)	2 (100)	4 (100)	2 (66.7)	2 (100)	2 (100)	1 (100)	1 (100)
	Intermediary								
	Resistant				1 (33.3)				
Tetracycline	**Sensitive**	4 (100)	2 (100)	3 (75.0)	3 (100)		2 (100)	1 (100)	1 (100)
	Intermediary			1 (25.0)					
	Resistant					2 (100)			
Trimethoprim	**Sensitive**	4 (100)	2 (100)	4 (100)	3 (100)	2 (100)	2 (100)	1 (100)	1 (100)
	Intermediary								
	Resistant								
Nitrofurantoin	**Sensitive**	2 (50.0)	2 (100)	3 (75.0)	3 (100)		2 (100)	1 (100)	1 (100)
	Intermediary	1 (25.0)		1 (25.0)					
	Resistant	1 (25.0)				2 (100)			

Blank spaces indicate that no MIC value was determined for that concentration.
Note: No *Escherichia coli* isolates were detected in WC tap, kitchen knob and stove buttons, therefore no results were here included.

3.7. Domestic survey

A survey (**Table 11**) was designed and some questions related with food safety and cleaning habits were administered to the persons responsible for the housework in each house, in attempts to correlate the microbiological results obtained for each domestic environment with the answers to the questionnaire applied at each house.

Demographic data including gender, age and educational background are given in **Table 10**. This survey constitutes a high number of female respondents (93.3%) and only one man was responsible for housework (House 1). This ratio of female participants to male participants reflects the ratio of people responsible for housework and preparing food in Portugal. Related to age group we can see that the most predominant group refers to people between 51 and 60 years old (33.3%) followed closely by those of more than 61 years of age (26.6%). Education rates show that 5 in 15 respondents have primary instruction while 4 in 15 have a university degree. This may have great importance since the contamination found in each house was not always in accordance with the answers given.

Table 10 - Descriptive characteristics of the respondents

House	Demographic characteristics		
	Gender	**Age Group**	**Education**
1	Male	≥ 61	University
2	Female	≥ 61	Technical course
3	Female	41-50	Primary
4	Female	31-40	University
5	Female	≥ 61	Primary
6	Female	41-50	University
7	Female	51-60	Primary
8	Female	31-40	University
9	Female	51-60	Primary
10	Female	51-60	Primary
11	Female	51-60	High school
12	Female	41-50	Junior high school
13	Female	51-60	Junior high school
14	Female	≥ 61	High school
15	Female	18-20	Technical course

Table 11 - Consumers cleaning habits and food safety knowledge in each house

House	Domestic Animal	Hand washing	Door knob	Taps	Kitchen counter	Stove buttons	Dishwasher handle	Refrigerator handle	Kitchen cloth	Cutting board	Food safety knowledge
1	Inside and out within kitchen	When using WC, handling raw, cooked or ready to eat products	No specific frequency with water and detergent	After using with detergent	After using with detergent	After using with detergent	No specific frequency with detergent	No specific frequency with water, detergent and disinfectant	Cloth, "Vileda" type and sponge	Separated for meat and fish	Yes
2	Inside and out within kitchen	When using WC, handling raw, cooked or ready to eat products	Every day with detergent	Every day with disinfectant	After using with disinfectant	After using with detergent	Every day with detergent	Every day with water	Cloth	One board only	Yes
3	Inside and out within kitchen	When using WC, handling raw, cooked or ready to eat products	No specific frequency with water and detergent	After using with water and detergent	After using with water and detergent	After using with water and detergent	N.A.**	Once a week with water and detergent	Cloth, "Vileda" type and sponge	One board only	No
4	Inside and out within kitchen	When using WC, handling raw, cooked or ready to eat products	No specific frequency with detergent	Every day with detergent	After using with detergent	Once a week with detergent	No specific frequency with detergent and disinfectant	No specific frequency with detergent	Cloth and sponge	One board only	Yes
5	Inside and out within kitchen	When using WC, handling raw, cooked or ready to eat products	Once a week with water	Every day with detergent	After using with detergent and disinfectant	N.A.*	Every day with water	Once a week with water and vinegar	"Vileda" type	Separated for meat, fish, vegetables, cooked food and bread	No
6	Inside and out within kitchen	When using WC, handling raw, cooked or ready to eat products	No specific frequency with detergent	After using with detergent	After using with detergent	After using with detergent	No specific frequency with detergent	No specific frequency with detergent	Cloth, "Vileda" type and sponge	One board only	Yes
7	Inside and out within kitchen	When using WC products	Once a week with disinfectant	Every day with detergent	Every day with detergent	N.A.*	Once a month with disinfectant	Once a month with disinfectant	Cloth	One board only	Yes
8	Inside and out within kitchen	When using WC, handling raw, cooked or ready to eat products	Once a month with detergent	Once a month with disinfectant	Once a week with disinfectant	Once a week with detergent	Once a week with disinfectant	Once a week with disinfectant	"Vileda" type	One board only	Yes

N.A. - House without domestic animal.
N.A.* - House with ceramic hob.
N.A.** - House without dishwasher.

Table 11 (Continuation) - Consumers cleaning habits and food safety knowledge in each house

House	Domestic Animal	Hand washing	Door knob	Taps	Kitchen counter	Stove buttons	Dishwasher handle	Refrigerator handle	Kitchen cloth	Cutting board	Food safety knowledge
9	Inside and out within kitchen	When using WC, handling raw, cooked or ready to eat products and when she feels the need	Never	Once a week with water and detergent	After using with water and detergent	After using with water and detergent	N.A.**	Once a week with water and detergent	Cloth and "Vileda" type	One board only	No
10	Inside and out within kitchen	When using WC, handling raw, cooked or ready to eat products	Once a week with water and detergent	After using with water and detergent	After using with water and detergent	After using with water and detergent	N.A.**	Once a week with water and detergent	Cloth and "Vileda" type	One board only	Yes
11	Inside and out within kitchen	When using WC, handling raw, cooked or ready to eat products and when she feels the need	3 in 3 months with detergent	Every day with disinfectant	After using with disinfectant	After using with disinfectant	N.A.**	Once a week with disinfectant	"Vileda" type	One board only	Yes
12	Inside and out within kitchen	When using WC, handling raw, cooked or ready to eat products	Once a week with disinfectant	Every day with disinfectant	Every day with disinfectant	After using with detergent	Once a month with disinfectant	Every day with disinfectant	"Vileda" type	One board only	Yes
13	Inside and out within kitchen	When using WC and handling ready to eat products	Once a week with disinfectant	Every day with detergent	After using with detergent	N.A.*	Every day with detergent	Once a week with detergent	"Vileda" type	One board only	Yes
14	N.A.	When using WC, handling cooked or ready to eat products	Once a week with detergent	After using with detergent	After using with detergent	After using with disinfectant	N.A.**	Once a week with disinfectant	Sponge	Separated for cooked and raw food	Yes
15	Inside and out within kitchen	When using WC and handling raw products	Once a month with detergent	Every day with detergent	After using with detergent	After using with detergent	Every day with detergent	Every day with detergent	Cloth	One board only	No

N.A. - House without domestic animal.
N.A.* - House with ceramic hob.
N.A.** - House without dishwasher.

Figures 5 to 8 exhibit foodborne pathogens found in the domestic setting for each house and in order to achieve some conclusions each house will be analysed separately with regard to the results of the questionnaire.

In **House 1**, no *E. coli* contamination was found and coagulase-positive *Staphylococcus* count showed levels lower than 10^2 CFU/swab. *L. seeligeri* was found in WC tap and knob while *Enterobacteriaceae* was detected in domestic animals' feet, cutting board, kitchen counter and kitchen cloth. Concerning antibiotic resistance, *Enterobacteriaceae* strains from domestic animal showed resistance to trimethoprim (MIC > 128 µg/mL), to ampicillin from cutting board (MIC of 64 µg/mL) and kitchen counter (MIC of 32 µg/mL). One *Salmonella* isolate from domestic animal presented resistance to nitrofurantoin (MIC of 128 µg/mL).
Concerning the survey, and comparing with food pathogens presence, we can conclude that domestic animals which are usually in the kitchen area may increase the contamination hazard to surfaces and food. Cleaning the kitchen counter with detergent after use doesn't seem to be effective and the presence of antibiotic-resistant *Enterobacteriaceae* strains on the cutting board may point to incorrect cleaning/disinfection.

Within **House 2**, no *E. coli* or *Listeria* spp. contamination was found and coagulase-positive *Staphylococcus* count showed levels lower than 10^2 CFU/swab. *Enterobacteriaceae* (*Salmonella* spp.) was detected in kitchen tap and kitchen cloth. Concerning antibiotic resistance, *Salmonella* strains showed high resistance to ampicillin only from the kitchen cloth (MIC of 256 µg/mL).
Concerning the survey, and comparing with food pathogens presence, we can conclude that in this house the problem lies in the kitchen towel material used (cloth) which can shelter numerous bacteria.

At **House 3**, *Listeria* spp. was absent and low rates (< 10^2 CFU/swab) of coagulase-positive *Staphylococcus* and *E. coli* were found. Several enterobacteria isolates were found on domestic animal, WC knob, Kitchen tap and counter, cutting board and kitchen cloth. Relating to antibiotic resistance, *Salmonella* strains from the cutting board, presented multiple resistance, specifically to ampicillin (MIC of 64 µg/mL), chloramphenicol (MIC of 32 µg/mL), tetracycline (MIC of 1286 µg/mL) and nitrofurantoin (MIC of 128 µg/mL). *Enterobacteriaceae* isolated from kitchen tap showed nitrofurantoin resistance (MIC of 64 µg/mL) and ampicillin resistance was detected in *E. coli* isolated from the kitchen cloth (MIC of 32 µg/mL).
With reference to the survey, multi-resistant *Salmonella* found in the single cutting board used seems to be the problem in this house.

House 4, also no *Listeria* was found, low rates (< 10^2 CFU/swab) of coagulase-positive *Staphylococcus* were found and *E. coli* were found on the kitchen counter (10^3 CFU/swab). Kitchen tap, counter, refrigerator and dishwasher handle were found to be highly contaminated with *Enterobacteriaceae* (10^3 to 10^4 CFU/swab). Ampicillin resistance was established in *Salmonella* from the kitchen tap (MIC of 32 µg/mL) and *Enterobacteriacea*e found on the dishwasher handle were

resistant to nitrofurantoin (MIC of 128 µg/mL). Resistance to ampicillin and nitrofurantoin were detected in *E. coli* isolated from the kitchen counter (MIC of 256 µg/mL and MIC of 128 µg/mL, respectively).
Concerning the domestic survey, special attention was given to *E. coli* found on the counter, usually cleaned after use with detergent, which doesn't seem to be effective in eradication of bacteria. Also taps that were declared to be cleaned every day with detergent presented *Salmonell*a contamination. Related to dishwasher handle no specific frequency in cleaning may explain the presence of enteric bacteria.

In **House 5,** low rates (< 10^2 CFU/swab) of coagulase-positive *Staphylococcus* were found, kitchen counter and dishwasher handle presented *E. coli* contamination (10^2 to 10^3 CFU/swab) while no *Listeria* was found. Enterobacteria were identified on WC knob, kitchen counter, dishwasher handle and kitchen cloth but no resistance to antibiotics was determined.
Regarding the survey, although after using kitchen counter cleaning with detergent and disinfectant *E. coli* was detected as well as on the dishwasher handle which was stated to be cleaned every day with water. Apparently this kind of action showed no efficient results and more actions should be taken.

Inside **House 6**, high rates of coagulase-positive *Staphylococcus* were detected (10^2 to 10^5 CFU/swab) but no *E. coli* was detected. Concerning *Listeria* presence, in fact *L. innocua* and *L. grayi* were found in kitchen tap and kitchen counter, in that order. Domestic animal (10^4 CFU/swab) and kitchen tap (10^3 CFU/swab) proved to be contaminated with enterobacteria but only pets paws isolates showed resistance to ampicillin (MIC of 32 µg/mL).
The survey informed us that domestic animals that are usually in the kitchen area may represent a hazard due to cross-contamination while taps after use and cleaning with detergent also does not seem to be effective.

At **House 7**, *Listeria* spp. was found in domestic animals' feet while coagulase-positive *Staphylococcus* and *E. coli* were detected at low rates (< 10^2 CFU/swab). *Enterobacteriaceae* contamination was revealed to be high (10^2 to 10^5 CFU/swab) on the kitchen tap, refrigerator and dishwasher handles and kitchen cloth, although only ampicillin resistance was found in *E. coli* isolates from the kitchen tap (MIC of 64 µg/mL) and refrigerator handle (MIC of 128 µg/mL).
In relation to the questionnaire, taps daily cleaning with detergent and monthly disinfection of refrigerator and dishwasher handle doesn't seem to be effective or this kind of actions aren't really taken in to action.

For **House 8**, the kitchen counter proved to be highly contaminated with coagulase-positive *Staphylococcus* but no *Listeria* was found. WC and kitchen knobs presented contamination with *Enterobacteriaceae* while cutting board and kitchen cloth were contaminated with *E. coli* but none of them showed antibiotic resistance.
The survey demonstrated that monthly knob cleaning is not enough to eliminate these foodborne pathogens and that different cutting boards should be used for different kinds of food.

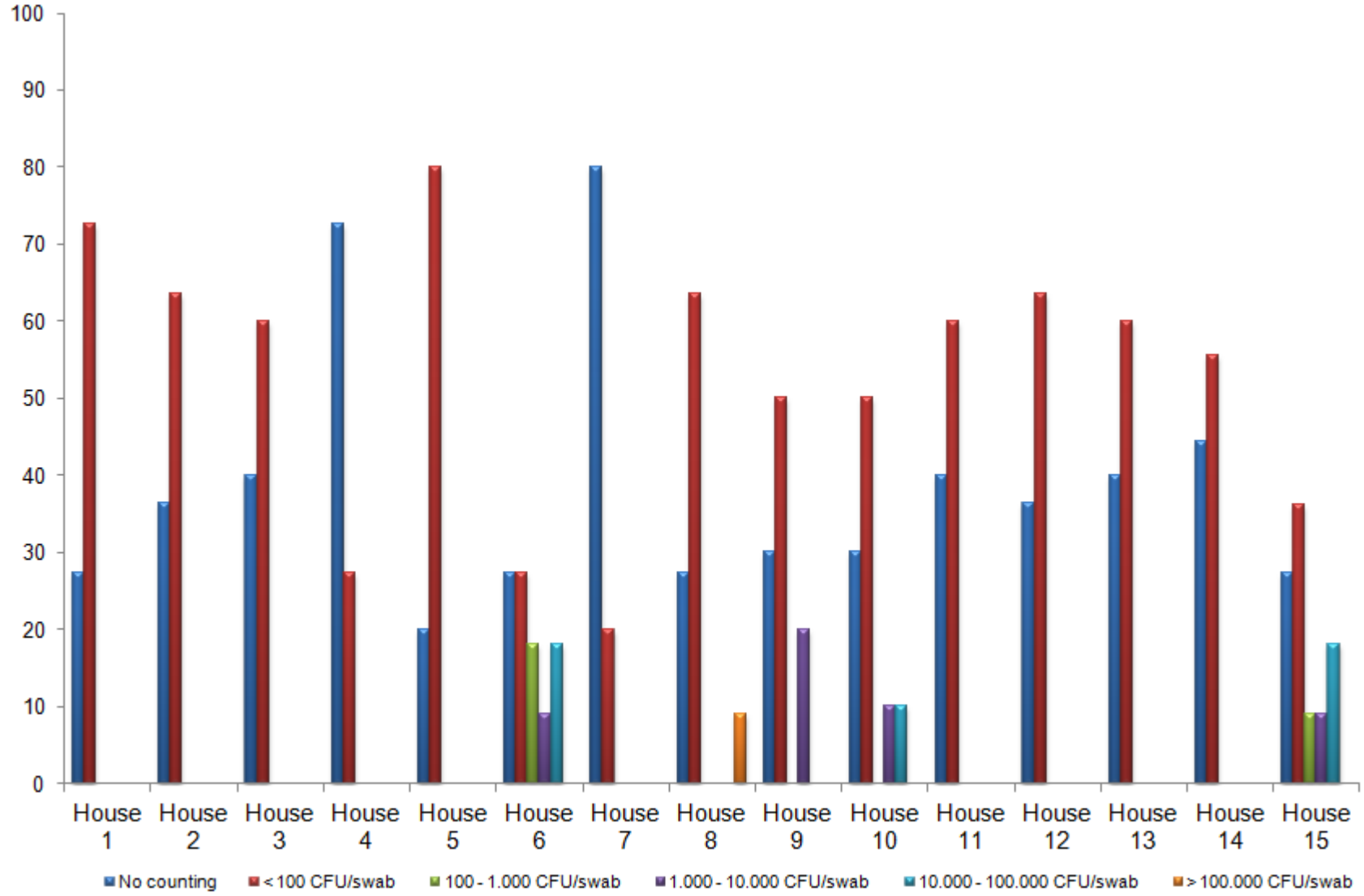


Figure 5 - Coagulase-positive *Staphylococcus* count (%) by house.

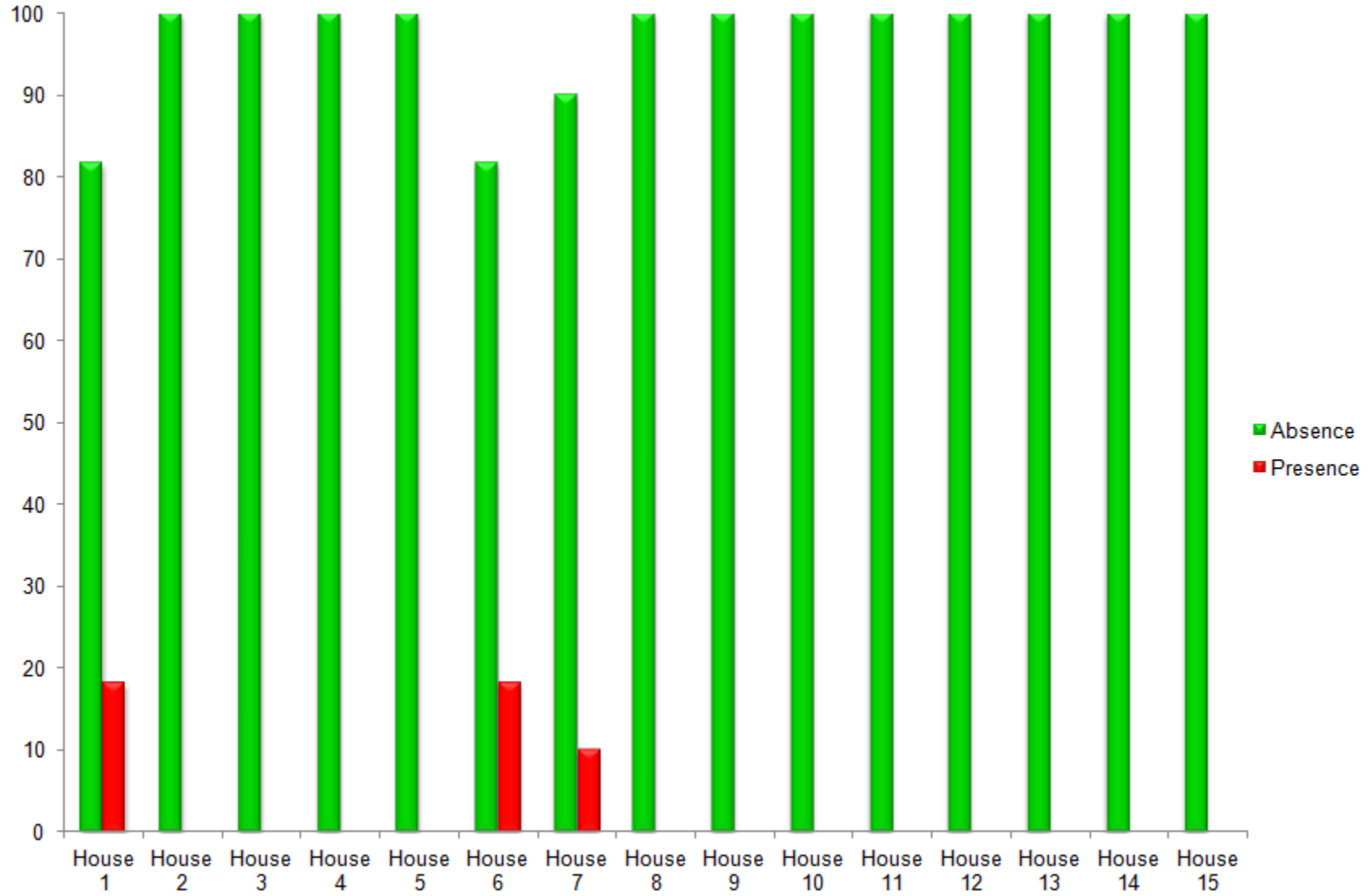
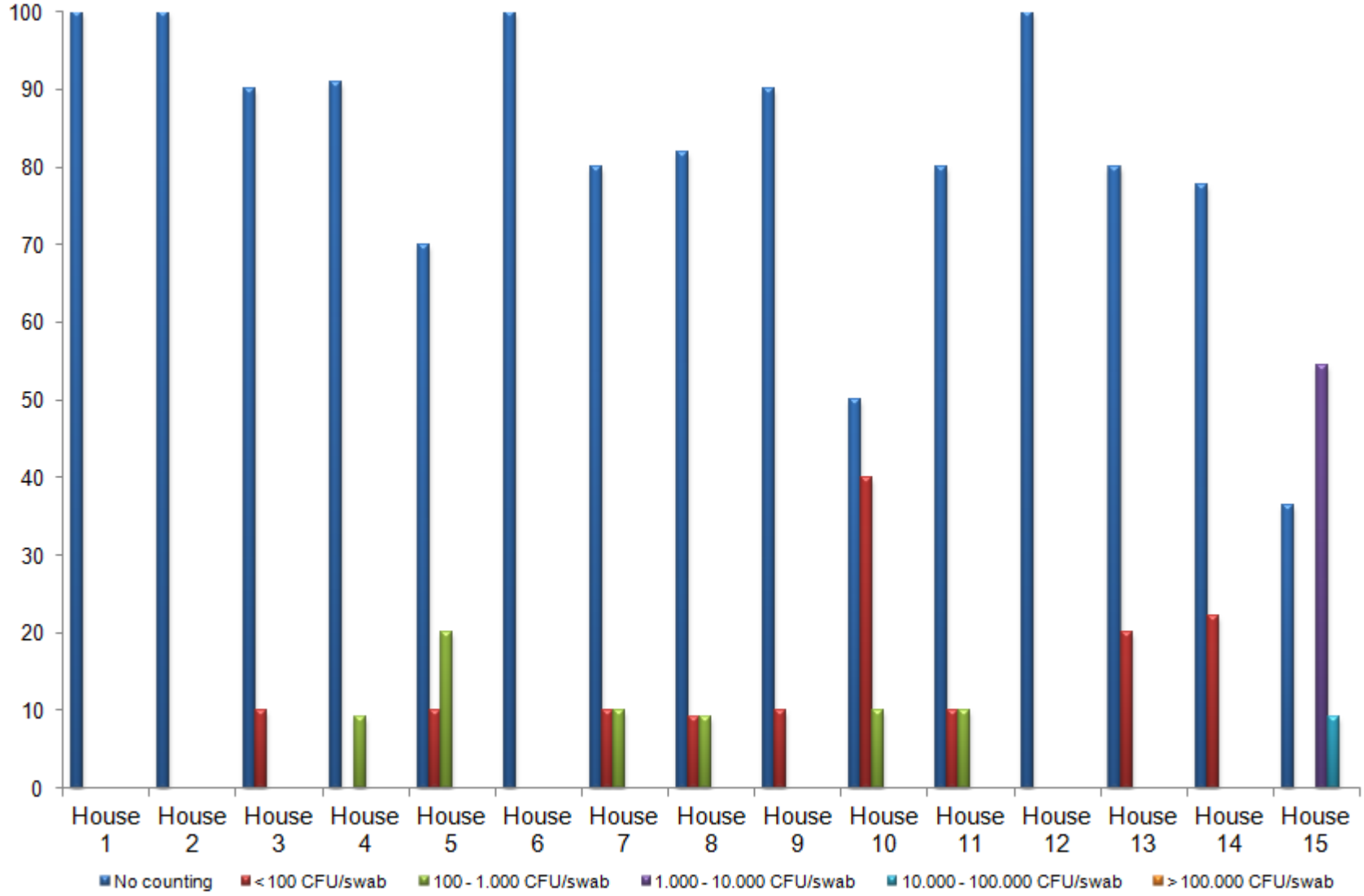


Figure 6 - *Listeria* spp. detection (%) by house.



. Figure 7 - *Escherichia coli* count (%) by house.

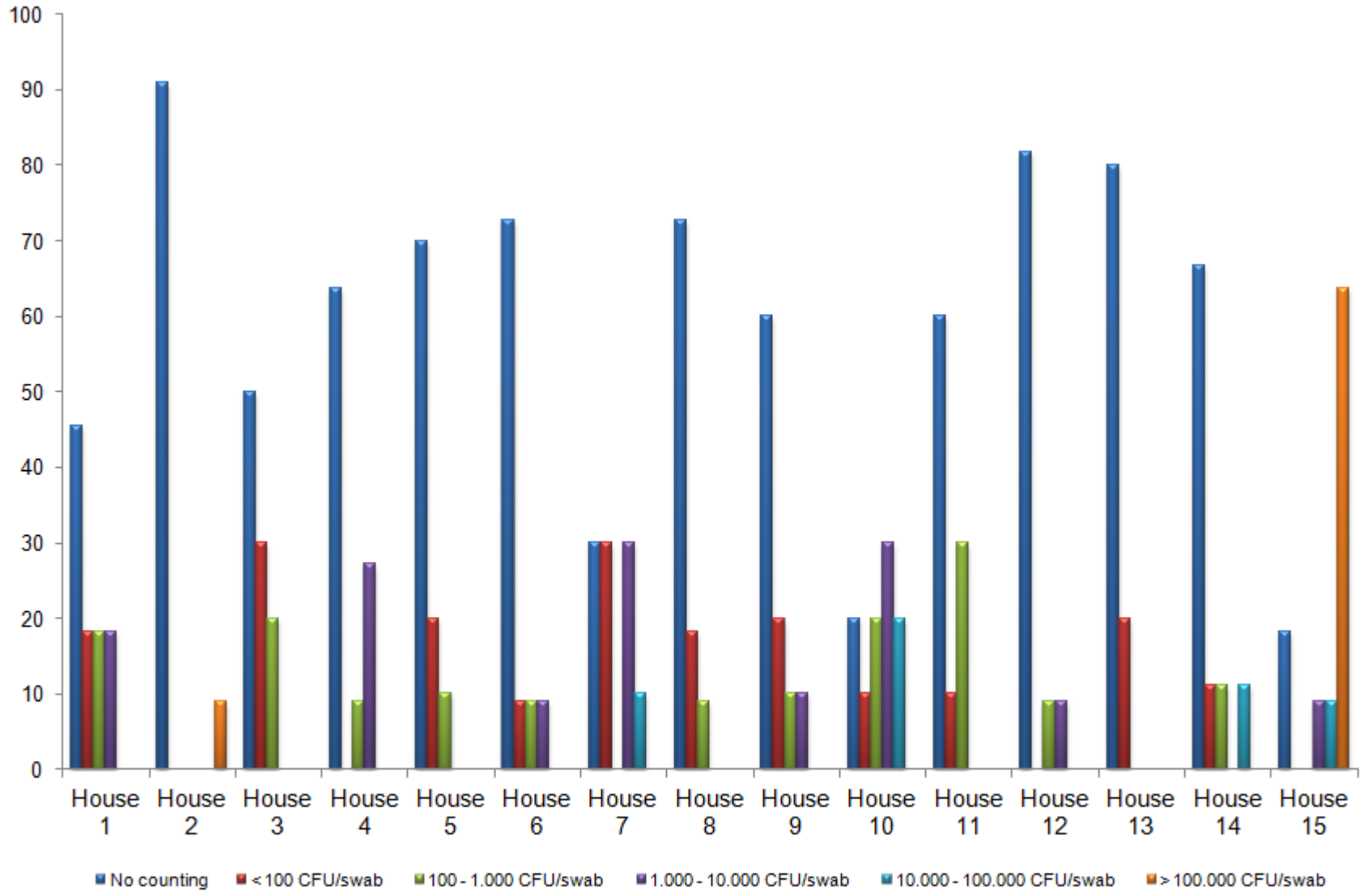


Figure 8 - *Enterobacteriaceae* count (%) by house.

In **House 9**, coagulase-positive *Staphylococcus* were present (10^4 CFU/swab), especially on stove buttons and cutting board, and *E. coli* contamination of kitchen knob ranged up to 10^3 CFU/swab while no *Listeria* was detected. Kitchen knob, counter and cloth demonstrated enteric contamination (ranging from 10^2 to 10^4 CFU/swab) and ampicillin resistance was found in isolates from the knob (MIC of 32 µg/mL) and counter (MIC of 32 µg/mL).
Knobs were assumed never to be cleaned, which can help explain *E. coli* contamination and antibiotic resistance, while kitchen counter cleaning is made using water and detergent.

House 10, shows contamination by coagulase-positive *Staphylococcus* (10^4 to 10^5 CFU/swab) and by *E. coli* (10^3 CFU/swab) although *Listeria* was not present. In this house enterobacteria seems to be widely spread, showing contamination levels *ca.* 10^5 CFU/swab, contaminating kitchen knob, kitchen tap, kitchen counter, cutting board, refrigerator handle, stove buttons and kitchen cloth. Resistance to ampicillin was found in *Enterobacteriaceae* isolated from kitchen knob (MIC of 64 µg/mL), kitchen tap (MIC of 64 µg/mL), cutting board (MIC of 128 µg/mL) and stove buttons (MIC of 64 µg/mL) while resistant isolates of *E. coli* were detected in WC tap (MIC of 32 µg/mL), cutting board (MIC of 128 µg/mL) and kitchen cloth (MIC of 256 µg/mL).
Tap and stove buttons were said to be cleaned after use and knobs once a week all of them using water and detergent which does not seem to be sufficient. A single cutting board is used for all types of food in this house meaning that cross-contamination can occur, while kitchen cloth, either fabric or "Vileda" type, may hold high levels of contamination.

At **House 11**, low rates of coagulase-positive *Staphylococcus* were found (< 10^2 CFU/swab) and no *Listeria* was detected. Regarding *E. coli*, 10^3 CFU/swab were detected on the cutting board but enteric pathogens were found on other surfaces, namely kitchen tap, kitchen counter and kitchen cloth. *Enterobacteriacea*e and *E. coli* resistant to ampicillin, were proven to exist in kitchen tap (MIC of 256 µg/mL and MIC of 512 µg/mL, correspondingly) while *Salmonella* spp. isolated from the kitchen counter presented resistance to nalidixic acid (MIC of 32 µg/mL).
In this house survey, taps were cleaned every day with disinfectant as well as kitchen counter which is cleaned after use although this routine does not appear to be effective. A single cutting board is in use and "Vileda" type kitchen cloth is used.

Within **House 12**, no *E. coli* nor *Listeria* were found and coagulase-positive *Staphylococcus* were found in low rates (< 10^2 CFU/swab). Despite this, high levels of enterobacteria were detected (10^3 to 10^4 CFU/swab) on the WC tap, kitchen counter and cloth. Resistance to ampicillin was identified for isolates from the WC tap and kitchen counter (MIC of 128 µg/mL and 32 µg/mL, respectively) and those from the kitchen cloth presented resistance to nitrofurantoin (MIC of 128 µg/mL).
This is in disagreement with the survey which indicates that both taps and counter are daily cleaned with disinfectant. The use of "Vileda" type kitchen cloth, which is wet most of the time, may be the cause of enteric bacteria presence.

About **House 13**, also no *Listeria* was found although *E. coli* and coagulase-positive *Staphylococcus* showed low rates (< 10^2 CFU/swab) of contamination. In relation to *Enterobacteriaceae*, these bacteria were detected on the dishwasher handle, cutting board and kitchen cloth but in low numbers (< 10^2 CFU/swab) even though multi-resistance was found. *E. coli* found on the dishwasher handle and kitchen cloth isolates presented high resistance to ampicillin (MIC of 256 µg/mL and 512 µg/mL), chloramphenicol (both with MIC of 256 µg/mL), nalidixic acid (both with MIC > 512 µg/mL) and tetracycline (both with MIC of 256 µg/mL).
These findings are not consistent with the survey where dishwasher handle was stated to be cleaned everyday with detergent. Although a single cutting board is used for different types of food the biggest problem is "Vileda" type of kitchen cloth that holds multi-resistant *E. coli*.

In **House 14**, as in the previous house, no *Listeria* was found although *E. coli* and coagulase-positive *Staphylococcus* showed low rates (< 10^2 CFU/swab) of contamination. High rates of enteric bacteria were detected (10^3 to 10^5 CFU/swab) namely on the cutting board, stove buttons and kitchen cloth. *E. coli* strains were determined to be resistant to ampicillin isolated from the cutting board (MIC of 32 µg/mL) and kitchen cloth (MIC of 256 µg/mL).
Once more this is not in accordance with the survey, where statements were made concerning daily cleaning of stove buttons with disinfectant. A single cutting board is used and sponge kind of kitchen cloth is obviously a good wet material for bacterial dissemination.

Inside **House 15** several pathogens were detected in high levels (10^3 to 10^5 CFU/swab), like coagulase-positive *Staphylococcus* and *E. coli* but no *Listeria* spp. was found. Concerning enteric contamination, this was the only house that presented extremely high rates of contamination (> 10^5 CFU/swab) on at least seven surfaces, namely domestic animal paws, kitchen tap, kitchen counter, refrigerator and dishwasher handles, stove buttons and kitchen cloth. Multiple resistance to ampicillin (MIC of 128 µg/mL) and nitrofurantoin (MIC of 128 µg/mL) was found in *E. coli* isolated from kitchen counter. Stove buttons, contaminated with *E. coli,* also presented multiple resistance to ampicillin (MIC of 64 µg/mL) and tetracycline (MIC of 16 µg/mL). *Enterobacteriaceae* isolates from the kitchen cloth were found to be resistant to both ampicillin (MIC of 32 µg/mL) and nitrofurantoin (MIC of 128 µg/mL) while from stove buttons, strains were resistant to ampicillin (MIC of 32 µg/mL).
From all that has been stated it seems hard to believe that taps are cleaned every day with detergent or that both refrigerator and dishwasher handles are daily cleaned with detergent. Furthermore, stove buttons and kitchen counter presented high enteric contamination and are stated to be cleaned after use with detergent. Kitchen cloth fabric and domestic animals in the kitchen area may contribute considerably to cross-contamination once high contamination rates are present.

Overall, consumers education or food knowledge does not give the impression that better or worse good food handling practices in a domestic environment are used. Therefore it seems necessary that enhanced information and training is given in order to develop improved behaviours in the domestic setting.

4. Conclusion

Taking into account all the obtained results it seems fundamental to comment that, in practice, cleaning the house is not the only important issue, knowing how to prevent contamination is just as crucial. In all cases “prevention is better than cure”.

According to results presented in this study and those previously published (Rusin *et al.*, 1998; Beumer and te Giffel, 1999; Scott, 2001; Adiga *et al.*, 2012;), the home is a multifunctional setting in which there is a constant transfer of pathogens into and out of the home. In the domestic setting various surfaces can harbour pathogenic organisms, thus being a potential source of food poisoning, possibly through cross-contamination. Potential pathogens from sources such as raw foods, persons, and animals can be transferred between inanimate and animate surfaces through either direct or indirect contact (Scott, 1999). The major factor contributing to foodborne illness, especially in the home, is the mishandling of food in the final preparation steps since consumers are not aware of their role in food poisoning. Epidemiological data suggests the home is a significant point of origin for food poisoning occurrences (Redmond and Griffith, 2003) and therefore it is important for consumers to be responsible for safe food-handling in their homes. Infectious diseases are a threat to public health and are often transmitted in the domestic setting. However, infectious disease spread in the home, as elsewhere, can be prevented through an effective hygiene strategy (Larson, 1999). Maintaining strictly hygienic practices, limiting cross-contamination, and regular cleaning of contact surfaces with detergents, hot water and sanitizers is indispensable to prevention of foodborne contamination.

This study illustrates that many pathogenic *Enterobacteriaceae* have developed resistance to frequently used antimicrobial agents, often presenting resistance to multiple antimicrobial classes simultaneously. Other recent studies have also reported on the declining activity of several antimicrobial groups against the *Enterobacteriaceae* including the fluoroquinolones (Levy, 2005; Rhomberg *et al.*, 2006; DiPersio and Dowzicky, 2007; Denton, 2007). In contrast to the early decades following the introduction of antibiotics, today's environments - hospitals, homes and communities - are replete with drug resistance genes, among both pathogens and commensals (Levy, 1998). Therefore, and as has been proved in this study, antibiotic resistance including multi-resistance, is not restricted to hospital environments since such strains can be found quite frequently in the domestic environment.

A consumer survey can be a useful instrument to collect information which yields concrete evidence for a requirement for educational measures. Some consumers do not have the necessary competence in handling food hygienically, and therefore public education is perceived as a key factor in improving food safety practices in the home and food preparers and consumers would benefit from home safety education, including information about temperature control, correct home food preparation practices and cross-contamination (Pfau and Piekarski, 2003; Wilcock *et al.*, 2004).

Overall, the information obtained from our survey revealed an urgent need for consumer education in Portugal regarding safe food handling practices and cleaning habits. It's also important to refer that most answers given in the questionnaire were not consistent with analytic data obtained in this study.

5. Future Work

It was a good challenge to perform this work by the fact that little information exists about real food safety and antibiotic susceptibility in the domestic environment. Therefore, several interesting work could be done such as:

- Identification at species level of all *Enterobacteriaceae* isolates that were mainly identified at genus level, by biochemistry or genotypic tests or appropriate PCR techniques;
- Identification and virulence factors of *Staphylococcus aureus* by PCR assay;
- Define an optimized protocol for *Campylobacter* spp. detection in order to increase the possibility of isolation in samples from the domestic environment;
- Provide information to consumers regarding better food handling practices and cleaning habits through education in Portugal;
- Perform an interdisciplinary investigation of microbial hazards during food preparation where domestic food safety practices can be evaluated *in situo*.

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Annex

Annex 1

MIC's Breakpoints

Class	Antibiotic	MIC[a] (µg/mL) Breakpoints S (Sensitive)	I (Intermediary)	R (Resistant)
Penicillins	Ampicillin (**AMP**)	≤ 8	16	≥ 32
Phenicols	Chloramphenicol (**CHL**)	≤ 8	16	≥ 32
Fluoroquinolones	Ciprofloxacin (**CIP**)	≤ 1	2	≥ 4
Aminoglycosides	Gentamicin (**GEN**)	≤ 4	8	≥ 16
Quinolones	Nalidixic acid (**NAL**)	≤ 16	---	≥ 32
Tetracyclines	Tetracycline (**TET**)	≤ 4	8	≥ 16
Folate pathway inhibitors	Trimethoprim (**TMP**)	≤ 8	---	≥ 16
Nitrofurantoins	Nitrofurantoin (**NIT**)	≤ 32	64	≥ 128

[a] - Classification according to the guidelines of NCCLS (2004).

HOUSE 1

Local	Microorganism	Antibiotic MIC's (µg/mL) Breakpoints AMP	CHL	CIP	GEN	NAL	TET	TMP	NIT
Domestic Animal (Dog)	*Enterobacteriaceae* spp.	8 (**S**)	2 (**S**)	0,03 (**S**)	0,5 (**S**)	4 (**S**)	1 (**S**)	0,25 (**S**)	64 (**I**)
		2 (**S**)	2 (**S**)	0,03 (**S**)	1 (**S**)	4 (**S**)	1 (**S**)	0,25 (**S**)	64 (**I**)
Domestic Animal (Dog)	*Enterobacteriaceae* spp.	4 (**S**)	2 (**S**)	< 0,015 (**S**)	0,5 (**S**)	2 (**S**)	0,5 (**S**)	0,125 (**S**)	64 (**I**)
		16 (**I**)	4 (**S**)	0,06 (**S**)	1 (**S**)	8 (**S**)	0,5 (**S**)	>128 (**R**)	32 (**S**)
Domestic Animal (Cat)	*Enterobacteriaceae* spp.	1(**S**)	2 (**S**)	< 0,015 (**S**)	1 (**S**)	4 (**S**)	2 (**S**)	0,5 (**S**)	32 (**S**)
		1 (**S**)	2 (**S**)	< 0,015 (**S**)	1 (**S**)	4 (**S**)	2 (**S**)	0,5 (**S**)	32 (**S**)
Domestic Animal (Cat)	*Salmonella* spp.	4 (**S**)	8 (**S**)	0,03 (**S**)	1 (**S**)	2 (**S**)	8 (**I**)	4 (**S**)	64 (**I**)
		4 (**S**)	8 (**S**)	0,03 (**S**)	0,5 (**S**)	2 (**S**)	8 (**I**)	4 (**S**)	128 (**R**)
Cutting Board	*Enterobacteriaceae* spp.	64 (**R**)	8 (**S**)	0,06 (**S**)	1 (**S**)	8 (**S**)	4 (**S**)	4 (**S**)	64 (**I**)
		64 (**R**)	8 (**S**)	0,06 (**S**)	2 (**S**)	8 (**S**)	4 (**S**)	8 (**S**)	64 (**I**)
Kitchen Counter	*Enterobacteriaceae* spp.	32 (**R**)	4 (**S**)	0,03 (**S**)	2 (**S**)	4 (**S**)	4 (**S**)	1 (**S**)	16 (**S**)
		32 (**R**)	8 (**S**)	0,06 (**S**)	1 (**S**)	8 (**S**)	4 (**S**)	8 (**S**)	64 (**I**)
Kitchen Cloth	*Enterobacteriaceae* spp.	8 (**S**)	8 (**S**)	0,03 (**S**)	1 (**S**)	4 (**S**)	2 (**S**)	1 (**S**)	32 (**S**)

HOUSE 2

Local	Microorganism	Antibiotic MIC's (µg/mL) Breakpoints AMP	CHL	CIP	GEN	NAL	TET	TMP	NIT
Kitchen Tap	*Salmonella* spp.	16 (**I**)	4 (**S**)	0,03 (**S**)	1 (**S**)	8 (**S**)	1 (**S**)	1 (**S**)	32 (**S**)
		4 (**S**)	8 (**S**)	0,03 (**S**)	1 (**S**)	8 (**S**)	2 (**S**)	1 (**S**)	32 (**S**)
Kitchen Cloth	*Salmonella* spp.	256 (**R**)	8 (**S**)	0,125 (**S**)	1 (**S**)	8 (**S**)	8 (**I**)	2 (**S**)	32 (**S**)
		4 (**S**)	16 (**I**)	< 0,015 (**S**)	1 (**S**)	8 (**S**)	2 (**S**)	1 (**S**)	32 (**S**)

HOUSE 3

Local	Microorganism	Antibiotic MIC's (µg/mL) Breakpoints							
		AMP	CHL	CIP	GEN	NAL	TET	TMP	NIT
Domestic Animal (Dog)	*Salmonella* spp.	8 (**S**)	8 (**S**)	< 0,015 (**S**)	1 (**S**)	8 (**S**)	4 (**S**)	0,5 (**S**)	32 (**S**)
		8 (**S**)	8 (**S**)	< 0,015 (**S**)	1 (**S**)	8 (**S**)	4 (**S**)	0,5 (**S**)	16 (**S**)
WC Knob	*Salmonella* spp.	8 (**S**)	8 (**S**)	0,03 (**S**)	1 (**S**)	8 (**S**)	4 (**S**)	0,5 (**S**)	16 (**S**)
		8 (**S**)	8 (**S**)	< 0,015 (**S**)	1 (**S**)	4 (**S**)	2 (**S**)	0,5 (**S**)	16 (**S**)
Kitchen Tap	*Enterobacteriaceae* spp.	8 (**S**)	8 (**S**)	0,03 (**S**)	0,5 (**S**)	8 (**S**)	2 (**S**)	0,25 (**S**)	16 (**S**)
		8 (**S**)	8 (**S**)	0,03 (**S**)	1 (**S**)	4 (**S**)	8 (**I**)	1 (**S**)	64 (**R**)
Kitchen Counter	*Salmonella* spp.	16 (**I**)	8 (**S**)	0,03 (**S**)	0,5 (**S**)	4 (**S**)	2 (**S**)	0,5 (**S**)	16 (**S**)
		16 (**I**)	8 (**S**)	0,03 (**S**)	0,5 (**S**)	4 (**S**)	4 (**S**)	0,25 (**S**)	32 (**S**)
Cutting Board	*Salmonella* spp.	64 (**R**)	16 (**I**)	0,25 (**S**)	2 (**S**)	4 (**S**)	128 (**R**)	2 (**S**)	128 (**R**)
		64 (**R**)	32 (**R**)	0,5 (**S**)	2 (**S**)	4 (**S**)	128 (**R**)	2 (**S**)	128 (**R**)
Kitchen Cloth	*Escherichia coli*	32 (**R**)	8 (**S**)	0,125 (**S**)	0,5 (**S**)	4 (**S**)	2 (**S**)	1 (**S**)	16 (**S**)
		32 (**R**)	4 (**S**)	0,03 (**S**)	0,5 (**S**)	4 (**S**)	2 (**S**)	1 (**S**)	16 (**S**)

HOUSE 4

Local	Microorganism	Antibiotic MIC's (µg/mL) Breakpoints							
		AMP	CHL	CIP	GEN	NAL	TET	TMP	NIT
Kitchen Tap	*Salmonella* spp.	32 (**R**)	8 (**S**)	0,03 (**S**)	1 (**S**)	4 (**S**)	8 (**I**)	4 (**S**)	64 (**I**)
	Enterobacteriaceae spp.	16 (**I**)	4 (**S**)	0,03 (**S**)	0,5 (**S**)	4 (**S**)	2 (**S**)	0,5 (**S**)	64 (**I**)
Kitchen Counter	*Enterobacteriaceae* spp.	8 (**S**)	2 (**S**)	0,03 (**S**)	1 (**S**)	4 (**S**)	2 (**S**)	0,25 (**S**)	16 (**S**)
	Escherichia coli	256 (**R**)	8 (**S**)	0,03 (**S**)	1 (**S**)	8 (**S**)	4 (**S**)	1 (**S**)	128 (**R**)
Refrigerator Handle	*Escherichia coli*	4 (**S**)	8 (**S**)	0,03 (**S**)	0,5 (**S**)	2 (**S**)	8 (**I**)	8 (**S**)	64 (**I**)
Dishwasher Handle	*Enterobacteriaceae* spp.	16 (**I**)	8 (**S**)	0,03 (**S**)	0,5 (**S**)	4 (**S**)	8 (**I**)	0,5 (**S**)	128 (**R**)

HOUSE 5

Local	Microorganism	Antibiotic MIC's (µg/mL) Breakpoints							
		AMP	CHL	CIP	GEN	NAL	TET	TMP	NIT
WC Knob	*Enterobacteriaceae* spp.	4 (**S**)	4 (**S**)	< 0,015 (**S**)	0,25 (**S**)	2 (**S**)	2 (**S**)	0,125 (**S**)	64 (**I**)
		4 (**S**)	4 (**S**)	< 0,015 (**S**)	0,25 (**S**)	2 (**S**)	2 (**S**)	0,125 (**S**)	64 (**I**)
Kitchen Counter	*Escherichia coli*	4 (**S**)	4 (**S**)	0,03 (**S**)	0,5 (**S**)	2 (**S**)	2 (**S**)	0,25 (**S**)	64 (**I**)
Dishwasher Handle	*Escherichia coli*	0,5 (**S**)	1 (**S**)	< 0,015 (**S**)	0,5 (**S**)	2 (**S**)	0,5 (**S**)	0,25 (**S**)	32 (**S**)
		0,5 (**S**)	1 (**S**)	< 0,015 (**S**)	0,5 (**S**)	4 (**S**)	0,5 (**S**)	0,5 (**S**)	16 (**S**)
Kitchen Cloth	*Escherichia coli*	16 (**I**)	4 (**S**)	< 0,015 (**S**)	0,5 (**S**)	2 (**S**)	0,5 (**S**)	0,25 (**S**)	32 (**S**)
	Enterobacteriaceae spp.	8 (**S**)	4 (**S**)	< 0,015 (**S**)	0,25 (**S**)	2 (**S**)	0,5 (**S**)	0,25 (**S**)	32 (**S**)

HOUSE 6

Local	Microorganism	Antibiotic MIC's (µg/mL) Breakpoints							
		AMP	CHL	CIP	GEN	NAL	TET	TMP	NIT
Domestic Animal (Cat)	*Enterobacteriaceae* spp.	32 (**R**)	8 (**S**)	0,06 (**S**)	1 (**S**)	4 (**S**)	2 (**S**)	0,5 (**S**)	64 (**I**)
		32 (**R**)	16 (**I**)	0,03 (**S**)	1 (**S**)	4 (**S**)	2 (**S**)	0,5 (**S**)	64 (**I**)
Kitchen Tap	*Enterobacteriaceae* spp.	16 (**I**)	2 (**S**)	< 0,015 (**S**)	1 (**S**)	2 (**S**)	1 (**S**)	0,5 (**S**)	8 (**S**)
		8 (**S**)	2 (**S**)	0,03 (**S**)	0,5 (**S**)	2 (**S**)	0,5 (**S**)	0,25 (**S**)	32 (**S**)

HOUSE 7

Local	Microorganism	Antibiotic MIC's (µg/mL) Breakpoints							
		AMP	CHL	CIP	GEN	NAL	TET	TMP	NIT
Kitchen Tap	*Escherichia coli*	64 (**R**)	4 (**S**)	< 0,015 (**S**)	1 (**S**)	4 (**S**)	2 (**S**)	1 (**S**)	16 (**S**)
		16 (**I**)	4 (**S**)	< 0,015 (**S**)	1 (**S**)	4 (**S**)	1 (**S**)	0,5 (**S**)	16 (**S**)
Refrigerator Handle	*Escherichia coli*	128 (**R**)	4 (**S**)	0,03 (**S**)	0,5 (**S**)	4 (**S**)	4 (**S**)	1 (**S**)	16 (**S**)
	Salmonella spp.	16 (**I**)	8 (**S**)	0,03 (**S**)	1 (**S**)	4 (**S**)	4 (**S**)	2 (**S**)	64 (**I**)
Dishwasher Handle	*Salmonella* spp.	8 (**S**)	2 (**S**)	< 0,015 (**S**)	0,5 (**S**)	1 (**S**)	1 (**S**)	0,5 (**S**)	32 (**S**)
	Enterobacteriaceae spp.	4 (**S**)	8 (**S**)	0,03 (**S**)	1 (**S**)	2 (**S**)	2 (**S**)	0,25 (**S**)	32 (**S**)
Kitchen Cloth	*Enterobacteriaceae* spp.	4 (**S**)	4 (**S**)	0,03 (**S**)	0,5 (**S**)	1 (**S**)	2 (**S**)	2 (**S**)	64 (**I**)
		4 (**S**)	4 (**S**)	0,03 (**S**)	0,5 (**S**)	0,5 (**S**)	2 (**S**)	1 (**S**)	64 (**I**)

HOUSE 8

Local	Microorganism	Antibiotic MIC's (µg/mL) Breakpoints							
		AMP	CHL	CIP	GEN	NAL	TET	TMP	NIT
WC Knob	*Enterobacteriaceae* spp.	2 (**S**)	4 (**S**)	0,06 (**S**)	0,5 (**S**)	4 (**S**)	2 (**S**)	0,5 (**S**)	128 (**R**)
		2 (**S**)	8 (**S**)	0,06 (**S**)	0,5 (**S**)	4 (**S**)	2 (**S**)	2 (**S**)	64 (**I**)
Kitchen Knob	*Enterobacteriaceae* spp.	1 (**S**)	2 (**S**)	< 0,015 (**S**)	0,5 (**S**)	2 (**S**)	0,5 (**S**)	0,5 (**S**)	32 (**S**)
		4 (**S**)	4 (**S**)	0,03 (**S**)	0,5 (**S**)	4 (**S**)	2 (**S**)	0,5 (**S**)	32 (**S**)
Cutting Board	*Escherichia coli*	0,5 (**S**)	2 (**S**)	< 0,015 (**S**)	0,5 (**S**)	2 (**S**)	0,5 (**S**)	0,5 (**S**)	32 (**S**)
		0,5 (**S**)	2 (**S**)	< 0,015 (**S**)	0,5 (**S**)	2 (**S**)	0,5 (**S**)	0,5 (**S**)	32 (**S**)
Kitchen Cloth	*Escherichia coli*	4 (**S**)	4 (**S**)	< 0,015 (**S**)	0,5 (**S**)	4 (**S**)	2 (**S**)	0,25 (**S**)	64 (**I**)
		4 (**S**)	2 (**S**)	< 0,015 (**S**)	0,5 (**S**)	2 (**S**)	0,5 (**S**)	0,25 (**S**)	16 (**S**)

HOUSE 9

Local	Microorganism	Antibiotic MIC's (µg/mL) Breakpoints							
		AMP	CHL	CIP	GEN	NAL	TET	TMP	NIT
Kitchen Knob	*Escherichia coli*	32 (**R**)	2 (**S**)	0,03 (**S**)	1 (**S**)	2 (**S**)	1 (**S**)	0,25 (**S**)	32 (**S**)
		32 (**R**)	2 (**S**)	0,03 (**S**)	0,5 (**S**)	2 (**S**)	1 (**S**)	0,25 (**S**)	32 (**S**)
Kitchen Counter	*Enterobacteriaceae* spp.	4 (**S**)	8 (**S**)	0,03 (**S**)	0,5 (**S**)	1 (**S**)	4 (**S**)	2 (**S**)	64 (**I**)
		32 (**R**)	2 (**S**)	< 0,015 (**S**)	0,5 (**S**)	2 (**S**)	0,5 (**S**)	0,25 (**S**)	16 (**S**)
Kitchen Cloth	*Enterobacteriaceae* spp.	4 (**S**)	8 (**S**)	0,03 (**S**)	0,5 (**S**)	1 (**S**)	4 (**S**)	2 (**S**)	64 (**I**)
		4 (**S**)	8 (**S**)	0,03 (**S**)	0,5 (**S**)	1 (**S**)	4 (**S**)	2 (**S**)	64 (**I**)

HOUSE 10

Local	Microorganism	Antibiotic MIC's (µg/mL) Breakpoints							
		AMP	CHL	CIP	GEN	NAL	TET	TMP	NIT
WC Tap	*Escherichia coli*	16 (**I**)	2 (**S**)	0,03 (**S**)	0,5 (**S**)	16 (**S**)	1 (**S**)	0,25 (**S**)	32 (**S**)
		32 (**R**)	2 (**S**)	< 0,015 (**S**)	0,5 (**S**)	2 (**S**)	1 (**S**)	0,25 (**S**)	32 (**S**)
Kitchen Knob	*Enterobacteriaceae* spp.	64 (**R**)	2 (**S**)	< 0,015 (**S**)	0,5 (**S**)	4 (**S**)	0,5 (**S**)	0,25 (**S**)	16 (**S**)
		8 (**S**)	2 (**S**)	< 0,015 (**S**)	1 (**S**)	2 (**S**)	1 (**S**)	0,25 (**S**)	16 (**S**)
Kitchen Tap	*Escherichia coli*	8 (**S**)	2 (**S**)	0,03 (**S**)	1 (**S**)	2 (**S**)	1 (**S**)	0,25 (**S**)	16 (**S**)
	Enterobacteriaceae spp.	64 (**R**)	4 (**S**)	< 0,015 (**S**)	1 (**S**)	2 (**S**)	1 (**S**)	0,25 (**S**)	16 (**S**)
Kitchen Counter	*Enterobacteriaceae* spp.	8 (**S**)	4 (**S**)	< 0,015 (**S**)	1 (**S**)	2 (**S**)	1 (**I**)	0,5 (**S**)	32 (**S**)
		8 (**S**)	4 (**S**)	0,06 (**S**)	1 (**S**)	2 (**S**)	1 (**I**)	0,25 (**S**)	32 (**S**)
Cutting Board	*Escherichia coli*	128 (**R**)	8 (**S**)	0,06 (**S**)	0,5 (**S**)	2 (**S**)	4 (**S**)	2 (**S**)	16 (**S**)
	Enterobacteriaceae spp.	128 (**R**)	8 (**S**)	0,125 (**S**)	1 (**S**)	2 (**S**)	4 (**S**)	2 (**S**)	16 (**S**)
Refrigerator Handle	*Enterobacteriaceae* spp.	8 (**S**)	2 (**S**)	< 0,015 (**S**)	1 (**S**)	2 (**S**)	1 (**S**)	0,25 (**S**)	16 (**S**)
		8 (**S**)	2 (**S**)	< 0,015 (**S**)	1 (**S**)	2 (**S**)	1 (**S**)	0,25 (**S**)	16 (**S**)
Stove Buttons	*Enterobacteriaceae* spp.	8 (**S**)	2 (**S**)	< 0,015 (**S**)	1 (**S**)	2 (**S**)	1 (**S**)	0,25 (**S**)	16 (**S**)
		64 (**R**)	2 (**S**)	0,03 (**S**)	1 (**S**)	2 (**S**)	1 (**S**)	0,25 (**S**)	16 (**S**)
Kitchen Cloth	*Escherichia coli*	256 (**R**)	4 (**S**)	< 0,015 (**S**)	1 (**S**)	2 (**S**)	2 (**S**)	0,5 (**S**)	16 (**S**)

HOUSE 11

Local	Microorganism	Antibiotic MIC's (µg/mL) Breakpoints							
		AMP	CHL	CIP	GEN	NAL	TET	TMP	NIT
Kitchen Tap	*Enterobacteriaceae* spp.	256 (**R**)	8 (**S**)	< 0,015 (**S**)	0,5 (**S**)	8 (**S**)	1 (**S**)	0,25 (**S**)	32 (**S**)
	Salmonella spp.	512 (**R**)	8 (**S**)	< 0,015 (**S**)	1 (**S**)	8 (**S**)	1 (**S**)	0,25 (**S**)	32 (**S**)
Kitchen Counter	*Salmonella* spp.	8 (**S**)	4 (**S**)	< 0,015 (**S**)	0,5 (**S**)	32 (**R**)	1 (**S**)	0,25 (**S**)	32 (**S**)
	Enterobacteriaceae spp.	8 (**S**)	2 (**S**)	< 0,015 (**S**)	0,5 (**S**)	2 (**S**)	1 (**S**)	0,25 (**S**)	64 (**I**)
Cutting Board	*Escherichia coli*	16 (**I**)	4 (**S**)	< 0,015 (**S**)	1 (**S**)	2 (**S**)	1 (**S**)	0,5 (**S**)	64 (**I**)
		16 (**I**)	4 (**S**)	0,03 (**S**)	1 (**S**)	2 (**S**)	1 (**S**)	0,5 (**S**)	64 (**I**)
Kitchen Cloth	*Escherichia coli*	16 (**I**)	4 (**S**)	0,06 (**S**)	1 (**S**)	2 (**S**)	1 (**S**)	0,5 (**S**)	64 (**I**)
	Enterobacteriaceae spp.	0,5 (**S**)	2 (**S**)	< 0,015 (**S**)	0,5 (**S**)	2 (**S**)	1 (**S**)	0,5 (**S**)	64 (**I**)

HOUSE 12

Local	Microorganism	Antibiotic MIC's (µg/mL) Breakpoints							
		AMP	CHL	CIP	GEN	NAL	TET	TMP	NIT
WC Tap	*Enterobacteriaceae* spp.	128 (**R**)	8 (**S**)	0,03 (**S**)	0,5 (**S**)	2 (**S**)	1 (**S**)	0,25 (**S**)	16 (**S**)
		128 (**R**)	4 (**S**)	0,03 (**S**)	0,5 (**S**)	2 (**S**)	1 (**S**)	0,25 (**S**)	16 (**S**)
Kitchen Counter	*Enterobacteriaceae* spp.	32 (**R**)	16 (**I**)	0,03 (**S**)	0,5 (**S**)	2 (**S**)	4 (**S**)	0,25 (**S**)	64 (**I**)
		32 (**R**)	16 (**I**)	< 0,015 (**S**)	0,5 (**S**)	2 (**S**)	4 (**S**)	0,25 (**S**)	64 (**I**)
Kitchen Cloth	*Enterobacteriaceae* spp.	1 (**S**)	8 (**S**)	< 0,015 (**S**)	1 (**S**)	2 (**S**)	4 (**S**)	0,25 (**S**)	128 (**R**)
		1 (**S**)	8 (**S**)	< 0,015 (**S**)	1 (**S**)	2 (**S**)	4 (**S**)	0,25 (**S**)	64 (**I**)

HOUSE 13

Local	Microorganism	Antibiotic MIC's (µg/mL) Breakpoints							
		AMP	CHL	CIP	GEN	NAL	TET	TMP	NIT
Dishwasher Handle	*Escherichia coli*	256 (**R**)	256 (**R**)	0,5 (**S**)	2 (**S**)	> 512 (**R**)	256 (**R**)	0,125 (**S**)	16 (**S**)
		8 (**S**)	8 (**S**)	0,125 (**S**)	1 (**S**)	4 (**S**)	4 (**S**)	0,5 (**S**)	16 (**S**)
Cutting Board	*Enterobacteriaceae* spp.	0,5 (**S**)	2 (**S**)	< 0,015 (**S**)	1 (**S**)	2 (**S**)	0,25 (**S**)	0,25 (**S**)	16 (**S**)
Kitchen Cloth	*Escherichia coli*	512 (**R**)	256 (**R**)	0,5 (**S**)	1 (**S**)	> 512 (**R**)	256 (**R**)	0,125 (**S**)	16 (**S**)
		4 (**S**)	8 (**S**)	0,125 (**S**)	1 (**S**)	> 512 (**R**)	1 (**S**)	0,125 (**S**)	32 (**S**)

HOUSE 14

Local	Microorganism	Antibiotic MIC's (µg/mL) Breakpoints							
		AMP	CHL	CIP	GEN	NAL	TET	TMP	NIT
Cutting Board	*Escherichia coli*	32 (**R**)	8 (**S**)	0,03 (**S**)	1 (**S**)	2 (**S**)	2 (**S**)	0,5 (**S**)	32 (**S**)
Stove Buttons	*Enterobacteriaceae* spp.	4 (**S**)	2 (**S**)	< 0,015 (**S**)	0,125 (**S**)	2 (**S**)	0,5 (**S**)	0,25 (**S**)	32 (**S**)
		16 (**I**)	4 (**S**)	0,06 (**S**)	0,5 (**S**)	4 (**S**)	2 (**S**)	1 (**S**)	32 (**S**)
Kitchen Cloth	*Escherichia coli*	256 (**R**)	2 (**S**)	< 0,015 (**S**)	1 (**S**)	4 (**S**)	0,5 (**S**)	0,5 (**S**)	16 (**S**)
		256 (**R**)	2 (**S**)	< 0,015 (**S**)	1 (**S**)	4 (**S**)	0,5(**S**)	0,5 (**S**)	16 (**S**)

HOUSE 15

Local	Microorganism	Antibiotic MIC's (µg/mL) Breakpoints							
		AMP	CHL	CIP	GEN	NAL	TET	TMP	NIT
Domestic Animal (Dog)	*Escherichia coli*	0,5 (**S**)	1 (**S**)	< 0,015 (**S**)	0,5 (**S**)	2 (**S**)	0,5 (**S**)	0,25 (**S**)	32 (**S**)
		2 (**S**)	4 (**S**)	< 0,015 (**S**)	0,5 (**S**)	4 (**S**)	2 (**S**)	0,25 (**S**)	16 (**S**)
WC Tap	*Escherichia coli*	1(**S**)	2 (**S**)	< 0,015 (**S**)	0,5 (**S**)	2 (**S**)	0,5 (**S**)	0,5 (**S**)	32 (**S**)
		1(**S**)	2 (**S**)	< 0,015 (**S**)	0,5 (**S**)	2 (**S**)	0,5 (**S**)	0,5 (**S**)	32 (**S**)
Kitchen Knob	*Escherichia coli*	1(**S**)	2 (**S**)	< 0,015 (**S**)	0,5 (**S**)	2 (**S**)	0,5 (**S**)	0,5 (**S**)	32 (**S**)
		1(**S**)	2 (**S**)	< 0,015 (**S**)	0,5 (**S**)	2 (**S**)	0,5 (**S**)	0,5 (**S**)	32 (**S**)
Kitchen Tap	*Enterobacteriaceae* spp.	1 (**S**)	4 (**S**)	< 0,015 (**S**)	1 (**S**)	4 (**S**)	0,5 (**S**)	0,25 (**S**)	32 (**S**)
	Escherichia coli	8 (**S**)	4 (**S**)	< 0,015 (**S**)	0,5 (**S**)	4 (**S**)	0,5 (**S**)	0,25 (**S**)	32 (**S**)
Kitchen Counter	*Escherichia coli*	128 (**R**)	4 (**S**)	< 0,015 (**S**)	1 (**S**)	1 (**S**)	0,5 (**S**)	0,5 (**S**)	128 (**R**)
		128 (**R**)	2 (**S**)	< 0,015 (**S**)	0,5 (**S**)	1 (**S**)	0,5 (**S**)	0,25 (**S**)	16 (**S**)
Refrigerator Handle	*Enterobacteriaceae* spp.	16 (**I**)	4 (**S**)	< 0,015 (**S**)	0,5 (**S**)	2 (**S**)	0,5 (**S**)	0,5 (**S**)	16 (**S**)
		8 (**S**)	4 (**S**)	< 0,015 (**S**)	0,5 (**S**)	4 (**S**)	0,5 (**S**)	0,5 (**S**)	16 (**S**)
Stove Buttons	*Escherichia coli*	64 (**R**)	2 (**S**)	< 0,015 (**S**)	1 (**S**)	4 (**S**)	16 (**R**)	0,5 (**S**)	32 (**S**)
	Enterobacteriaceae spp.	32 (**R**)	4 (**S**)	0,03 (**S**)	1 (**S**)	4 (**S**)	2 (**S**)	0,25 (**S**)	16 (**S**)
Dishwasher Handle	*Escherichia coli*	0,5 (**S**)	1 (**S**)	< 0,015 (**S**)	0,5 (**S**)	2 (**S**)	0,5 (**S**)	0,5 (**S**)	32 (**S**)
		4 (**S**)	4 (**S**)	< 0,015 (**S**)	0,5 (**S**)	2 (**S**)	0,5 (**S**)	0,5 (**S**)	32 (**S**)
Kitchen Cloth	*Enterobacteriaceae* spp.	32 (**R**)	4 (**S**)	< 0,015 (**S**)	0,5 (**S**)	4 (**S**)	1 (**S**)	0,5 (**S**)	128 (**R**)
		8 (**S**)	2 (**S**)	< 0,015 (**S**)	0,5 (**S**)	2 (**S**)	0,5 (**S**)	0,25 (**S**)	16 (**S**)