SENSITIVITY PATTERNS OF *LISTERIA MONOCYTOGENES* AN OTHER AEROBIC BACTERIA IN SCAVENGING CHICKENS AN DUCKS."

By:

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DECLARATION

This thesis is my original work; it has not been presented for a degree in any other university.

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This thesis has been submitted for examination with our approval as university supervisors.

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Dedication

This thesis is dedicated to my parents, Ephantus Njagi Rugea and Kellen Gichuku Njagi.

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ABSTRACT

The carrier status of *Listeria monocytogenes* organisms in indigenous chicken and ducks may have great impact on human health because of their zoonotic nature, particularly in an environment where humans, chicken and ducks share the same ecosystems. In Kenya, the epidemiological issues and the dynamics of carrier status for *Listeria monocytogenes* in local indigenous chickens and ducks have not been documented.

In this study, 175 indigenous birds (55 chickens from farms, 40 chickens from market and trading centres, 41 chickens from slaughterhouses and 39 ducks from farms) were sampled. Samples taken included oropharyngeal and faecal swabs (cloacal and intestinal swabs). Bacteriological isolation and characterisation of *Listeria monocytogenes*, other *Listeria* species and other aerobic bacteria was carried out. The recovered *Listeria* isolates were tested for antibiotic and disinfectant sensitivities. Eight commonly used antibiotics and 7 disinfectants were tested. Disinfectant sensitivity was also done on pooled bacterial cultures from farms.

Listeria species isolated were *Listeria monocytogenes* (2), L. *innocua* (3), *L.seeligeri* (2), *L. grayi* (1) and *L. murrayi* (1). They were recovered from both farms and markets and only from chickens; not from ducks. Other bacteria that were isolated from both chicken and ducks and from farms, market and slaughterhouses, belonged to the genera: *Staphylococcus; Streptococcus; Escherichia* and *Erysipelothrix.*

All *Listeria* tested for antibiotic sensitivity were 100% sensitive to gentamycin; sensitivities to kenamycin, tetracycline, cotrimoxazole and chloramphenicol were; 88.9%, 77.8%, 66.7%, and 66.7% respectively. All the *Listeria* isolates were resistant to ampicillin, augmentin and cefuroxime. The difference in the antibiotic sensitivity of various *Listeria* isolates and *Listeria monocytogenes* type strains was not significant (P=0.2639).

All *Listeria* isolates were sensitive to Omnicide® at the recommended user – dilution. For Bromosept® 50, Lavik®, and Dettol®, 88.9%; 77.8%; 77.8% of the isolates, respectively, were sensitive at user – dilution. All of the isolates were resistant to Sodium hypochlorite, Kleenol®, and Lysol®. The results indicated that a slightly higher concentration than the recommended user – dilution may be preferred.

Sensitivity of the pooled bacterial culture to the disinfectants used indicated Bromosept 50® and Omnicide® as the most efficient with a mean sensitivity at user dilution of 80% and 60% respectively. The others namely: Dettol®, Lavik® and Sodium hypochlorite had each a mean of 30 % susceptibility. Kleenol® and Lysol® were ineffective at both user and higher concentrations. Bromosept 50® and Omnicide®, are recommended for disinfection of chicken and duck coops at the village level. Kleenol and Lysol® are not to be recommended.

The study showed conclusively, for the first time, that indigenous chickens in Kenya are carriers of *Listeria monocytogenes* and other potentially pathogenic bacteria. The study also indicates the need to carry out antibiotic and disinfectant sensitivity testing in order to identify the effective ones.

CHAPTER 1: INTRODUCTION AND JUSTIFICATION

1.1: Introduction

Listeria monocytogenes bacteria are frequently overlooked as a possible causative agent of food poisoning (Paustian, 2000). This bacterium has a growth range in temperatures of 4^0C to 37^0C, and is commonly found in refrigerated, processed and unprocessed foods (Muriana, 1996). Thus, possibility of exposure to consumers is high. Extensive studies on prevention and further experiments to uncover the toxigenicity mechanisms of this bacterium are needed. *Listeria monocytogenes* produces an endotoxin. These microorganisms are often found on vegetables, domestic and wild animals (Paustian, 2000).

The scavenging local chicken (SLC) and ducks interact in their scavenging activities, and are exposed to and experience similar conditions, such as scanty feed, predators and disease. They are usually not vaccinated. Thus, diseases are among the most important constraints to their production (MLD, 1989; Mbugua, *et.al.*, 1994; Sa'idu , *et. al.*,1994; Nyaga *et al.*, 2002; Mbuthia, *et al.*, 2002a). *Listeria monocytogenes* is zoonotic; the infection may be acquired by consumption of undercooked poultry meat (Calnek, *et al.*, 1997).

Listeria monocytogenes is widely distributed in animals and humans and has been isolated from different mammalian and avian species (Ryser *et. al.*, 1997). Scavenging local chickens and ducks may serve as symptomless carriers of *Listeria monocytogenes* (Zander *et al.*, 1997; Marsden, 1994). This has been documented in other countries (Zander *et al.*,

1997), but the nature and extent of this carrrier status in SLC and ducks and their zoonotic potential in Kenya is not known. This study was aimed at investigating this carrier status in Kenya.

Hypotheses of this study were:

- that indigenous chicken and ducks are carriers of *Listeria monocytogenes*
- that indigenous chicken and ducks shed *Listeria* and other organisms into the environment.

The objectives of the study were to:

1. investigate the occurrence of *Listeria monocytogenes* in local scavenging chicken and ducks in selected farms, markets and slaughterhouses in Kenya.
2. investigate the carrier status of other aerobic bacteria in local scavenging chickens and ducks in selected farms, markets and slaughterhouses in Kenya.
3. investigate the antibiotic and disinfectant sensitivity patterns of the recovered *Listeria* isolates.
4. determine the efficacy of selected disinfectants in decontaminating rural chicken and duck coops.

1.2.Justification of the study

At the village level, humans and indigenous chickens and ducks live in close proximity in houses, especially at night. The chickens and ducks may contaminate the area they inhabit at night. They may also contaminate food, cooked food and water with bacteria and mainly through faeces and oropharyngeal discharges. There is therefore tendency for the respective bacterial concentrations in these areas to increase with time. Apart from infection through direct contact with infected birds and / or premises, humans can get infected through ingestion of the contaminated feed and / or water. Most of these bacteria, including *Listeria* species, *Staphylococcus* species, *Streptococcus* species, are zoonotic, hence of public health importance. *Listeria monocytogenes* is of particular importance because it causes neurologic, intestinal and respiratory tract infections in humans. Knowing the respective bacterial carrier status of the indigenous chicken and ducks will help in the development of control programmes for the respective diseases caused.

The increased prevalence of bacterial resistance to commonly prescribed antimicrobials and disinfectants in developing countries poses a major concern in the management of poultry and human infections. Occurrence of isolates resistant to antibiotics and disinfectants represents an emerging public health problem. Thus, the assessment of antibiotic and disinfectant resistance patterns of the *Listeria* isolates recovered from the indigenous chickens and ducks would give some picture of the general antibiotic / disinfectant sensitivity status of the recovered organisms. This will also contribute towards development of efficient management and control measures.

Under normal circumstances, various concentrations of various bacteria are deposited onto the environment (premises) by the birds, excreted from the gastrointestinal, urinary and respiratory tracts. Thus it is important to study the effect of this heterologous culture in terms of interbacterial protectiveness and also in terms of effect of bacterial concentration on antimicrobial agents. This will make it possible to establish whether the agent is effective in killing the bacteria / stopping bacteria from growing; if so, the number of times and respective intervals it will need to be applied in order to clear the premises of the organisms. Since farmers are advised to disinfect areas where birds inhabit at night, and also to disinfect coops and egg trays, used for transporting chicken and eggs, respectively, disinfectant sensitivity testing (monitoring) gives a more representative picture of this control system than antibiotic sensitivity testing

CHAPTER 2: LITERATURE REVIEW

2.1. *Listeria monocytogenes*

2.1.1. General comments on *Listeria* organisms

Listeria species are medium sized Gram positive rods, non – spore forming and non – acid fast, measuring about 0.4 – 0.5 µm in diameter by 0.5 – 2.0 µm in length. From rapidly growing cultures or tissues the cells appear coccoid (Quinn *et al.* 1994). Based on DNA – DNA hybridization analysis and 16s rRNA studies, *Listeria* have been divided into seven species, namely: *Listeria monocytogenes*, *Listeria ivanovii*, *Listeria innocua*, *Listeria seeligeri*, *Listeria welshimeri*, *Listeria grayi* and *Listeria murrayi* (Bille and Doyle, 1991; Cooper and Arthur, 1998).

2.1.2. Isolation and identification of *Listeria monocytogenes*

Listeria monocytogenes grows on blood agar containing 0.05% cysteine tellurite (which inhibits the growth of many gram-negative bacteria). Holding the media at 4°C (cold enrichment) also increases the chances of isolating *Listeria* since most of the competing organisms are inhibited at 4°C while *Listeria* continues to multiply. The cold enrichment technique is useful for culturing contaminated specimens (Carter, 1994). Grossly contaminated specimens such as faeces or environmental samples are cultured with a two-stage enrichment procedure that relies on nalidixic acid and acriflavin for inhibition of competing organisms. The sample is incubated at 30°C for 24 hours in a primary enrichment broth and then subcultured to a secondary enrichment broth at 30°C for 24 hours (Quinn *et al.*, 1994, Mackie and Mc cartney, 1996). A portion of the enrichment broth is transferred on to lithium chloride-phenylethanol-moxalactam (LPM) agar, which

contains lithium chloride and moxalactam as inhibitors (Bille and Doyle, 1991). Other selective media commonly used for the isolation of *L.monocytogenes* from contaminated specimens include oxford agar, modified oxford agar and polymyxin-acriflavin-lithium chloride-ceftazidime-esculin-mannitol (PALCAM) agar (Hirsh and Zee, 1999)

After 24-48 hours of growth, small, smooth, grey translucent colonies, 0 2-0 4 mm in diameter, are formed and on blood agar a narrow zone of B-hemolysis is usually produced. On clear media, such as lithium chloride-phenylethanol-moxalactam (LPM) agar, the colonies appear blue when viewed with an oblique light source. In broth cultures, turbidity is produced, which tends to settle on standing (Carter, 1994, Hirsh and Zee, 1999)

Listeria monocytogenes produces acid without gas from glucose, maltose, lactose, sucrose, trehalose, rhamnose and salicin It does not ferment arabinose, dulcitol, inulin, raffinose or xylose The organism is usually methyl-red positive, Voges-Proskauer positive, catalase-positive, esculin-positive, and produces a positive reaction on the Christie, Atkins and Muench-Peterson (CAMP) test with *Staphylococcus aureus*. The bacterium is urease-negative, gelatinase-negative, and does not produce hydrogen sulphide in triple sugar iron agar. A miniaturized biochemical multitest kit is available commercially for identifying all seven species of *Listeria* (Bille and Doyle, 1991; Bailey and Scotts, 1994; Holt *et al.*, 1994)

Other strain - specific typing methods including, serotyping (Donachie *et al.* 1992; Jacquet *et al.* 1993), phage typing (Jacquet *et al.* 1993), plasmid profiles (Fistrovici and Collins – Thompson, 1990), multilocus enzyme electrophoresis (Donachie *et al.* 1992; Harvey and Gilmour, 1994), restriction enzyme analysis (Donachie *et al.* 1992; Harvey and Gilmour, 1994), Pulsed – field gel electrophoresis (Brosch *et al.* 1994), and ribotyping (Nocera *et al.* 1993) have proven useful in characterizing *Listeria monocytogenes* isolates associated with human cases of food - borne listeriosis.

2.1.3. Serotyping of *Listeria monocytogenes*

Listeria monocytogenes has been subdivided into 16 serotypes, or serovars, based on the possession of different flagellar (H) and somatic (O) antigens. More than 90% of all cases of listeriosis reported worldwide in humans and animals (ruminants and poultry) are produced by serotypes 4B, $1\frac{1}{2a}$ and $1\frac{1}{2b}$ (Bille and Doyle, 1991). New methodologies used to characterise various strains of *L.monocytogenes* include ribotyping, multilocus electrophoresis, and DNA restriction enzyme analysis (Bille and Doyle, 1991).

2.1.4. Epidemiology of *Listeria monocytogenes*

A considerable rise in the number of listeriosis cases in renal transplant patients has been noted in recent years. In addition to newborns, elderly people are also at risk of contracting listeriosis, especially those suffering from malignancies or other debilitating diseases or conditions (Bojsen - Moller, 1972). Human infection can result from contact with affected birds or by consumption of their products and unpasteurised milk (Marsden, 1994). Infection can follow inhalation, ingestion, or wound contamination. Food borne

listeriosis in humans has been reported in United States, with about 2,500 cases occurring each year (Stapleton, 2002). Most cases, and most deaths, occur in pregnant women, newborns, the elderly and adults with weakened immune systems (Stapleton, 2002).

Listeria monocytogenes also affects cattle. On the farm, *Listeria* can enter raw milk by direct contact with contaminated milking equipment, manure, or bedding material (Sanaa *et al.*1993). Dairy cattle can also shed *Listeria monocytogenes* in milk asymptomatically as well as symptomatically as a result of *Listeria* – related mastitis (Farber *et al.* 1990, Ryser and Marth, 1991), abortion or encephalitis (Ryser and Marth, 1991).

Mice, rabbits, and guinea pigs are susceptible to experimental *Listeria monocytogenes* infection, and have been used to isolate the organism and to evaluate pathogenicity. The Anton test has been used as a presumptive test, wherein a purulent keratoconjunctivitis occurs 24 – 36 hours after the instillation of a drop of broth culture in the conjunctival sac of a rabbit or guinea pig (Hirsh and Zee, 1999).

2.1.5. Disease caused by *Listeria monocytogenes*

2.1.5.1. Listeriosis in poultry

The most common avian hosts of *Listeria monocytogenes* are chickens, ducks, turkeys, geese, and canaries (Barnes, 1991). Infection in other avian species has been reported less frequently. All age groups are susceptible, but the disease is primarily one of the young birds, where it may cause a septicemic infection, with focal necrosis in the liver and

myocardium, pericarditis and, occasionally, encephalitis. Outbreaks of the encephalitic form have been seen in commercial broiler flocks, where the predominant clinical sign is torticollis (Cooper, 1989).

Lesions seen histologically include focal microabscesses, gliosis, and perivascular lymphocytic cuffing in the medulla of the brain. In the septicemic form, multiple focal hepatic abscesses and myocardial degeneration are common. Bacteria may be found in Kupffer's cells of liver or in the periphery of brain lesions. Infiltration of plasma cells, lymphoid cells, and macrophages are characteristic of the lesions (Cooper and Arthur, 1998).

2.1.5.2. Listeriosis in humans

The major cause of contraction among adults is ingestion of contaminated foods; among infants it is transplacental infection. Those most susceptible to infection are pregnant women, alcoholics, extremes of age, and immunocompromised individuals (Bailey and Scotts, 1990, Stapleton, 2002).

In humans, meningitis is the most common of the three forms of listeriosis. Other disease manifestations include infective endocarditis, oculoglandular disease, and dermatitis (Walker, 1999). It also manifests as encephalitis, uterine infections with abortion, stillbirths, and a neonatal septicemic form called granulomatosis infantiseptica; febrile pharyngitis; and septicemia (Bailey and Scotts, 1994). Cutaneous listeriosis has been

reported in a 64 – year – old male who acquired the organism from a cow while assisting in the delivery of a stillborn calf (Busch, 1971)

Stapleton (2002), describes the initial symptoms of human listeriosis as being similar to the flu and can include fever, muscle aches, nausea or diarrhoea. If the infection spreads to the nervous system, symptoms include headache, stiff neck, confusion, loss of balance, or convulsions.

2.1.5.3. Listeriosis in other domestic animals

Septicemia, meningoencephalitis, and abortion are the major disease forms. The septicemic form marked by depression, inappetence, fever, and death is the most common in monogastric animals and in neonates (Walker, 1999). Septicemia in neonates is the most common presentation in horses. Chinchillas are particularly susceptible to listerial septicemia (Walker, 1999)

The encephalitic form, sometimes called, " circling disease," is the most common form in ruminants. In cattle, it is subacute to chronic. Signs include depression, anorexia, and tendency to circle in one direction, head pressing or turning of the head to one side, unilateral facial paralysis, and bilateral keratoconjunctivitis. Similar signs are seen in sheep and goats, but the course is more acute and frequently fatal (Walker, 1999)

Abortion is common in ruminants, but also occurs in other species. Abortion is usually late term – after 7 months in cattle and 12 weeks in sheep. Retained placenta and metritis

may result. Systemic signs are rare in the cow unless the fetus is retained and triggers a fatal septicemia. Although abortion is usually sporadic, abortion rates of upto 10% have been noted (Ryser and Marth, 1991). Dairy cattle can also shed *Listeria monocytogenes* in milk asymptomatically as well as symptomatically as a result of *Listeria* – related mastitis (Farber *et al* 1990; Ryser and Marth, 1991)

2.1.6. Prevention and Control of listeriosis

Control measures for listeriosis include reduction or elimination of feeding of silage, particularly poor quality silage, and improving general hygiene. All forms of stress should be minimized. Affected animals should be slaughtered and either buried or burnt and buildings thoroughly disinfected. Since antibody production by infected farm animals is so variable, serological tests are of little value for the detection of infected individuals (Buxton and Fraser, 1977). Vaccination has not proven to be highly successful and may not be warranted due to the sporadic nature of the disease (Walker, 1999)

2.2.Other aerobic bacteria isolated from chicken and ducks

Bacteria other than Listeria species isolated from chicken and ducks include: *Campylobacter jejuni* (Osano and Arimi, 1999; Shane, 2000; *Uyttendele et al.*, 1999), *Pasteurella multocida* (Mbuthia *et al.*, 2002), *Escherichia coli* (Wooley *et al*., 1994), *Staphylococcus spp.* (Kibenge *et al*., 1982; Notermans *et al.*, 1982; Chen *et al.*, 1984; Jensen *et al*., 1987), *Streptococcus spp.* (Alaboudi *et al.*, 1992), *Erysipelothrix rhusiopathiae* (Bricker and Saif, 1997) and *Mycobacterium avium* (Denis, 1994; Falkingham, 1994).

Staphylococcus species and other *staphylococci* have been associated with poultry throughout the world, including Argentina, Australia, Belgium, Bulgaria, Canada, China, Costa rica, France, Germany, Hungary, India, Italy, Japan, Netherlands, Pakistan, Poland, Romania, Taiwan, the United Kingdom and the United States of America (Kibenge *et al*., 1982; Notermans *et al*., 1982; Chen *et al*., 1984; Jensen *et al*., 1987). Morbidity and mortality due to staphyloccosis is usually low, unless there has been massive contamination of chicks, because of exposure to unusually high numbers of bacteria in the hatchery through environmental contamination, vaccination or servicing procedures (Willet, 1992).

Streptococcus zooepidemicus occurs almost exclusively in healthy appearing chickens, it has been documented as a cause of mortality in wild birds (Ivanics *et al*., 1984). *Enterococcus faecalis* affects species of all ages, it is the most serious disease occurring in embryos and young chicks from fecal contaminated eggs (Alaboudi *et al*., 1992). *Streptococcus faecium* and *S. mutans* have been identified as causes of mortality in ducklings and goslings, respectively (Rudy, 1991).

Colibacillosis of poultry is a common systemic infection caused by *Escherichia coli*. The disease is economically important to poultry production worldwide. The various serotypes of *E. coli* are intestinal inhabitants of animals, including humans, and probably infect most mammals and birds; therefore, they have a cosmopolitan distribution. Clinical disease is reported most often in chickens, turkeys, and ducks. Coliform bacteria can be

found in litter and fecal matter. Dust in poultry houses may contain $10^5 - 10^6$ *E. coli* / gram (Wooley *et al.*, 1994). These bacteria persist for long periods, particularly when in dry matter (Leitner and Heller, 1992)

Erysipelothrix rhusiopathiae is ubiquitous in nature and has been isolated from a variety of environmental sources, including soil, food, and water. Contamination of the environment is thought to occur secondary to excretion of the organism by infected or colonized animals. Outbreaks of erysipelas in poultry occur sporadically, although there are areas in the world where the disease is endemic (Bricker and Saif, 1997). Although the disease in turkeys has been reported more frequently among males than females, there is no evidence of differing susceptibility between sexes (Bricker and Saif, 1997)

2.3. Antibiotic sensitivity / resistance

2.3.1. General remarks

Antibiotics are broadly defined to include all antibacterial drugs in conformity with the classification in the WHO model list of essential drugs (WHO, 1995). The term antibiotic has been defined as a chemical produced by a microorganism that inhibits the growth of other microorganisms (Bailey and Scotts, 1990). Antibiotics may be natural or synthetic. They range from sulphonamides, penicillin, streptomycin, tetracyclines, chloramphenicol to other antibacterial substances (Bailey and Scotts, 1990).

Antimicrobial drugs are divided into two classes, based upon their general effects on bacterial populations That is, bactericidal and bacteriostatic drugs The major antimicrobial drugs are grouped according to their mechanism of action as follows: (i) inhibition of growth by analogues for instance, sulfonamides and sulfones; (ii) Inhibition of cell wall synthesis, which includes, semisynthetic penicillins, cephalosporins, cycloserine, bacitracin and vancomycin; (iii) Inhibition of protein synthesis for instance, aminoglycosides, streptomycin, neomycin, kenamycin, gentamicin, tobramycin, spectinomycin, amikacin, tetracyclines, chloramphenicol, erythromycins, tylosin, clindamycin and lincomycin; (iv) impairment of membrane function which encompasses polymyxins (B and E); (v) inhibition of nucleic acid synthesis, includes quinolones, rifampin and novobiocin (Carter and Chengappa, 1991)

Antibiotic resistance in bacteria is widespread in Kenya (Bebora, 1987, Senerwa, 1988) Being part of the bacterial family, *L. monocytogenes* is no exception to this. Specific reporting of antibiotic resistance to *L. monocytogenes* has been made by a number of workers (Courvalin, 1996; Gray and Killinger, 1966) *Listeria monocytogenes* was shown to be resistant to sulphonamides, penicillin G, amoxycillin, chloramphenicol and streptomycin (Courvalin, 1996).

In certain circumstances the application of an antibiotic may co – select for resistance to other related or non – related antibiotics. For example, London *et al.* (1994) observed an increase in prevalence of amoxycillin and tetracycline resistant faecal flora of patients

treated with amoxycillin. Similarly, treatment of patients with duocycline resulted in an increased prevalence of resistance to both tetracycline and amoxycillin.

Cross – resistance to different antibiotics is a common phenomenon. A bacterium can become simultaneously resistant to several β - lactams by the acquisition of a single β - lactamase (Fisher, 1985). This is referred to as positive cross – resistance since the pleiotropic effect of the β - lactamase is to increase resistance to these β - lactams. Similarly, negative cross – resistance can develop if a resistance gene has a pleiotropic effect of increasing resistance to one antibiotic while decreasing resistance to a second one. For example, resistance to isoniazid and ethionamide in *Mycobacterium tuberculosis* are negatively correlated, as are resistance to rifampicin and novobiocin in a *Microspora* species and resistance to tetracycline and fusaric acid in both *Salmonella typhimurium* and *Escherichia coli* (Maloy and Nunn, 1981; Gado *et al.*, 1982 and Canetti, 1965).

The increasing prevalence of bacterial resistance to commonly prescribed antimicrobials especially in developing countries poses a major concern in the management of infections (Shanaham, *et al.*, 1993; Nijsten, *et al.*, 1996a). This has mainly resulted from extensive use and often misuse of antimicrobials in both human and veterinary medicine (Kayser, 1993; Mitsuhashi, 1993). The finding that plasmids, which can transmit the antibiotic resistance factor, can spread between different species and genera has led to the concept of 'epidemic plasmids' (O' Brien *et al.*, 1997). Bacteria have evolved mechanisms to withstand the attack of antibiotics and in parallel, mechanisms for the spread of these

characters to other bacteria by means of exchange of genetic material (Perra – Lopez *et al.* 1993; Wieldemann, 1993; Rasmussen *et al.* 1993)

2.3.2. Testing for susceptibility to various antibiotics

There are two main methods for testing the susceptibility of the antibiotics; namely disc diffusion method and dilution test as discussed below. They measure the ability of the respective drug to inhibit the growth of microorganism (s).

2.3.2.1. Disc diffusion method

This method involves use of disks impregnated with specific antibiotics, which are placed on agar plates previously inoculated with the organism to be tested (Bauer *et al.*, 1966). The plates are then incubated overnight and examined for zones of inhibition (Washington, 1985). The sizes of zones of growth inhibition determine the level of resistance, whereby the smaller the zone or none at all the more resistant the bacterium is. However, it is not advisable to compare the zonal sizes of different drugs acting on the same microorganisms. The zonal sizes are supposed to be compared with a standard culture, provided the media, innoculum sizes and other conditions are carefully standardized. The respective minimum zone sizes may then be listed (Jawetz *et al.*, 1970). The results are interpreted according to the critical diameters set by Mc Ghie and Finch (1975), as follows: inhibition zones upto 2 millimetres from the edge of the disc to the inhibition zone front (13mm diameter) are taken as resistant, and those beyond 2 millimetres (13mm diameter) are taken as sensitive.

Tablets may also be used in place of discs. Each tablet is made aseptically and contains a standard concentration of the desired drug (Bou cassals, 1980) Jawetz *et al.*, (1970) compared impregnated paper discs with tablets and found a larger safety margin in interpretation of results with tablets than with paper discs.

The single disc method used in most clinical laboratories has been shown to correlate well with results of dilution techniques. The method is suitable for most rapidly growing pathogens (Bou cassals, 1980)

2.3.2.2. Dilution method: Minimum inhibitory concentration (MIC) test

The broth minimum inhibitory concentration (M I C.) tests are often considered to be the standard reference method for the evaluation of antibiotic resistance according to Waterworth (1980). In the MIC test, the antibiotic is diluted and the effect of the various dilutions on bacterial growth is noted: if susceptible there is no turbidity; if resistant there is turbidity. The dilution normally used is two – fold. In general, dilution tests are considered satisfactory if the MIC'S vary no more than +1 or – 1 dilution step around the mean on repeated tests. Conventional MIC testing procedures are not able to differentiate satisfactorily between cultures that are suppressed overnight but residual turbidity remains; those unaffected by antibiotic; and those that are inhibited by antibiotic but regrowth occurs after antibiotic inactivation (Greenwood and Eley, 1982)

For most purposes, a concentration of 128 µg/ml is a satisfactory upper limit for routine testing with any antimicrobial agent. The lowest concentration selected will vary according to the type of microbial agent In general, however, this concentration should be below the

upper limit of a high degree of susceptibility. The range of concentrations should include the end-point for appropriate standard strains such as *E. coli* ATCC 25922 to permit adequate control (Washington, 1985).

In addition to broth dilution MIC's, another method for testing the MIC's of a large number of isolates against many concetrations of several antimicrobial agents is that of agar dilution. For this method, concetrations of antimicrobial agents are incorporated into agar plates, one plate for each concentration to be tested. The organisms to be tested are diluted to a slightly greater turbidity than that of a Mc Farland 0.5 standard, and an aliquot of each suspension is placed into one well of a replicating inoculator device (called a Steers – Foltz replicator). This device has metal prongs that are calibrated to pick up a small amount of the bacterial suspension (usually 0.001 ml) and deliver it to the agar surface. At least 25 different strains plus controls can be placed in the wells of the inoculator for delivery to each plate in a single manual movement. In this manner, approximately 1×10^4 colony forming units (CFU) are delivered in a discrete drop to the surface of agar plates containing different concentrations of antimicrobial agents. After overnight incubation, the organisms will grow on those plates that do not contain enough antimicrobial agents to inhibit them. The lowest concetration of agent that allows no more than one or two CFU or only a slight haze to grow is the MIC. Minimum bactericidal concentration (MBC) results cannot be determined using this technique (Bailey and Scotts, 1990).

Although agar dilution susceptibility testing is not commonly used by clinical laboratories for testing aerobic organisms, it is a good research laboratory technique for testing

anaerobic bacteria and also is used as a reference method against which other methods are compared for testing anaerobic bacteria (Bailey and Scotts, 1990). The agar dilution method has advantages of being able to test a number of strains simultaneously, detect microbial heterogeneity or contamination, and has a slightly better reproducibility than broth dilution method (Ericsson and Sherris, 1971).

2.4. Bacterial disinfectant sensitivity

2.4.1. General remarks on disinfectants

Disinfectants are chemical agents, which provide the most widespread means of chemical decontamination and, depending on intended use, are formulated with solubilising, emulsifying or suspending agents (Spooner and Sykes, 1972). The European committee for the standardisation of disinfectants has defined disinfection as 'the selective elimination of certain undesirable organisms in order to prevent their transmission, achieved by action on their structure or metabolism, irrespective of their functional state' (Reber, 1973). The British standard defines disinfection as the destruction of microorganisms, but not usually bacterial spores. Disinfection does not necessarily kill all microorganisms but reduces them to a level acceptable for a defined purpose (British standards Institution, 1991; Zander *et al.*, 1997). Some disinfectants, notably formaldehyde, glutaraldehyde and chlorine – containing agents, give off irritant toxic or allergenic vapours and the occupational exposure limits for these substances must not be exceeded (Health and Safety Executive, 1991). These levels are revised annually. Disinfectants range from phenolics, hypochlorides, glutaraldehydes, ethyl alcohols, pine fluids, quartenary ammonium compounds, and ampholytes to bisguanides (Health and safety Executive, 1991). They may

kill vegetative bacteria, fungi, and viruses and, occasionally, spores by the destruction of proteins, lipids or nucleic acids in the cell or its cytoplasmic membrane (British Standards Institution, 1991)

2.4.2. Methods for testing the efficacy of disinfectants

A great variety of techniques have been employed in the laboratory by microbiologists studying the effects of physical agents and chemicals on fungi, bacteria and viruses since their role in the initiation of infection and disease was established in the latter part of 19th century by Semmelweiss, Pasteur, Koch and Lister (Stuart, 1968) Of the many *in vitro* laboratory methods presently employed in comparing germicidal chemicals one with another and providing an index to the concentration of products which can be employed in disinfecting inanimate surfaces where infectious organisms are suspected of being present, the Association of Official Agricultural Chemists' (AOAC) phenol coefficient method (1965) has been appraised more for its precision and accuracy (Stuart, 1968, Spooner and Sykes, 1972). However, it has been shown that, considering precision or reproducibility of results, variations ranging from - or + 12% to – or + 23% have been reported (Stuart., 1968)

There are two main methods for testing the efficacy of disinfectants. The first one is the phenol coefficient method and the second is the agar well technique (Spooner and Sykes, 1972). The phenol dilution method is basically a dilution tube technique and it is affected by various factors including culture media, test culture maintenance routines, test culture exposure manipulations, subculture routines, and temperature for test organism exposure

and subculture incubations (Stuart, 1968) This method is designed to determine the highest dilution of a germicidal chemical, which kills the test organism within a series of time intervals under specified conditions From the test results, and comparable results obtained at the same time with the pure chemical, phenol, a specific calculation is made to yield a product known as "the phenol coefficient number". This number is employed to calculate the dilution which might be presumed to be equivalent in germicidal activity to a 5% solution of the pure chemical (phenol) or the maximum dilution that can be relied upon to disinfect under conditions commonly encountered in actual use The maximum dilution calculated is taken as that required for practical disinfecting applications (Bass and Stuart, 1968). The phenol coefficient method yields a result, which can be interpreted only indirectly, in terms of the concentration of the product necessary for actual disinfection (Bass and Stuart, 1968)

In the agar well method, a plate of sterile agar, poured to a depth of about 4 millimetres is allowed to set and a uniform lawn of the relevant bacteria is spread on the surface. Then a single well or several wells, each of 15-millimetre diameters, cut from the centre of the plate with a sterile cork borer. Then, the wells are filled with the respective disinfectants The disinfectant is allowed to diffuse into the agar and produce a zone of inhibition of growth. In the other method: the disinfectant is incorporated into the agar and the ability of an organism to grow on the surface of such a medium is assessed by direct observation for colonial growth (Spooner and Sykes, 1972)

CHAPTER 3: MATERIALS AND METHODS

3.1. Study area

This study was carried out in Embakasi and Dagorretti Divisions in Nairobi District; Athi River Division in Machakos District and Ngong Division in Kajiado District, in Kenya. Indigenous chickens were sampled from 4 farms in Embakasi division and 3 farms from Athi river division. Indigenous ducks were sampled from two farms each in Embakasi, Ngong and Dagorretti divisions

The study was also carried out at Kariokor and Burma - Maziwa slaughterhouses, Jogoo road and Kariokor open - air markets, and Nairobi South and Westlands shopping centres. At the time of sampling the bird's rural district of origin was recorded

3.2. Birds sampled and other experimental animals used

3.2.1. Chicken and ducks

Indigenous chicken and ducks were sampled. Chicken included: cocks, hens, pullets and chicks. Chickens were sampled from the farms and slaughterhouses. Others were obtained from city markets in Nairobi and transported to the laboratory where sample taking was done. Ducks sampled comprised drakes, mature ducks, pullet ducks and ducklings. They were swabbed at the farm.

3.2.2. Mice

Three week old, Balb – C mice, prescreened and confirmed to be ***Listeria*** – free, were used for this study. Both males and females were used for *Listeria* isolation and for pathogenicity testing. Mice colonies were raised and maintained within the university premises.

3.3. Type cultures used in this study

Three type cultures were used in this study. These were; *Listeria monocytogenes* type strains (L028 and DGH) from Denmark and *Staphylococcus aureus* NCTC 6571 and *Escherichia coli* ATCC 25922 from Kenya Medical Research Institute (KEMRI). *Listeria monocytogenes* type cultures were used for identification and characterization of *Listeria* isolates and also for antibiotic and disinfectant sensitivity tests. The *Staphylococcus aureus* NCTC 6571 and *Escherichia coli* ATCC 25922 were used as controls for antibiotic sensitivity tests.

3.4. Sample collection and handling

Forty-one (41) indigenous local village chickens were sampled from slaughterhouses, 40 from traders and 55 from farms. Similarly 39 ducks were sampled from farms. Cloacal and oro -pharyngeal swabs were taken separately from each bird using sterile cotton - tipped applicator swabs. The swabs were placed in 2 ml of sterile physiological saline and transported on ice in a cool box to the laboratory for bacterial isolation. Intestinal swabs taken from 41 slaughtered birds were treated as cloacal swabs.

Random (probability) sampling was carried out with the sample size for estimation of a proportion (π) worked out as follows:

$n = (Z_{\alpha/2})^2 \quad P(1-P)/L^2$

L was the required precision

P was the anticipated prevalence or proportion of attribute.

$(Z_{\alpha/2})^2$ was approximately $(1.96)^2$ so that the estimate was at the 95% level of confidence.

The proportion could not be greater than 0.35, hence, P= 0,35.

At 95% confidence interval, L= 0.05

Hence,

$N = (1.96)^2 (0.35)(0.65)/(0.05)^2 \approx 350$ samples.

These were estimated to be 175 birds since two samples (oropharngeal and cloacal swabs) were taken from each bird.

The choice of study area was governed by availability of transport so that areas nearest to the department of veterinary pathology and microbiology were chosen. The ducks population (N) within the sampling frame was quite low, hence the required sample size (n) was lowered.

3.5. Bacterial isolation and characterisation

Samples were processed for the isolation and characterisation of *Listeria monocytogenes* and other aerobic bacteria presumed to be resident flora as described below.

Within one hour of sampling, each swab was processed in two ways:

(1) **Direct isolation**: done by streaking the swabs onto 3.5 % potassium tellurite blood agar (PTBA) (BDH Chemicals Ltd. Poole, England) plate, to isolate *Listeria species.* The plates were then incubated at 37^0C for 48 hours. The swabs were also streaked onto Mc Conkey (Lab M. BL 96 AU. Topley Hse, Bury, England) and blood agar base (CM 55: Oxoid Ltd., Basingstoke, Hampshire, England) with 5% citrated sheep blood, and then incubated at 37^0C aerobically for 24 hours inorder to isolate the other aerobic bacteria

(2) Indirect isolation done to increase the chance of isolating *Listeria*

The contents of each swab were thoroughly vortexed and 0.5 ml inoculated intraperitoneally into *Listeria* – free 21 day – old Balb / c mice. Inoculated mice (each with material from a different specimen) were observed for 48 hours. The dead mice were then opened up and the living ones sacrificed. Liver and spleen were aseptically removed and macerated using separate sterile wire loop for each and the macerated materials streaked onto potassium tellurite blood agar plates separately. Plates were incubated aerobically at 37^0C for 48 hours.

After 24 - 48 hours, all the colonies from all the media used were subcultured onto sheep blood agar (CM 55: Oxoid Ltd. Basingstoke. Hampshire. England) and incubated

aerobically at 37^0C overnight, to obtain pure cultures for characterization. The isolates were characterised following the criteria given by Bergey's manual of determinative bacteriology (Holt *et al.*, (1994) and Cowan and Steel's Manual for the identification of medical bacteria (Barrow and Feltham, 1993).

3.6. Viable counting of *Listeria monocytogenes*

Viable counting was done to standardize seeding for antibiotic and disinfectant sensitivity tests and also establish the dose rate for the mice pathogenicity tests.

The viable counting, using an 18 – hour culture in Brain – Heart Infusion (BHI) broth, was done following the method of Miles and Misra (1938). Ten fold dilutions were made ranging from 10^{-2} to 10^{-10}. Two drops from each dilution were dropped separately onto blood agar base (CM 55: Oxoid Ltd., Basingstoke, Hampshire, England) with 5% citrated sheep blood. This was done using a calibrated dropper, which drops 40 drops per millilitre of broth (that is, each drop = 25 microlitres). The drops were allowed to dry and the plates were incubated aerobically at 37°C overnight.

The plates were then examined and visible colony counts taken per drop, at a dilution where the colonies were about 30 and below. Noting the average number of colonies (N) in one drop, at a particular dilution 10^x, the concentration of the original harvested culture was computed using the following formula;

$N \times 40 \times 10^x$ organisms / ml.

Where N is the average number of colony forming units per drop and 10^x is the dilution factor.

3.7. Mice pathogenicity testing for *Listeria* isolates

Mice pathogenicity testing was carried out as an additional criterium towards *Listeria* characterisation and identification (Hirsh and Zee, 1999). A final concentration of 10^9 cfu/ ml of each isolate was worked out and then inoculated at a dose rate of 0.5 ml into each of the five *Listeria* - free 21 day - old Balb / c mice intraperitoneally. The mice were kept in separate cages correspondingly labelled for the respective *Listeria* isolate or type culture. The mice were observed for at least 5 days for any clinical signs and mortality. Any mouse that remained alive was euthanized. From each mouse, the liver and the spleen were first examined thoroughly for necrotic foci and splenomegally, respectively. They were then aseptically removed, macerated and a loopful of the macerated materials streaked onto marked potassium tellurite blood agar plates and subsequently incubated at 37°C for 48 hours inorder to reisolate the *Listeria* organisms.

3.8. Antibiotic sensitivity testing of *Listeria* isolates including the *Listeria monocytogenes* type strains (L028 and DGH) from Denmark.

The test was done using modified controlled disk diffusion technique (Stokes and Waterhouse, 1973) on Mueller – Hinton agar (Oxoid Limited, Basingstoke, Hampshire, England). The two *Listeria monocytogenes* isolates and the seven other *Listeria species* isolated were subcultured from egg yolk agar slants onto sheep blood agar and incubated

aerobically at 37°C overnight. Single colonies from respective isolates were then inoculated into Brain – heart infusion broth (Oxoid, Basingstoke, and Hampshire, England) and incubated at 37°C aerobically for 18 hours.

To prepare the bacterial lawn on petri dishes, 150 microlitres of the bacterial suspension containing 10^7 cfu / ml were dropped onto each Mueller – Hinton agar plate and then spread out using a sterile glass rod. Antibiotic discs (manufactured by ABTEK Biologicals), were then placed on the inoculated plates using a pair of sterile forceps, which were also used to gently press the discs onto the surface. The plates were then incubated at 37^0C overnight and the diameters of each zone of inhibition, including the diameter of the disc, measured in millimetres. The results were interpreted according to critical diameters set by National Committee for Clinical Laboratory Standards (NCCLS), USA, as illustrated on table 1. *Staphylococcus aureus* NCTC 6571 and *Escherichia coli* ATCC 25922 from Kenya Medical Research Institute (KEMRI) were used as controls. In the interpretation of the results, only those that showed full susceptibility as given by NCCLS tables were considered; hence the intermediate diameters were ignored.

The antibiotics that were used in the test included: ampicillin (25 μg); tetracycline (25 μg); cotrimoxazole (25 μg); augmentin (30 μg); kenamycin (30 μg); gentamicin (10 μg); cefuroxime (30 μg) and chloramphenicol (30 μg). Ampicillin, tetracycline and cotrimoxazole were in higher concentrations than the recommended minimum inhibitory concentration by NCCLS.

Table 1: Interpretation of disc susceptibility tests.

Antimicrobial agent	Disc content (μg)	Disc diameter (mm)		
		Resistance	Intermediate	Susceptible
Ampicillin	10	≤ 20	21 - 28	≥ 29
Tetracycline	10	≤ 14	15 - 18	≥ 19
Cotrimoxazole	23.75	≤ 10	11 -15	≥ 16
Augmentin	30	≤ 19	0	≥ 20
Kenamycin	30	≤ 13	14 - 17	≥ 18
Gentamicin	10	≤ 12	13 - 14	≥ 17
Cefuroxime	30	≤ 14	15 – 17	≥ 18
Chloramphenicol	30	≤ 12	13 - 17	≥ 18
Oxacillin	1	≤ 10	11 - 15	≥ 16
Cephtazidime	30	≤ 14	15 – 17	≥ 18
Erythromycin	15	≤ 13	14 - 17	≥ 18
Vancomycin	30	≤ 9	10 - 11	≥ 12
Amoxyclav	30	≤ 19	0	≥ 20

Source: National clinical control of Laboratory standards (NCCLS), USA. (1987) as given by Oxoid manual, 1987.

3.9. Disinfectant sensitivity testing of *Listeria monocytogenes* (2) and other *Listeria* (7) isolates including the *Listeria monocytogenes* type strains (L028 and DGH) from Denmark.

A diffusion technique using wells punched into Mueller – Hinton agar was used, following the method of Bebora (1987). The bacteria were seeded in the same way as for antibiotic sensitivity testing, given in section 3.7. After seeding, wells were punched using a sterile 6 mm diameter well – cutter. Five different dilutions of 7 commonly used disinfectants (two folds of double dilution below and two folds above the Manufacturer's user - dilution), as shown on table 2, were dispensed using a micropipette into different wells, using one petri – dish per disinfectant. Each well was loaded with 50 μl of the

relevant disinfectant dilution. The plates were then incubated upright at 37^0c overnight and the diameters of each inhibition zone measured and recorded in millimetres. The results were interpreted as follows: inhibition zones of upto 2 millimetres from the edge of the well to the inhibition zone front (10mm diameter) were taken as resistant, and beyond 2 millimetres (10mm. diameter) were taken as sensitive.

3.10. Disinfectant sensitivity of pooled pharyngeal and cloacal cultures.

A total of 14 (8 from Ruai and 6 from Katani) pooled pharyngeal and cloacal swabs were tested. To make the pooled culture preparation, individual cloacal and pharyngeal swab extracts were mixed in equal volumes after which 150 µl of the undiluted mixture was placed on a Mueller – Hinton agar plate and spread using a sterile glass rod. Wells punched in the inoculated plates were filled with respective disinfectants and the plates incubated at 37^0c overnight. Interpretation of the results was as per section 3.9. Total clearance of lawn around the respective disc (no growth) was taken as complete sensitivity; no change in growth density (no clearance), was taken as resistance; any degree of reduction of growth density (partial clearance) was taken as partial sensitivity; it indicates that some bacterial organisms were killed or rendered static. Bacteria growing at the partial sensitivity area were subcultured and disinfectant sensitivity testing redone on them.

Table 2: Dilutions of the various disinfectants

Disinfectant type	Dilutions				
	1	2	3*	4	5
Omnicide®	1%	0.5%	0.25%	0.125%	0.0625%
Bromosept 50®	0.2%	0.1%	0.05%	0.025%	0.0125%
Sodium hypochloride	10 %	5%	2.5%	1.25%	0.625%
Lavik®	16%	8%	4%	2%	1%
Dettol®	24%	12%	6%	3%	1.5%
Kleenol®	5.3%	2.6%	1.3%	0.67%	.33%
Lysol®	20%	10%	5%	2.5%	1.25%

Legend:
3*: Recommended user dilution
Omnicide® (Cooper Kenya Ltd., Kaptagat Road off Waiyaki way, - 0.25% Glutaraldehyde and Coco - benzyl dimethyl ammonium chloride (a QAC); **Bromosept 50®** (ABIC Ltd., Netanya, Israel, - Didecyldimethyl ammonium bromide 50% w\v); **Jik®** (Reckitt Benckiser Easrt Africa Ltd., Likoni Road, Nairobi, - 10% Sodium hypochlorite); **Lavik®** (United chemical industries Ltd.; Unichem, - Pine disinfectant and antiseptic); **Dettol®** (Reckitt Benckiser East Africa, Nairobi – 4.8% chloroxylenol); **Kleenol®** (Biodeal Laboratories Ltd., Nairobi, 7 – 12% phenol content) and **Lysol®** (Alpha chemicals Ltd., Lunga Lunga Road, Nairobi – cresol and soap solution).

3.11. Statistical analysis

Paired F- test in STATISTIX computer package was used to analyse the data. The proportions of antimicrobial resistant *Listeria* isolates were compared with the two *Listeria monocytogenes* reference strains ((L028 and DGH) from Denmark. The proportions of *Listeria* isolates resistant to the various disinfectants were also compared between the various disinfectants dilutions and their recommended – user dilutions.

CHAPTER 4: RESULTS

4.1. *Listeria* isolation

Tables 3 and 4 show the *Listeria* organisms isolated from farms, markets and trading centres, and slaughterhouses with respect to indigenous chickens and ducks.

From the market and trading centres, 2 (5%) *Listeria monocytogenes* and 6 (15%) other *Listeria* isolates namely, *L. innocua* (3); *L. seeligeri* (1); *L.grayi* (1) and *L. murrayi* (1) were recovered from a total of 40 indigenous chickens sampled. *Listeria seeligeri* was isolated from oropharyngeal region of a farm chicken and two *Listeria monocytogenes* isolates were recovered also from the oro -pharyngeal regions of two market chickens. No recovery of *Listeria* was made from the cloaca samples. Ducks and slaughter chickens were all negative for *Listeria* (appendices 1, 2, 3 and 4).

4.2: Mice pathogenicity test for *Listeria* isolates

After inoculating with 0.5 ml of *Listeria* isolates, intraperitoneally, one *Listeria monocytogenes* and one *L.innocua* were found to be pathogenic, with *L. monocytogenes* (47piv) killing 80% of the mice within 72 hours, and *L. innocua* (45pi) killing 60% of the mice within 72 hours. The main clinical signs observed were, dullness and diarrhoea. Surprisingly, one *L. monocytogenes* isolate did not affect the mice. No further studies were carried out on this isolate to unravel the unexpected behaviour. Both *Listeria monocytogenes* type strains were pathogenic to mice. The other seven *Listeria* isolates were non- pathogenic to mice (Table 5).

Table 3: *Listeria* isolations from farm chickens, market chickens, slaughter chickens and farm ducks

Type of birds	Source of birds		Number of birds sampled	Samples taken		*Listeria* spp. isolation	
				C	P	C	P
Chickens	Farm	Nairobi	26	26	26	0/26	1/26
	Farm	Machakos	29	29	29	0/29	0/29
	Total Farm		**55**	**55**	**55**	**0/55**	**1/55**
	Market	Nairobi	40	40	40	0/40	8/40
	Slaughterhouse	Nairobi	41	41	41	0/41	0/41
Total chickens			**136**	**136**	**136**	**0/136**	**9/136**
Ducks	Farm	Nairobi	34	34	34	0/34	0/34
	Farm	Kajiado	5	5	5	0/5	0/5
Total ducks			**39**	**39**	**39**	**0/39**	**0/39**
Total chickens and ducks			**175**	**175**	**175**	**0/175**	**9/175**

Legends:

C: Cloacal

P: Oro- pharyngeal

Table 4: The type of *Listeria* isolates from farms and markets chickens

Source	No. of birds	*Listeria* isolates
Farms	55	*Listeria seeligeri* (1)
Markets	40	*Listeria monocytogenes* (2)
		Listeria seeligeri (1)
		Listeria innocua (3)
		Listeria grayi (1)
		Listeria murrayi (1)

Table 5: Mice pathogenicity test results

***Listeria* isolates**	**Clinical signs**	**Mortality (%)**	**Re–isolation of *Listeria***
L.monocytogenes^{T} *1* (L028)	Dull, diarrhoea	100	+
L.monocytogenes ^{T} 2 (DGH)	Dull, diarrhoea	80	+
L.monocytogenes (47piv)	Dull, Diarrhoea	80	+
L.monocytogenes (46pv)	Bright	0	-
L.seeligeri (8piv)	Bright	0	-
L.seeligeri (48pi)	Bright	0	-
L.innocua (53pii)	Bright	0	-
L.innocua (45pi)	Dull	60	+
L.innocua (51pv)	Brightl	0	-
L.grayi (11pii)	Bright	0	-
L.murrayi (11piii)	Bright	0	-

Legends:

+: Reisolated

-: No reisolation

L.monocytogenes^{T}1 (L028): Reference strain from Denmark

L.monocytogenes ^{T} 2 (DGH): Reference strain from Denmark

4.3: Other bacteria isolated

Tables 6 and 7 show other aerobic bacteria isolated from farms, markets and trading centres, and abattoirs, with respect to indigenous chicken and ducks. For comparison purposes the proportions of *Listeria* organisms recovered were included in the two tables

From the market and trading centres, *Staphylococcus aureus* (20.1%); *Streptococcus spp.* (14.3%); *Escherichia coli* (33.9%) and unidentified bacteria (11.7%) were recovered from a total of 40 indigenous chicken sampled.

From the farms, the following bacterial pathogens were isolated: *Erysipelothrix spp.* (1.8%); *Staphylococcus aureus* (23.1%); *Streptococcus spp* (19.7%); and *Escherichia coli* (48.1%); 5.5% chicken yielded other bacteria, which were not identified, including 3 *Campylobacter* – like bacteria. The isolates recovered from the ducks included: *Erysipelothrix spp.* (2.6%); *Staphylococcus aureus* (20.1%); *Streptococcus spp.* (21.2%); and *Escherichia coli* (40.2%) and 15.9% ducks yielded other bacteria.

From the forty-one indigenous chicken sampled from slaughterhouses, 4.9% had *Erysipelothrix spp.;* 28.4% had *Staphylococcus aureus;* 22.5% had *Streptococcus spp.*; 40.1% had *Escherichia coli* while 4.1 % had other bacteria, which were not identified (appendix 5).

Table 6: Chickens yielding *Listeria species* and other bacteria.

Source of chickens	No. Of birds	*Listeria monocytogenes* (%)	Other *Listeria* (%)	*Erysipelothrix* spp. (%)	*Staphylococcus aureus* (%)	*Streptococcus* spp. (%)	*E.coli* (%)	Others (%)
Farms	55	0	1.8	1.8	23.1	19.7	48.1	5.5
Slaughter houses	41	0	0	4.9	28.4	22.5	40.1	4.1
Markets	40	5	15	0	20.1	14.3	33.9	11.7

Table 7: Ducks yielding *Listeria* and other bacteria species

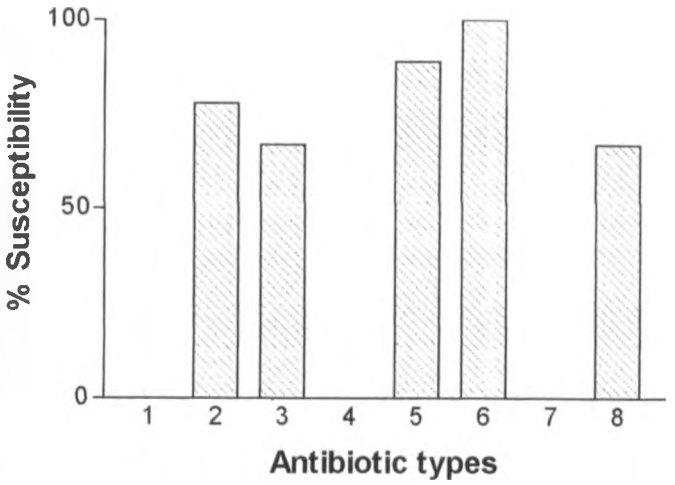
Source of ducks	No.of birds	*Listeria monocytogenes* (%)	Other *Listeria* (%)	*Erysipelothrix* spp. (%)	*Staphylococcus aureus* (%)	*Streptococcus* spp. (%)	*E.coli* (%)	Others (%)
Farms	39	0	0	2.6	20.1	21.2	40.2	15.9

4.4. Antibiotic sensitivity testing on *Listeria monocytogenes* and other *Listeria spp.*

Table 8 and figure 1 give the sensitivity patterns for the 9 *Listeria* isolates tested. All the isolates were resistant to ampicilin, augmentin and cefuroxime. They were 100% susceptible to gentamicin. The difference between percentage resistances of the *Listeria* isolates and the *Listeria monocytogenes* type strains was found to be insignificant (P = 0.263886). Figure 2 gives the antibiotic multiple resistance patterns for the isolates tested All the 9 *Listeria* isolates showed multiple resistances to at least 3 antibiotics while a few were resistant to more than 5 antibiotics. The detailed results are given in appendix 6.

Table 8: Antibiotic sensitivity testing for all *Listeria* isolates recovered from chicken.

Antibiotic type	No. Sensitive	%Sensitive	No. Resistant	% Resistant	Total isolates tested
Ampicillin	0	0	9	100	9
Tetracycline	7	77.8	2	22.2	9
Cotrimoxazole	6	66.7	3	33.3	9
Augmentin	0	0	9	100	9
Kenamycin	8	88.9	1	11.1	9
Gentamycin	9	100	0	0	9
Cefuroxime	0	0	9	100	9
Chloramphenicol	6	66.7	3	33.3	9



Legend:

1: Ampicillin, 2: Tetracycline, 3: Cotrimoxazole, 4: Augmentin, 5: Kenamycin, 6: Gentamycin, 7: Cefuroxime, 8: chloramphenicol

Figure 1:Susceptibility of *Listeria* species to 8 common antibiotics

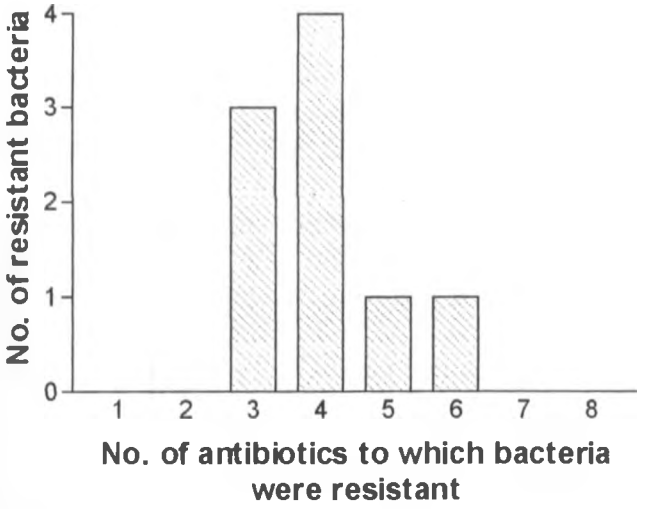


Figure 2: Frequency of multiple resistances to tested antibiotics among *Listeria* isolates

Figure 3 shows the control strain (*Escherichia coli* ATCC 25922) was resistant to ampicillin, augmentin and cefuroxime. It was highly sensitive to chloramphenicol, gentamycin, kenamycin, tetracycline and cotrimoxazole. The *Listeria* isolate, 51pv, was highly sensitive to chloramphenicol and tetracycline followed by kenamycin, gentamycin and cotrimoxazole The other isolate, 47piv, was sensitive to chloramphenicol, gentamicin and cotrimoxazole. It was resistant to tetracycline, kenamycin, ampicillin, augmentin and cefuroxime The *Listeria* isolate 8piv, showed sensitivity to kenamycin and gentamycin. They were all resistant to ampicillin, augmentin and cefuroxime.

Figure 4 shows *Staphylococcus aureus* (NCTC 6571) control strain. It was sensitive to tetracycline, cotrimoxazole, kenamycin, gentamycin and choramphenicol. It was resistant to augmentin, ampicillin and cefuroxime

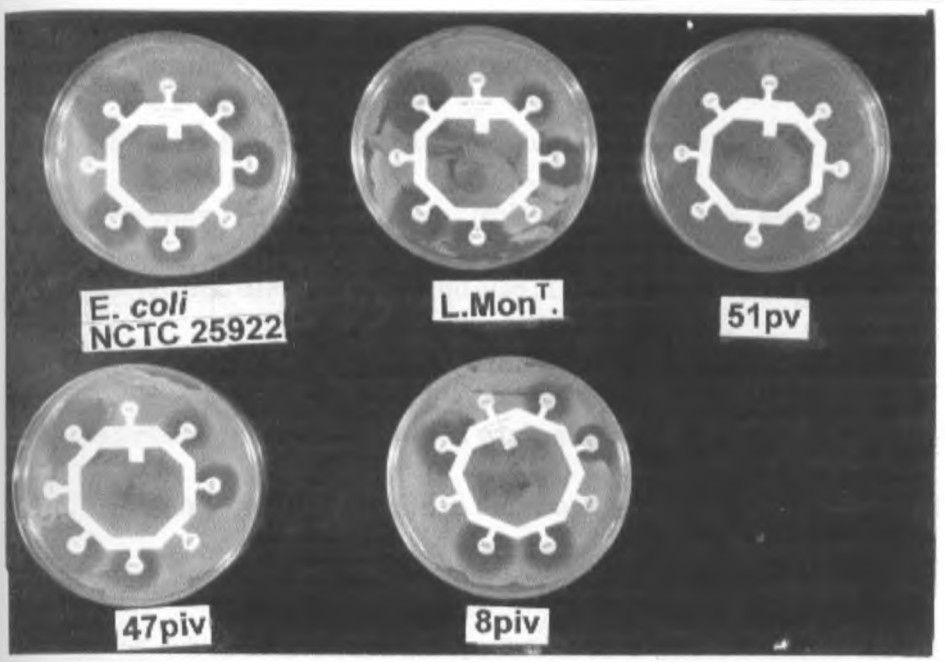


Figure 3: Photograph showing respective reactions for the Escherichia coli control strain (ATCC 25922) and Listeria isolates.

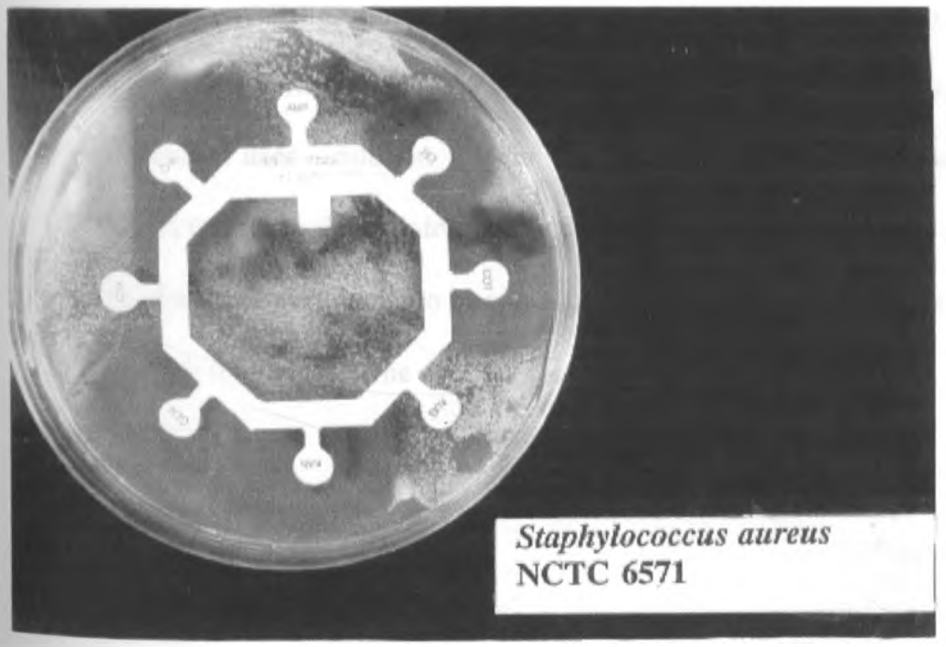


Figure 4: Photograph showing respective reactions for the Staphylococcus aureus control strain (NCTC 6571).

4.5: Disinfectant sensitivity testing on *Listeria spp.*

Table 9 and figure 5 give the sensitivity patterns for the 9 isolates tested, using the 7 disinfectants. All the *Listeria* isolates tested were sensitive to Omnicide® at the recommended user – dilution and also at lower dilutions. Eighty nine percent (89%) of the isolates tested were susceptible to Bromosept 50® at the recommended user dilution, 66.7% were sensitive at a higher dilution while all the isolates were 100% sensitive to Bromosept 50® at higher concentration than the recommended user - dilution. All the isolates were sensitive to Lavik® and Dettol® at higher concentration than the recommended user – dilution, while 77.8% were susceptible to both disinfectants at the recommended user – dilution. All the isolates showed resistance to Kleenol® at the recommended user – dilution, with more than 50% showing susceptibility at higher concentration than the recommended user – dilution. All the isolates were resistant to Sodium hypochlorite at the recommended – user dilution, with only 22.2% to 11.1% being sensitive at dilutions lower than the recommended user – dilution. All the isolates were resistant to Lysol® at all dilutions covered in this study. The relative sensitivities of the *Listeria* isolates to the disinfectants at various dilutions were found to be significant when compared to the recommended user – dilution (P= 0.002931). The detailed results are given in appendix 7

Table 9: Sensitivity of the *Listeria* isolates to disinfectants

Disinfectant	Dilutions														
	1			2			3*			4			5		
	S	R	%S	S	R	%S	S	R	%S	S	R	%S	S	R	%S
Omnicide®	9	0	100	9	0	100	9	0	100	9	0	100	8	1	88.9
Bromosept 50®	9	0	100	9	0	100	8	1	88.9	6	3	66.7	5	4	55.6
Sodium hypochlorite	2	7	22.2	1	8	11.1	0	9	0	0	9	0	0	9	0
Lavik®	9	0	100	9	0	100	7	2	77.8	5	4	55.6	4	5	44.4
Dettol®	9	0	100	9	0	100	7	2	77.8	7	2	77.8	3	6	33.3
Kleenol®	6	3	66.7	5	4	55.6	0	9	0	0	9	0	0	9	0
Lysol®	0	9	0	0	9	0	0	9	0	0	9	0	0	9	0

Legend:

3*: recommended user - dilution (Table 2)

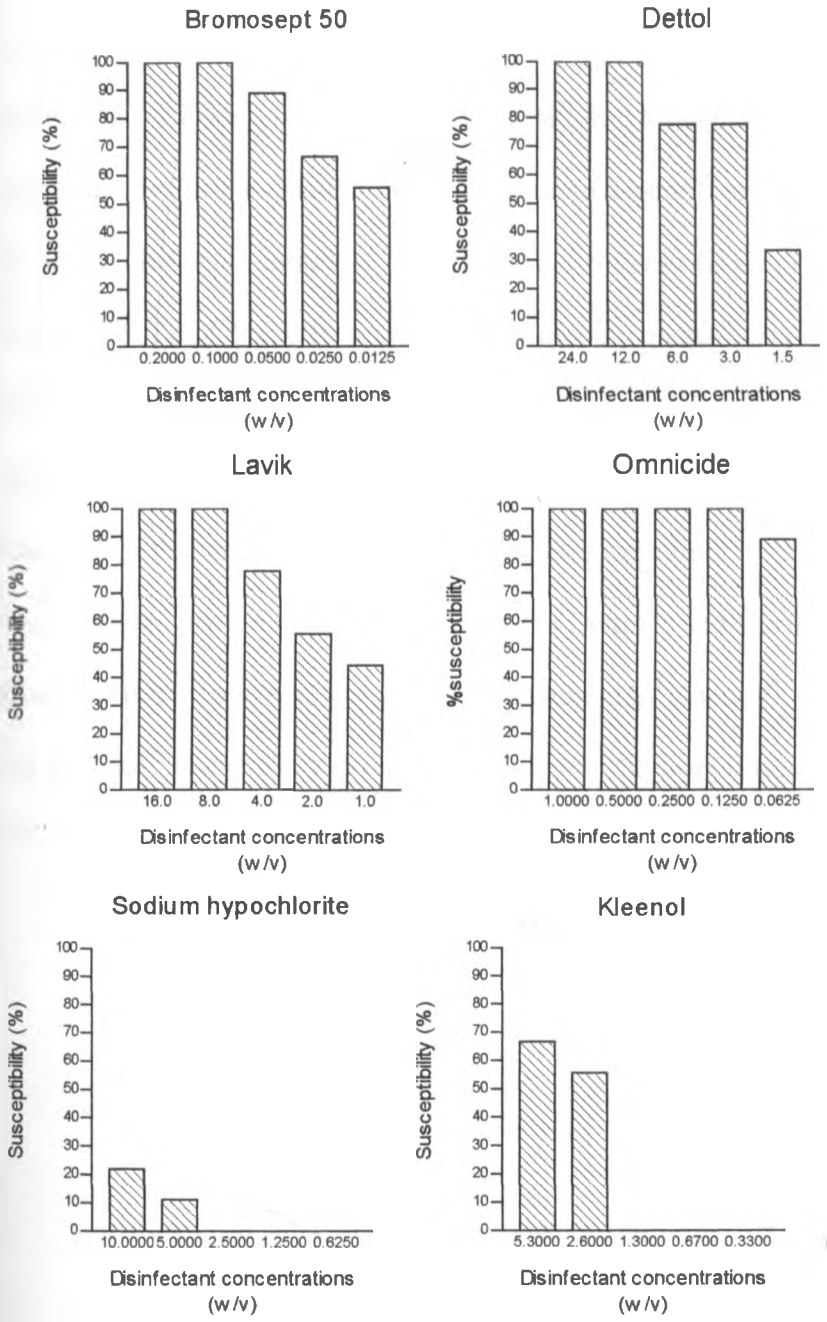
Interpretation: > 10 mm were regarded as sensitive; < 10mm were regarded as resistant

S: Susceptible

R: Resistant

%S: Percentage susceptible

1, 2, 3*, 4 and 5 represent doubling dilutions (Table 2)

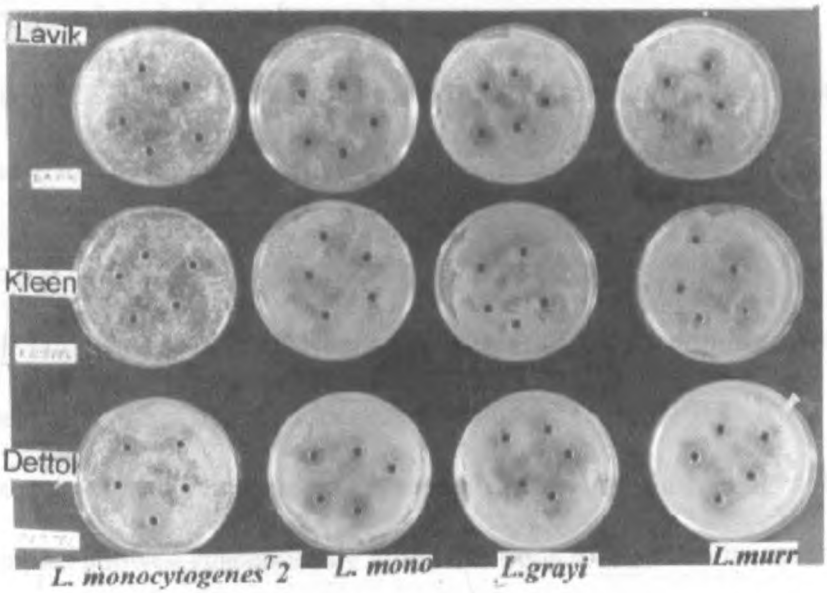


Legend:
All the isolates were resistant to Lysol® at all the dilutions covered in this study.

Figure 5: Disinfectant profile for *Listeria* isolates

Figures 6 (a) and (b) show inhibition zones for disinfectant activity against *Listeria monocytogenes* type strain and *Listeria* isolates. The type strain was more sensitive to Omnicide®, followed by Lavik®, then Dettol®, and finally Bromosept 50® at the recommended user – dilution. It was resistant to Kleenol® and Sodium hypochlorite at the recommended user – dilution. *Listeria monocytogenes* (isolate) was sensitive to Omnicide®, Lavik®, and Bromosept 50® at recommended user dilution. It was resistant to sodium hypochlorite, Kleenol and Dettol® at recommended user dilution. *Listeria seeligeri* showed higher sensitivity to Bromosept 50®, followed by Omnicide®, Lavik®, Dettol® and Kleenol® in this order at the recommended user - dilution. It was not sensitive to sodium hypochlorite. *Listeria grayi* was sensitive to Dettol® and Lavik® at recommended user dilution. *Listeria murrayi* was sensitive to Omnicide®, Dettol®, Bromosept 50® , Lavik®, and Kleenol® respectively at recommended user dilution. It was resistant to Sodium hypochlorite at recommended user dilution.

(a)



(b)

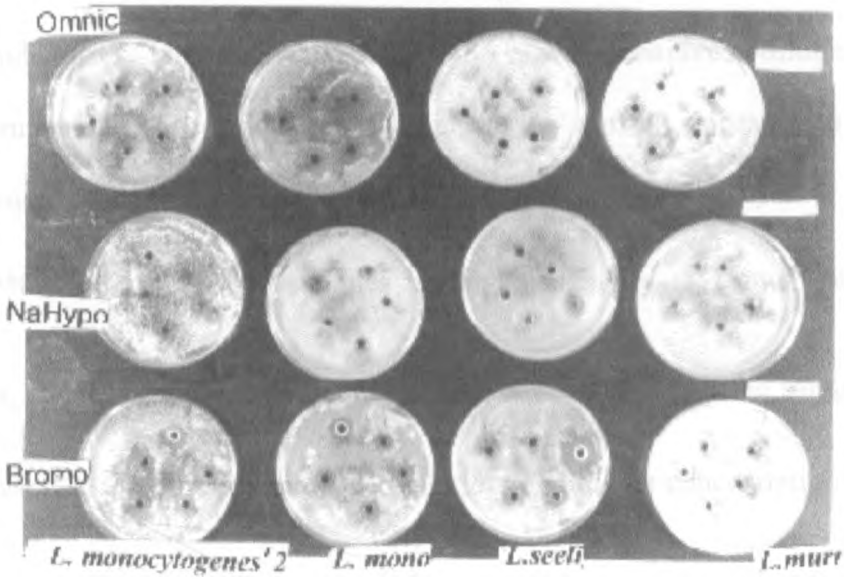


Figure 6 (a,b): Photographs showing *Listeria monocytogenes* type strains and *Listeria* isolates inhibition zones to disinfectant activity.

4.6. Disinfectant sensitivity testing on Ruai and Katani pooled pharyngeal and cloacal samples

Tables 10, 11 and 12 show the proportions of samples from Katani and Ruai sensitive to the 7 disinfectants for the 5 dilutions covered in this study.

None of the 8 samples tested from Ruai showed a 100% susceptibility to the seven disinfectants tested at the recommended user – dilution (Table 10). None of the 8 samples were susceptible to omnicide® at the recommended user - dilution, while 75% to 25% of the samples showed susceptibility at the dilutions lower than the recommended user - dilutions. Twenty five percent of the samples were sensitive to Bromosept 50® at the recommended user – dilution, while 50 % to 86 % were sensitive at dilutions lower than the recommended user – dilutions. All the samples showed resistance to Dettol®, Lavik®, Kleenol® and sodium hypochlorite at the recommended user – dilution. However, 63% and 75% of the samples showed sensitivity to Dettol® at higher concentration; and 13% showed partial sensitivity to Kleenol®. All the samples were resistant to Lysol® at both the recommended dilutions and higher concentrations.

The six samples from Katani, showed 50% sensitivity to Omnicide®, while 17% had partial resistance to the same disinfectant at recommended user dilution (Table 11). At a higher concentration than the recommended user dilution, all the pooled samples were sensitive to Omnicide®. The pooled samples showed slightly higher sensitivity to

Bromosept 50® at recommended user dilution with 83% being sensitive and 17% showing partial resistance. All the samples were sensitive to Bromosept 50® at higher concentrations than the recommended user dilution. Thirty three percent of the samples were sensitive to Sodium hypochlorite at the recommended user dilution, while 50% of the samples were sensitive at a lower dilution than the recommended user dilution. Fifty percent of the samples were sensitive to Lavik® at recommended user dilution, while 83% were sensitive at a higher concentration than the recommended user dilution. In addition, 17% showed partial sensitivity to Lavik® at the recommended user dilution. Thirty three percent of the samples showed sensitivity to Dettol®, while 17% had partial resistance at recommended user dilution. All the samples were resistant to Kleenol® at recommended user dilution, with 50% being sensitive to Kleenol® at higher concentration. All the samples were resistant to Lysol® at the dilutions used in this study. The microorganisms showing partial resistance were highly susceptible to the respective disinfectants when retested individually following subculturing.

When relative disinfectant sensitivities of Katani and Ruai pooled pharyngeal and cloacal samples were combined, samples showed a lower sensitivity to all the disinfectants tested (Table 12). Twenty one percent were sensitive to Omnicide® at the recommended user dilution and 7% showed partial resistance to Omnicide®. Fifty percent of the samples were sensitive to Bromosept 50® at the recommended user dilution, while 14% showed partial resistance. Fourteen percent were sensitive to Sodium hypochlorite at the recommended user dilution. Twenty one percent of the samples were resistant to Lavik® at the recommended user dilution. Fourteen percent showed partial resistance to Lavik®

at the recommended user dilution. Fourteen percent of the samples were sensitive to Dettol® at the recommended user dilution while 7% showed partial resistance. All the samples were resistant to Kleenol® and Lysol® at the recommended user dilution

Some disinfectants were shown to be bactericidal since there was no growth when subcultured from the zone of inhibition while others were bacteriostatic. On reseeding from the area of partial sensitivity, the resultant bacterial growth showed sensitivity to the respective disinfectants. The detailed results are given in appendices, 8 and 9.

Table 10: Disinfectant sensitivity testing on Ruai pooled pharyngeal and cloacal samples

Disinfectant	Dilutions												
	1			2			3*			4			5
	%S	%PR	%TR	%S	%PR	%TR	%S	%PR	%TR	%S	%PR	%	%S
Omnicide®	75	0	25	25	0	75	0	0	100	0	0	100	0
Bromosept 50®	86	0	14	50	0	50	25	0	75	38	0	62	0
Sodium hypochlorite®	13	0	87	0	0	100	0	0	100	0	0	100	0
Lavik®	50	0	50	0	0	100	0	0	100	0	0	100	0
Dettol®	75	0	25	63	0	37	0	0	100	0	0	100	0
Kleenol®	0	13	87	0	0	100	0	0	100	0	0	100	0
Lysol®	0	0	100	0	0	100	0	0	100	0	0	100	0

Legends:

3*: recommended user - dilution (Table 2)

%S: Percentage susceptible

%TR: Percentage total resistant

% PR: Percentage partial resistant

1, 2, 3*, 4 and 5 represent doubling dilutions (Table 2)

Table 11: Disinfectant sensitivity testing on Katani pooled pharyngeal and cloacal samples

Disinfetant	Dilutions												
	1			2			3*			4			5
	%S	%PR	%TR	%S	%PR	%TR	%S	%PR	%TR	%S	%PR	%TR	%S
Omnicide®	100	0	0	67	17	16	50	17	33	50	17	33	33
Bromosept 50®	100	0	0	100	0	0	83	17	0	50	17	33	33
Sodium hypochlorite®	50	33	17	50	0	50	33	0	67	0	0	100	0
Lavik®	83	17	0	67	33	0	50	17	33	17	17	66	0
Dettol®	83	17	0	67	17	16	33	17	50	33	0	67	17
Kleenol®	50	17	33	0	0	100	0	0	100	0	0	100	0
Lysol®	0	0	100	0	0	100	0	0	100	0	0	100	0

Legend:

3*: recommended user - dilution (Table 2)

%S: Percentage susceptible

%TR: Percentage total resistant

% PR: Percentage partial resistant

1, 2, 3*, 4 and 5 represent doubling dilutions (Table 2)

Table 12: Disinfectant sensitivity patterns for Katani and Ruai pooled pharyngeal and cloacal samples

Disinfectant	Dilutions												
	1			2			3*			4			5
	%S	%PR	%TR	%S	%PR	%TR	%S	%PR	%TR	%S	%PR	%TR	%S
Omnicide®	86	14	0	43	7	50	21	7	72	21	7	72	14
Bromosept 50®	93	7	0	71	7	22	50	14	36	50	7	43	14
Sodium hypochlorite®	29	14	43	21	0	79	14	0	86	14	0	86	0
Lavik®	64	7	29	29	21	50	21	14	65	21	7	72	0
Dettol®	79	7	14	64	7	29	14	7	79	14	0	86	7
Kleenol®	21	14	65	0	0	100	0	0	100	0	0	100	0
Lysol®	0	0	100	0	0	100	0	0	100	0	0	100	0

Legend:

3*: recommended user - dilution (Table 2)

%S: Percentage susceptible

%TR: Percentage total resistant

% PR: Percentage partial resistant

1, 2, 3*, 4 and 5 represent doubling dilutions (Table 2)

Comparison of percentage sensitivity of pooled bacteria from Ruai, Katani and a supermix of all the samples from the two regions is given on Table 13. Generally, Ruai pooled isolates seemed to show more resistance to respective disinfectants than the Katani ones; they appeared to be slightly sensitive to Bromosept 50® (25%) at user dilution, but were resistant to all other disinfectants. Pooled isolates of Katani showed sensitivity to Omnicide® (67%), Bromosept 50® (100%), Lavik® (67%), Dettol® (50%) and Sodium hypochlorite (33 %). They were resistant to Kleenol® and Lysol®.

On pooling all the bacteria (Ruai and Katani ones), the percentage sensitivity of the bacteria was reduced to: Bromosept 50® (64%), Lavik® (35%), Omnicide® (28%), Dettol® (21%) and Sodium hypochlorite (14%). The isolates remained resistant to Kleenol® and Lysol®.

Table 13: Comparison of percent sensitivity of pooled bacteria from Ruai, Katani, and both Katani and Ruai together at user – dilution.

Region	1*	2*	3*	4*	5*	6*	7*
Ruai	0	25	0	0	0	0	0
Katani	67	100	33	67	50	0	0
Ruai and Katani together	28	64	14	35	21	0	0

Legend:

1*: Omnicide®

2*: Bromosept 50®

3*: Sodium hypochlorite

4*: Lavik®

5*: Dettol®

6*: Kleenol®

7*: Lysol®

Other numbers: % Sensitivity

When the pooled bacteria were compared per farm (Tables 14 and 15), pooled organisms of farm 1 (Ruai) were resistant to all disinfectants at user dilution. They however, showed sensitivities to Bromosept 50® (100%), Omnicide® (50%), Dettol® (50%) at higher concentration. Pooled organisms from farm 2 (also in Ruai) showed sensitivities to Bromosept 50® (100%) and Omnicide® (50%), but not to the other disinfectants. Pooled organisms from Katani (farms, 3, 4 and 5) showed more sensitivity to the respective disinfectants than Ruai farms (1 and 2). Farm 5 was the most resistant of the three, being sensitive to Bromosept 50® (100%), Omnicide® (50%), Lavik® (50%), Sodium hypochlorite (0%) and Dettol® (0%) at user dilution. The organisms were resistant to Kleenol® and Lysol®. Farm 4 was the next less sensitive, being sensitive to Bromosept 50® (100%), Omnicide® (100%), Lavik® (50%), Sodium hypochlorite (50%) and Dettol® (50%). These organisms were also resistant to Kleenol® and Lysol®. Farm 3 had the most sensitive organisms showing 100% sensitivity to Bromosept 50®, Omnicide® and Sodium hypochlorite and Dettol® , and 50% sensitivity to Lavik® . These organisms were also resistant to Kleenol® and Lysol®.

Table 14: Disinfectant sensitivity for Katani and Ruai pharyngeal and cloacal pooled samples in different farms.

Farm	Omnicide®			Bromosept 50®			Sodium Hypochloride			Dettol®			Lavik®			Kleenol®			Lysol®		
	2*	3**	4*	2*	3*	4*	2*	3*	4*	2*	3*	4*	2*	3*	4*	2*	3*	4*	2*	3**	4*
Farm 1 (Ruai)	50	0	0	100	0	0	0	0	0	50	0	0	0	0	0	0	0	0	0	0	0
Farm 2 (Ruai)	100	50	0	100	100	50	50	0	0	100	0	0	0	0	0	0	0	0	0	0	0
Farm 3 (Katani)	100	100	100	100	100	100	100	100	50	100	100	50	50	50	0	0	0	0	0	0	0
Farm 4 (Katani)	50	100	50	100	100	100	50	50	50	100	50	50	100	50	50	0	0	0	0	0	0
Farm 5 (Katani)	50	50	0	100	100	100	0	0	0	50	0	0	100	50	50	0	0	0	0	0	0
Φ (mean)	70	60	30	100	80	70	40	30	20	80	30	20	50	30	30	0	0	0	0	0	0

Legends:

2*, 3**, 4*: are the dilutions one fold below, the recommended – user dilution, and one fold above the user dilution, respectively.

Other numbers: % Sensitivity

Table 15: Disinfectant sensitivity for Katani and Ruai pharyngeal and cloacal pooled samples in different farms (at user dilution).

Farm	% Sensitivity						
	Omnicide®	Bromosept 50®	Sodium hypochlorite	Dettol®	Lavik®	Kleenol®	Lysol®
Farm 1 (Ruai)	0	0	0	0	0	0	0
Farm 2 (Ruai)	50	100	0	0	0	0	0
Farm 3 (Katani)	100	100	100	100	50	0	0
Farm 4 (Katani)	100	100	50	50	50	0	0
Farm 5 (Katani)	50	100	0	0	50	0	0
Φ (mean)	60	80	30	30	30	0	0

A breakdown of the bacterial isolates recovered from the various farms is given on table 16. Detailed results are given in appendices 10 and 11.

Figure 7 shows inhibition zones for disinfectant activity against pooled pharyngeal and cloacal samples K, L, M and N. The three samples were sensitive to Bromosept 50® at recommended user dilution. Only sample K was sensitive to Omnicide® at recommended user dilution. All the 4 samples were resistant to sodium hypochlorite.

Table 16: Bacterial isolates recovered from Katani and Ruai pooled pharyngeal and cloacal samples

Microorganisms	Ruai samples								Katani samples					
	A	B	C	D	E	F	G	H	I	J	K	L	M	N
S.aureus	+	+	-	-	+	+	-	+	+	+	+	+	+	-
S. epidermidis	-	-	-	-	-	+	+	-	-	-	-	-	-	-
Streptococcus spp.	+	-	+	+	+	+	+	+	+	-	+	+	-	-
Listeria seeligeri	-	-	-	-	-	-	-	+	-	-	-	-	-	-
Bacillus spp.	-	-	-	-	-	-	-	-	-	-	+	+	-	+
E.coli	-	+	+	+	+	+	+	-	+	+	+	+	+	-
Klebsiella spp.	-	-	-	+	-	-	-	-	-	-	-	-	-	-
Pseudomonas spp.	-	-	+	-	+	+	-	-	-	-	-	+	-	-
Proteus spp.	-	-	-	-	-	-	-	-	+	+	-	-	-	-

Legends:

+: Bacterium recovered

-: Bacterium not recovered

A to N: Sample codes.

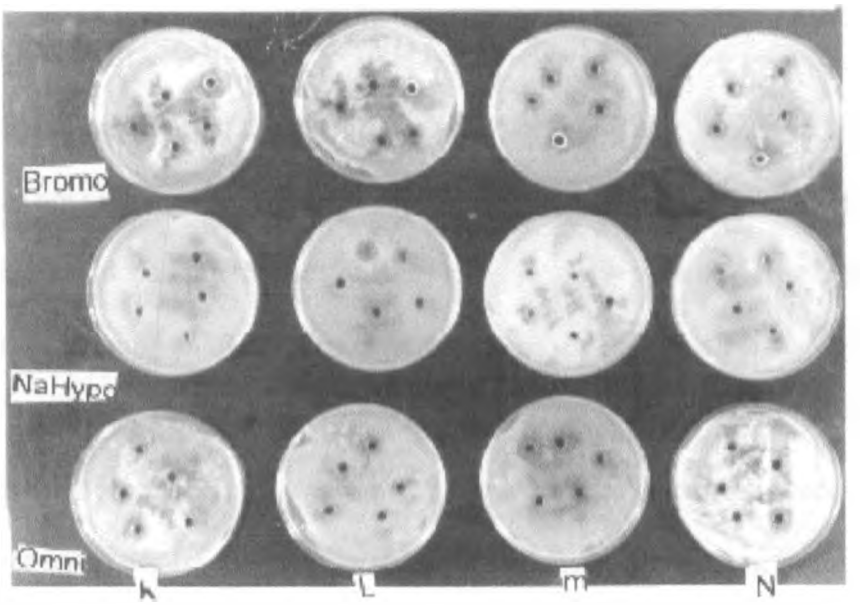


Figure 7: Photograph showing disinfectant sensitivities for Katani and Ruai pooled pharyngeal and cloacal samples

CHAPTER 5: DISCUSSION AND CONCLUSION

Systematic investigation of the occurrence of *Listeria monocytogenes* and other *Listeria* species has not previously been done on healthy appearing chickens and ducks from farms, market places and slaughterhouses in Kenya. In this study, five different *Listeria* isolates from local scavenging chickens were isolated. These were *Listeria monocytogenes*, *Listeria innocua*, *Listeria seeligeri*, *Listeria grayi* and *Listeria murrayi*. All these isolates were recovered from oropharyngeal swabs and none from the cloacal. The recovery rate was quite low mainly because the samples were not enriched. This finding is supported by the results of Ryser *et al.* (1996), who found that isolation rates for *Listeria monocytogenes* and the other *Listeria spp.* typically improve when samples are enriched in more than one primary enrichment medium. More isolates were recovered from market birds than farmed birds. This may be because of birds being from different locations and crowding in small premises, thus cross transmission between infeccted birds and clean occurs easily. In scavenging situation birds are dispersed hence pathogens do not get concentrated at one point. This is confirmation that healthy appearing scavenging indigenous chicken and possibly ducks are carriers of the organism. This observation agrees with the work of Quinn *et al.*, (1994); Bolin and Turn, (1951) who reported the occurrence of asymptomatic faecal carriers in man and domestic animals.

Epidemics of human listeriosis have been traced to ingestion of contaminated milk (Quinn *et al.*, 1994; Fleming *et al.*, 1985), cheese (James *et al.*, 1985; Malinvemi *et al.*, 1985; Linnan *et al.*, 1988, Schlech *et al.*, 1983), vegetables (Ho *et al.*, 1986), poultry and other

meat products (Schwartz *et al.*, 1988). The majority of human cases of listeriosis occur in immunosupressed individuals, the elderly, the very young, and pregnant women (Hirsh and Zee, 1999). The few *Listeria* isolates recovered in this study show that there is a potential risk to the farming, trading and poultry consuming communities in Kenya. The carrier birds can also transmit the *Listeria* species to other birds as reported by Cooper and Arthur, (1998), through environmental faecal contamination, especially the commercial breeds, causing losses of upto 40%.

Listeriosis occurs in animals other than man and poultry. Seeliger and Jones (1986) have documented that *L. ivanovii* is pathogenic for sheep. Although, *L. innocua; L. seeligeri; L. grayi* and *L. welshmeri* are considered to be generally non-pathogenic, there is some evidence suggesting that some strains of both *L. seeligeri* (Rocourt et *al.*, 1986) and *L. innocua* ((Rocourt and Seeliger, 1985) are occasionally pathogenic for man and animals, respectively. *Listeria innocua* has also been isolated from cases of encephalitis in ruminants: Rocourt and Seeliger, (1985) reported one case of *L.innocua* in a deer and one in a cow; Nicolas *et al.*, (1986) reported seven cases of encephalitis in sheep caused by *L. innocua*. However, this organism has also been isolated from brain tissue of clinically normal sheep (Gronstol *et al.*, 1986). Hence, scavenging local chicken and ducks, having been incriminated as carriers of *Listeria species*, can actually play a major role in the transmission of these organisms to other livestock, especially ruminants, through fecal contamination of the feed, or the use of poultry manure as cattle feed.

Mice pathogenicity results for *Listeria* isolates from chickens showed that, 2 isolates out of 9 were pathogenic to mice. The two were identified as *Listeria monocytogenes* (47piv) and *Listeria innocua* (45Pi) respectively. These, findings are similar to those of Hirsh and Zee (1999), who found *Listeria monocytogenes* and *Listeria ivanovii* to be important pathogens of mice. Rocourt and Seeliger (1985) also reported some pathogenic strains of *Listeria innocua*, which is in agreement with this study's findings. One of the isolates (46pv) with biochemical reactions similar to *Listeria monocytogenes* type strains was not pathogenic to mice. This could be due to mutations leading to loss of pathogenicity of the strain. This is a field that needs further studies.

Other bacteria isolated from this study were *Staphylococcus*, *Streptococcus*, *Erysipelothrix*, *Escherichia coli* and others that were not fully identified. *Staphylococcus aureus* carrrier rate was observed as 23.1% in farms, 28.4% from slaughterhouses and 20.1% from the market scavenging local chickens and ducks sampled in this study. *Staphylococcus aureus* is common in poultry and is most frequent in bones, tendon sheaths, and joints of the leg (Cheville *et al.*, 1988). Carrier birds have been reported, with *Staphylococcus aureus*, *Staphylococcus epidermidis* and *Staphylococcus gallinarum* being isolated from processed poultry (Devriese *et al.* 1983). For staphylococcal disease to occur, a breakdown in the natural defense mechanisms of the host must occur (Anderson, 1986). In most cases, this would involve damage to an environmental barrier, such as a skin wound or inflammed mucous membrane. In newly hatched chicks, the open wound at the attachment of the remnant of the yolk sac provides a portal of entry leading to omphalitis and other types of

infections. Minor surgical procedures, for instance, trimming of toes, beak, or comb, removal of snood, and parenteral vaccinations may offer additional means of entry for *staphylococci*. Another type of host defense impairment occurs following infectious bursal disease (Santivatr *et al.*, 1981), chicken infectious anemia, or possibly Marek's disease, where the bursa of Fabricius or thymus is damaged and the immune system is compromised (Santivatr *et al.*, 1981). Under these conditions, septicaemic staphylococcal infections can occur and cause acute death. Hence the carrier status in this study is of economic importance to the poultry farmers.

In addition to causing disease in poultry, approximately 50% of typical and atypical *Staphylococcus aureus* strains produce enterotoxins that cause food poisoning in humans (Evans *et al.*, 1983; Harvey *et al.*, 1982, Skeels, 1997).

In this study, *Streptococcus* organisms were isolated from local chicken and ducks at the rates of 19.7 % from farms, 22.5% from slaughterhouses and 14.3 % from the market. As far as *Streptococci* carrier status is concerned, *streptococci* form part of the normal intestinal flora of most avian species, including wild birds (Brittingham, *et al.*, 1988). A low percentage (16.67%) of poultry meat contamination with *Streptococcus faecalis* has been found in ready – to – cook products (Brittingham, *et al.*, 1988).

Streptococcosis in avian species is worldwide in distribution, occurring as both acute septicaemic and chronic infections with mortality ranging from 0.5% to 50%.

Enterococcus fecalis affects species of all ages; it is one of the disease causing organisms in embryos and young chicks, mainly from faecal contaminated eggs (Alaboudi *et al.*, 1992; Mbuthia *et al.*, 2002a). *Streptococcus fecium* and *Streptococcus mutans* have been identified as causes of mortality in ducklings and goslings, respectively (Rudy, 1991). On the other hand, a low percentage (16.67%) of poultry meat contamination with *Streptococcus fecalis* has been found in ready- to- cook products. However, no incrimination of food poisoning in humans has been found (Vaillancourt *et al.*, 1992).

Erysipelothrix rhusiopathiae (insidiosa) is the only species in the genus and is a well – known animal pathogen of considerable economic importance (Bailey and Scotts, 1994). Erysipelas in birds is generally an acute, fulminating infection of individuals within a flock. Outbreaks of economic significance are infrequent in avian species other than turkeys. Occasional losses of individual birds within a flock have been reported (Bricker and Saif, 1997), and a few economically significant outbreaks in chicken and ducks have occurred (Bricker and Saif, 1997). *Erysipelothrix rhusiopathiae* occurs in pheasants, ducks, geese, guinea fowl, chukars, grebes, and emus (Bricker and Saif, 1997).

In this study, E*rysipelothrix spp.* was isolated from 1 out of the 94 (1.8) indigenous chicken and ducks sampled from the farms and 2 out of 41 (4.9%) slaughter chicken. This means that local scavenging chickens and ducks are potential sources of *Erysipelothrix* infection to other animals. Human infection is an occupational hazard among groups

handling raw meat or fish, including butchers, poultry workers, veterinary surgeons and fish mongers, who may suffer contaminated cuts or scratches (Reboli and Farrar, 1989).

Escherichia coli is a common inhabitant in the intestinal tract of poultry at concentrations upto 10^6 per gram. Higher numbers are found in younger birds without an established normal flora and in the lower intestinal tract (Wooley *et al.*, 1994). The carrier rate of *Escherichia coli* was observed to be 48.1%, 40.1% and 33.9% from farmed, slaughter and market birds respectively.

Colibacillosis occurs when skin or mucosal barriers are compromised (for instance, wounds; mucosal damage from viral; bacterial; or parasitic infections and lack of normal flora). It also occurs when the mononuclear – phagocytic systems are impaired (for instance, in cases of viral infections, toxaemia and nutritional deficiencies). Faecal contamination of eggs is considered to be the most important source of infection. Barnes and Gross (1997) observed that, the incidence of infection increases shortly after hatching and gets reduced after about 6 days.

Most *E.coli* serotypes isolated from poultry have been taken to be pathogenic only for birds and are supposed not to be important causes of disease in other animals including man (Barnes and Gross, 1997). However, Bebora *et al.* (1993) have differing view to this theorem. Considering the fact that production of colonisation factors (attachment fimbriae) is coded for by plasmids (Echeverria *et al.*, 1986); the fact that Bebora *et al.* (1993)

demonstrated easy plasmid transfer between bacteria in close proximity, and the fact that bacteria present in the gut are normally in close proximity, they argue that there is always a possibility of the poultry / human strains acquiring the respective plasmids and thus be able to produce the relevant fimbriae for attachment. This is supported by the fact that Bebora *et al.* (1993) isolated serotypes 0127, 0158, 078, 063, 0126, 01 and one isolate of 0157, which are documented human pathogens, from cases of septicaemia and omphalitis in chicks (Honda, 1992). However, 56.8% of the isolates screened were untypable using the antisera available.

The realisation that chicken can carry *E.coli* 0157: H7 (Bebora *et al.*, 1993) is significant because *E.coli* 0157: H7 is an important enterohemorrhagic pathogen of humans. Bebora *et al.* (1993) isolated it from a case of septicaemia in chicks. Chicken meat can therefore be contaminated with this organism. However, in an effort to look for it in our study, 30 *E. coli* isolates were tested using fermentation of sorbitol; none were *E.coli* 0157. A foodborne outbreak of diarrhoeal disease due to *Escherichia coli* strain 0157: H7 has been associated with contaminated turkey meat (Stavric *et al.*, 1993).

In this study, 3 isolates had characteristics similar to *Campylobacter*. They were Gram negative, thin curved rods (pleomorphic). It was, however, not possible to follow them further as they fizzled out fast. The carrier status of *Campylobacter* in chickens is documented to be at 77% (Osano and Arimi, 1999). Upto 90% of broiler chickens may be infected (Blaser, 1982), while 100% of turkeys (Lammerding *et al.*, 1988) and 88% of

domestic ducks (Prescott and Bruin –Mosch, 1981) may harbor the organisms. Infection has been recorded among game birds, including partridges, pheasants and quail (Volkheimer and Wuthe, 1986). Thermophilic *Campylobacters* are among the leading causes of acute self limiting diarrhoea and Guillain – Barre syndrome (which is a disorder of peripheral nerves characterised by ascending paralysis) in humans worldwide, with *C. jejuni* being the most commonly isolated species from faeces (Hughes and Rees, 1997). Chicken showing signs of being carriers for *Campylobacter* serve as a possible source of infection to other animals and man (Osano and Arimi, 1999).

Antibiotics are being used extensively, around the world, to treat bacterial diseases, listeriosis included (Wieldmann et *al.*, 1994; Blenden *et al.*, 1987; Report of working group, standing medical advisory committee, 1992).). In this study, *Listeria monocytogenes* and other *Listeria species* isolated were found to be sensitive to gentamicin (100%), while a few showed resistances to cotrimoxazole, tetracycline, kenamycin and chloramphenicol. All the isolates showed resistance to ampicillin, augmentin and cefuroxime. The *Listeria monocytogenes* type strains (L028 and DGH) showed similar antibiotic sensitivity patterns to those of *Listeria* field isolates.

Poyart – Salmeron *et al.* (1990) reported that, *Listeria monocytogenes* was sensitive to penicillin, amoxycillin, gentamicin, chloramphenicol, trimethoprim, cotrimoxazole, erythromycin, vancomycin, rifampicin and tetracyclines. The same authors reported that, *Listeria monocytogenes* is resistant to most cephalosporins but appears sensitive to

imipenem. The resistance could be through plasmid (Courtie and Courvalin, 1990). Poyart – Salmeron *et al.* (1990), have documented this type of transmission in tetracycline, erythromycin, chloramphenicol and streptomycin. This drug resistance, single or multiple, has also been reported in Gram – positive bacteria by a number of workers (Barlow and Mc Corum, 1985; Farber and Peterkin, 1991). The fact that, the type strains and most of the *Listeria* isolates showed multiple resistances poses a problem in treating listeriosis in humans and domestic animals.

For cleaning the chicken houses and other surroundings, various disinfectants can be used (Pomeroy, 1972; Snoeyenbos, 1972). These include acids, alkalis, salts, heavy metals, halogens, formaldehyde, ethylene oxide, cationic detergents like quarternary ammonium compounds, and phenols (Davis *et al.*, 1973; Hugo, 1970). This study tested the sensitivity of *Listeria* isolates to 7 disinfectants: namely; Omnicide®, Bromosept 50®, Dettol®, Lavik®, Kleenol®, Sodium hypochlorite and Lysol®. Of these, Omnicide® was found to be the most efficient disinfectant, followed by Bromosept 50®. Dettol® and Lavik® had similar sensitivity patterns while Kleenol® and Sodium hypochlorite were effective at dilutions higher than the recommended user dilution. Omnicide® was the only one that was 100% effective at the recommended user dilution while for the others; higher dilutions were needed for any significant sensitivity to be seen. Lysol® had no effect at all the dilutions studied.

Listeria monocytogenes (L028 and DGH) type cultures gave reactions similar to the local isolates recovered in this study, when tested against Sodium hypochlorite, Kleenol®, Lysol®, and Dettol®, but showed greater sensitivity to Omnicide®, Bromosept 50®, and Lavik® using both the recommended user – dilution and higher ones. Bebora (1987) and Karaba (1991) carried out similar disinfectant sensitivity studies using *Salmonella gallinarum* and the following disinfectants; Lysol®, Pynol – 5, Kerol®, Biodan®, Bromosept® 50 and Municipal fluid. They also found kerol® and Lysol® not being effective at the recommended user dilution. These observations emphasise the importance of ascertaining the efficacy of a disinfectant on a respective organism for one to effectively eliminate it from the disinfected area.

The fact that, as mentioned earlier, the throat and cloacal swabs from birds in this study have yielded a number of bacteria including, *Listeria monocytogenes*, other *Listeria spps.*, *Staphylococcus spps.*, *Streptococcus spp.*, *E. coli*, *Proteus spps.*, *Bacillus spps.* *Pastreurella spp.* and *Pseudomonas spp.*, there is a possibility that these bacteria are shed into the surroundings of the bird, more so in the poultry house and the coops (tengas), which are used to confine birds and also for transportation etc. This shedding has been confirmed in cross – reaction studies using *Pastreurella multocida* organisms (Mbuthia 2002c, personal communication). It was, therefore, felt that pooled cultures (throat and cloacal) from various farms be taken as representatives of the bacteria contaminating the various environments, and disinfectant sensitivities be run using the same disinfectants as for pure *Listeria* cultures, to assess the composite action of the disinfectants. This was

taken as the most ideal way to assess disinfectant sensitivity, rather than the pure cultures, since this is how it is in natural circumstances

When the disinfectants were tested, the samples showed varied disinfectant sensitivity profiles. The microbes in farms 3 and 4 (Katani) were sensitive to all disinfectants except Kleenol® and Lysol®, while microbes in farm 5 were only sensitive to Omnicide®, Bromosept 50®, and Lavik® at the recommended user – dilution. Ruai pooled isolates showed a wide spread resistance to the seven disinfectants tested. Thus, of the seven disinfectants tested, only Bromosept 50 and Omnicide can be used to disinfect all the farms except farm 1 (Ruai), while others had variable sensitivities. Assuming that the user dilution was scientifically set, after various test – runs, the picture given here is that of some bacteria having developed resistance towards the disinfectants, following the same principle as that for antibiotic resistance. The resistance may be natural or by adaptation.

The discrepancy between pooled bacteria from Ruai and Katani regions is great (Tables 12 and 13). This is because the Ruai farmers are practising a lot more hygiene – routinely using various types of disinfectants. The organisms, thus, seem to have developed some resistance towards these commonly used disinfectants. In doing disinfectant sensitivity testing, most of the disinfectants showed partial sensitivity over 24 hour's incubation. However, on subculturing of the growing bacteria and redoing of the test, all the respective disinfectants cleared the bacteria. This may be due to bacterial concentration reduction, removing the shielding effect amongst the bacteria. Assaying the modes of action of the

disinfectants by scraping through the clear areas (zones of inhibition) and plating on suitable media, Omnicide®, Sodium hypochlorite, Dettol® and Bromosept 50®, were found to be bacteriocidal (there was no growth on the medium). Lavik® and Kleenol® were bacteriostatic (there was growth on the medium). The observation that there was inhibition on reseeding indicates that, in most cases, two consecutive treatments are needed for proper disinfection to occur

This study has shown that local scavenging chicken and possibly ducks are carriers of *Listeria monocytogenes*, other *Listeria* species; *Erysipelothrix* species, *Escherichia coli*, *Staphylococcus aureus* and *Streptococcus* spp; Local scavenging chickens and ducks are therefore potential reservoirs of the zoonotic infections to humans. *Listeria monocytogenes* and other *Listeria* species have shown some degree of resistance to some of the commonly used antibiotics like tetracycline, cotrimoxazole, kenamycin, chloramphenicol, amoxycillin and ampicillin; For disinfection of *Listeria monocytogenes* and other *Listeria* species, a slightly higher concentration than the recommended user – dilution is needed for effectiveness; Bromosept 50® and Omnicide® were the most effective at user – dilution, for disinfection of chicken and duck coops at the village level. The others will need to be used at higher concentrations than the user dilution. Also, when the other disinfectants are used, farmers are recommended to apply them twice, within an interval of 18 hours. Kleenol® and Lysol® were, however found to be mostly useless.

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APPENDICES

Appendix 1: *Listeria* isolates from samples of birds at farm level

District	Division	Animal type	Farm code	Flock size	Total birds sampled	Samples taken		*Listeria* species isolated	
						C	P	C	P
Nairobi	Embakasi	Chicken	1	31	6	6	6	0/6	0/6
Nairobi	Embakasi	Chicken	2	20	6	6	6	0/6	0/6
Nairobi	Embakasi	Chicken	3	36	6	6	6	0/6	0/6
Nairobi	Embakasi	Chicken	4	52	6	6	6	0/6	0/6
Nairobi	Embakasi	Chicken	5	12	2	2	2	0/2	½
Nairobi	Embakasi	Ducks	6	30	12	12	12	0/12	0/12
Nairobi	Embakasi	Ducks	7	18	12	12	12	0/12	0/12
Nairobi	Embakasi	Ducks	8	32	5	5	0	0/5	-
Nairobi	Dagoretti	Ducks	9	11	5	5	0	0/5	-
Kajiado	Ngong	Ducks	10	19	5	5	0	0/5	-
Subtotal				**261**	**65**	**65**	**50**	**0/65**	**1/50**
Machakos	**Athi river**	Chicken	11	25	12	12	12	0/12	0/12
Machakos	**Athi river**	Chicken	12	56	11	11	11	0/11	0/11
Machakos	Athi river	Chicken	13*1	74	6	6	6	0/6	0/6
Subtotal				**130**	**29**	**29**	**29**	**0/29**	**0/29**
Total				**391**	**94**	**94**	**79**	**0/94**	**1/79**

Legends:

Farms: 1- Mubangi; 2 – Kibuthu; 3 – Alex; 4 –K. Muthemba; 5 – M. Njuguna; 6 – Mureithi; 11- Maranga; 12 – Nzoya 13 Nzuki

1 - The chickens in this farm belong to three family members but treated as one farm

C - Cloacal swab

P - Oro – pharyngeal swab

Appendix 2: *Listeria* isolation from market birds

Trading market or centre	District of origin	Total chicken sampled	Samples taken		*Listeria* species isolated	
			C	P	C	P
Jogoo road	Bomet	4	4	4	0/4	¼
Jogoo road	Kisii	3	3	3	0/3	2/3
Jogoo road	Makueni	7	7	7	0/7	2/7
Kariokor	Kitui	1	1	1	0/1	0/1
Kariokor	Makueni	2	2	2	0/2	0/2
Kariokor	Mwingi	2	2	2	0/2	0/2
Kariokor	Uasin Gishu	2	2	2	0/2	0/2
Nairobi south	Machakos	14	14	14	0/14	3/14
Nairobi south	Makueni	3	3	3	0/3	0/3
Westlands	Mwingi	2	2	2	0/2	0/2
Total		**40**	**40**	**40**	**0/40**	**8/40**

Legends:

C - Cloacal swab

P - Oro – pharyngeal swab

Appendix 3: *Listeria* isolation from slaughter birds

Slaughtering house	District of origin	Total chicken sampled	Samples taken		*Listeria* spp. isolated	
			C	P	C	P
Kariokor	Kitui	12	12	12	0/12	0/12
Kariokor	Bomet	10	10	10	0/10	0/10
Kariokor	Kericho	6	6	6	0/6	0/6
Kariokor	Uasin – Gishu	4	4	4	0/4	0/4
Burma – Maziwa	**Makueni**	**9**	**9**	**9**	**0/9**	**0/9**
Total		**41**	**41**	**41**	**0/41**	**0/41**

Legends:

C - Cloacal swab
P - Oro – pharyngeal swab

Appendix 4: Characteristics of the *Listeria species isolated*

Sample code	L. mon 1	L. mon. T 2	*L. mon. (47piv)*	*L. mon.(46pv)*	*L. seel.* (8piv)	*L.seel.* (48pi)	*L. innoc.*(53pii)	*L. innoc.* (45pi)	*L. innoc.* (51pv)	*L. grayi* (11pii)	*L.murrayi* (piii)
Gram	G +ve s. rods	G+ve Coccob.	G +ve s. rods	G +ve s. rods	G +ve s. rods	G +ve s. rods	G +ve s. rods	G +ve s. rods	G +ve s. rods	G +ve s. rods	G +ve s. rods
Oxidase	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
Catalase	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
SACVBA	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG
Coagulase	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
CAMP	+ve	+ve	+ve	+ve	+ve	+ve	-ve	+ve	-ve	-ve	-ve
Motility at 25°C	+ve	+ve	+ve	+ve	+ve	-ve	+ve	+ve	+ve	+ve	+ve
Rhamnose	+ve	+ve	+ve	+ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
Mannitol	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
Xylose	+ve	+ve	-ve	-ve	-ve	-ve	-ve	+ve	-ve	-ve	-ve
Salicin	+ve	+ve	+ve	+ve	-ve	-ve	-ve	+ve	-ve	+ve	+ve
VP	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
Urea utilization	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
Nitrate reduction	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
Mice pathogenicity	+ve	+ve	+ve	-ve	-ve	-ve	-ve	+ve	-ve	-ve	-ve

Legend:
L.mon. : *Listeria monocytogenes*
L. seel. : *Listeria seeligeri*
L. innoc. : *Listeria innocua*
L. murr.: *Listeria murrayi*
G+ve s. rods: Gram positive short rods
G+ve coccob.:Gram positive coccobacillus
NG: No growth
+ve: Positive reaction
-ve : Negative reaction

Appendix 5: Isolation of *Escherichia coli; Staphylococcus aureus; Streptococcus spp. and Erysipelothrix spp.* from farm, market and slaughter chickens / ducks

District	Division	Animal type	Farm code	Flock size	Total birds sampled	*E.coli*		*S. aureus*		*Streptococcus spp.*		*Erysipelothrix spp.*	
						Total No.	%	Total No.	%	Total No.	%	Total No.	%
Nairobi	Emb	Chic	1	31	6	2	33.3	1	16.7	2	33.3	2	33.3
Nairobi	Emb	Chic	2	20	6	2	33.3	2	33.3	1	16.7	0	0
Nairobi	Emb	Chic	3	36	6	3	50	2	33.3	2	33.3	0	0
Nairobi	Emb	Chic	4	52	6	1	16.7	2	33.3	2	33.3	0	0
Nairobi	Emb	Chic	5	12	2	1	50	1	50	1	50	0	0
Nairobi	Emb.	Duc.	6	30	12	5	41.7	4	33.3	4	33.3	0	0
Nairobi	Emb.	Duc.	7	18	12	5	41.7	6	41.7	5	41.7	0	0
Nairobi	Emb.	Duc.	8	32	5	2	40	4	80	1	20	0	0
Nairobi	Dag.	Duc.	9	11	5	2	40	2	40	2	40	0	0
Kajiado	Ngo.	Duc.	10	19	5	1	20	3	2	2	40	40	0
Subtotal				**261**	**65**	**24**		**26**		**22**		**2**	
Machak.	Athi Riv.	Chic.	11	25	12	5	41.7	4	33.3	3	25	0	0
Machak.	Athi Riv.	Chic.	12	56	11	4	36.4	3	27.3	3	27.3	0	0
Machak.	Athi Riv.	Chic	13*[2]	74	6	3	50	2	33.3	1	16.7	0	0
Subtotal				**130**	**29**	**12**	**41.4**	**9**	**31.0**	**7**	**24.1**	**0**	**0**
Total				**391**	**94**	**36**	**38.3**	**35**	**37.2**	**29**	**30.9**	**2**	**2.1**

Legend:

Emb. : Embakasi **Athi Riv.** : Athi river
Dag. : Dagorretti **Chic.** : chicken
Ngo. : Ngong **Duc.** : Duck

Appendix 6: Antibiotic sensitivity testing for *Listeria monocytogenes* and other *Listeria* isolates

Sample code	Antibiotic Type	Diameter (Sensitivity zone in mm)
Ox – *Staphylococcus aureus* NCTC 6571	Ampicillin	0.0
	Tetracycline	20
	Cotrimoxazole	20
	Augumentin	0.0
	Kenanycin	23
	Gentamicin	26
	Cefuroxime	0.0
	Chloramphenicol	30
L. monocytogenes[1] 1	Ampicillin	0.0
	Tetracycline	16
	Cotrimoxazole	18
	Augumentin	0.0
	Kenanycin	19
	Gentamicin	19
	Cefuroxime	0.0
	Chloramphenicol	26
L. monocytogenes (47piv)	Ampicillin	0.0
	Tetracycline	17
	Cotrimoxazole	17
	Augumentin	0.0
	Kenanycin	17
	Gentamicin	19
	Cefuroxime	0.0
	Chloramphenicol	28
L. monocytogenes (46pv)	Ampicillin	0.0
	Tetracycline	20
	Cotrimoxazole	20
	Augumentin	0.0
	Kenanycin	20
	Gentamicin	20
	Cefuroxime	0.0
	Chloramphenicol	10
L. seeligeri (8piv)	Ampicillin	0.0
	Tetracycline	7
	Cotrimoxazole	11
	Augumentin	0.0
	Kenanycin	19
	Gentamicin	21
	Cefuroxime	0.0
	Chloramphenicol	16
L.seeligeri (48pi)	Ampicillin	0.0
	Tetracycline	22
	Cotrimoxazole	12
	Augumentin	0.0

	Kenanycin	32
	Gentamicin	32
	Cefuroxime	0.0
	Chloramphenicol	40
L. innocua (53pii)	Ampicillin	0.0
	Tetracycline	38
	Cotrimoxazole	18
	Augumentin	0.0
	Kenanycin	30
	Gentamicin	32
	Cefuroxime	0.0
	Chloramphenicol	40
L. innocua (45pi)	Ampicillin	0.0
	Tetracycline	32
	Cotrimoxazole	18
	Augumentin	0.0
	Kenanycin	30
	Gentamicin	30
	Cefuroxime	0.0
	Chloramphenicol	42
L. innocua (51pv)	Ampicillin	0.0
	Tetracycline	38
	Cotrimoxazole	10
	Augumentin	0.0
	Kenanycin	32
	Gentamicin	30
	Cefuroxime	0.0
	Chloramphenicol	38
L. grayi (11pii)	Ampicillin	0.0
	Tetracycline	20
	Cotrimoxazole	20
	Augumentin	0.0
	Kenanycin	21
	Gentamicin	20
	Cefuroxime	0.0
	Chloramphenicol	16
L. murrayi (11piii)	Ampicillin	0.0
	Tetracycline	21
	Cotrimoxazole	19
	Augumentin	0.0
	Kenanycin	25
	Gentamicin	24
	Cefuroxime	0.0
	Chloramphenicol	29

Appendix 7: Disinfectant sensitivity testing on *Listeria monocytogenes* and other *Listeria* isolates

***Listeria monocytogenes* isolates**	**DISINFECTANTS**	**DILUTIONS**				
		1	2	3	4	5
Listeria monocytogenes^{T} 1	Omnicide®	3.0 cm	2.7 cm	2.5 cm	2.5 cm	1.2 cm
	Sodium Hypochlorite	1.9 cm	0.0	0.0	0.0	0.0
	Bromosept 50®	1.7 cm	1.7 cm	1.7 cm	1.7 cm	1.5 cm
	Lavik®	2.5 cm	2.5 cm	2.2 cm	2.0 cm	1.8 cm
	Dettol®	2.4 cm	2.4 cm	1.9 cm	1.3 cm	0.0
	Kleenol®	0.0	0.0	0.0	0.0	0.0
	Lysol®	0.0	0.0	0.0	0.0	0.0
Listeria monocytogenes^{T} 2	Omnicide®	2.1 cm	1.8 cm	1.7 cm	1.7 cm	1.7 cm
	Sodium Hypochlorite	1.8 cm	1.0 cm	0.4 cm	0.4 cm	0.0
	Bromosept 50®	1.7 cm	1.6 cm	1.6 cm	1.6 cm	1.6 cm
	Lavik®	2.3 cm	1.9 cm	1.8 cm	1.5 cm	1.4 cm
	Dettol®	1.0 cm	0.9 cm	0.0	0.0	0.0
	Kleenol®	0.6 cm	0.0	0.0	0.0	0.0
	Lysol®	0.0	0.0	0.0	0.0	0.0
47 p ii	Omnicide®	3.5 cm	3.0 cm	2.7 cm	2.2 cm	1.1 cm
	Sodium Hypochlorite	0.0	0.0	0.0	0.0	0.0
	Bromosept 50®	1.7 cm	1.7 cm	1.5 cm	1.5 cm	1.5 cm
	Lavik®	2.5 cm	2.5 cm	2.1 cm	1.7 cm	1.1 cm
	Dettol®	1.3 cm	1.3 cm	1.2 cm	0.0	0.0
	Kleenol®	0.7 cm	0.7 cm	0.0	0.0	0.0
	Lysol®	0.0	0.0	0.0	0.0	0.0

47 p iv (*Listeria monocytogenes*)	Omnicide®	3.0 cm	2.0 cm	2.0 cm	1.2 cm	1.2 cm
	Sodium Hypochlorite	1.0 cm	0.4 cm	0.4 cm	0.4 cm	0.4 cm
	Bromosept 50®	2.4 cm	2.2 cm	2.0 cm	2.0 cm	2.0 cm
	Lavik®	3.4 cm	3.0 cm	2.4 cm	2.0 cm	1.4 cm
	Dettol®	2.4 cm	1.1 cm	0.8 cm	0.0	0.0
	Kleenol®	0.0	0.0	0.0	0.0	0.0
	Lysol®	0.0	0.0	0.0	0.0	0.0
46 p v (*Listeria monocytogenes)*	Omnicide®	2.2 cm	2.1 cm	1.9 cm	1.7 cm	1.1 cm
	Sodium Hypochlorite	0.9 cm	0.9 cm	0.7 cm	0.0	0.0
	Bromosept 50®	1.7 cm	1.6 cm	1.2 cm	0.9 cm	0.9 cm
	Lavik®	1.7 cm	1.6 cm	0.9 cm	0.5 cm	0.0 cm
	Dettol®	2.6 cm	2.6 cm	2.5 cm	2.2 cm	1.9 cm
	Kleenol®	1.0 cm	0.9 cm	0.0 cm	0.0 cm	0.0
	Lysol®	0.0	0.0	0.0	0.0	0.0
8Piv *(Listeria seeligeri)*	Omnicide®	2.0 cm	1.8 cm	1.6 cm	1.1 cm	0.9 cm
	Sodium Hypochlorite	1.0 cm	0.0	0.0	0.0	0.0
	Bromosept 50®	1.5 cm	1.4 cm	1.0 cm	0.9 cm	0.7 cm
	Lavik®	1.5 cm	1.5 cm	0.9 cm	0.6 cm	0.0
	Dettol®	1.4 cm	1.3 cm	0.9 cm	0.7 cm	0.0
	Kleenol®	0.0	0.0	0.0	0.0	0.0
	Lysol®					
48Pi *(Listeria seeeligeri)*	Omnicide®	2.5 cm	2.5 cm	2.3 cm	2.3 cm	2.3 cm
	Sodium Hypochlorite	0.6 cm	0.0	0.0	0.0	0.0
	Bromosept 50®	3.2 cm	3.0 cm	3.0 cm	2.1 cm	2.0 cm
	Lavik®	2.6 cm	2.4 cm	2.2 cm	2.2 cm	2.2 cm

	Dettol®	2.2 cm	1.8 cm	1.5 cm	1.2 cm	0.0
	Kleenol®	2.5 cm	1.5 cm	0.7 cm	0.7 cm	0.0
	Lysol®	0.0	0.0	0.0	0.0	0.0
45Pi *(Listeria innocua)*	Omnicide®	3.8 cm	3.2 cm	3.2 cm	3.0 cm	3.0 cm
	Sodium Hypochlorite®	2.4 cm	1.6 cm	0.0	0.0	0.0
	Bromosept 50®	3.8 cm	3.0 cm	2.8 cm	2.8 cm	2.8 cm
	Lavik®	3.0 cm	2.8 cm	2.4 cm	2.3 cm	1.0 cm
	Dettol®	2.1 cm	2.1 cm	2.0 cm	1.8 cm	0.7 cm
	Kleenol®	2.0 cm	1.7 cm	0.9 cm	0.0	0.0
	Lysol®	0.0	0.0	0.0	0.0	0.0
51Pv *(Listeria innocua)*	Omnicide®	3.8 cm	3.7 cm	3.7 cm	3.4 cm	3.4 cm
	Sodium Hypochlorite	0.9 cm	0.6 cm	0.0	0.0	0.0
	Bromosept 50®	3.6 cm	2.4 cm	2.4 cm	2.4 cm	2.4 cm
	Lavik®	4.0 cm	3.8 cm	3.8 cm	3.0 cm	3.0 cm
	Dettol®	1.9 cm	1.4 cm	1.2 cm	0.7 cm	0.7 cm
	Kleenol®	1.7 cm	1.7 cm	0.7 cm	0.0	0.0
	Lysol®	0.0	0.0	0.0	0.0	0.0
53Pii *(Listeria innocua)*	Omnicide®	3.4 cm	3.2 cm	1.8 cm	1.8 cm	1.8 cm
	Sodium Hypochlorite	1.0 cm	0.7 cm	0.7 cm	0.0	0.0
	Bromosept 50®	3.2 cm	2.4 cm	1.4 cm	1.0 cm	1.0 cm
	Lavik®	2.8 cm	2.8 cm	2.5 cm	1.5 cm	1.2 cm
	Dettol®	2.4 cm	1.9 cm	1.8 cm	1.4 cm	1.2 cm
	Kleenol®	1.8 cm	1.2 cm	1.0 cm	0.0	0.0
	Lysol®	0.0	0.0	0.0	0.0	0.0

11Pii *(Lsteira grayi)*	Omnicide®	2.5 cm	2.4 cm	2.0 cm	1.7 cm	1.4 cm
	Sodium Hypochlorite	1.0 cm	0.5 cm	0.0	0.0	0.0
	Bromosept 50®	1.9 cm	1.4 cm	1.2 cm	1.4 cm	1.0 cm
	Lavik®	1.6 cm	1.5 cm	1.1 cm	0.7 cm	0.0
	Dettol®	2.7 cm	2.5 cm	2.5 cm	2.0 cm	1.1 cm
	Kleenol®	1.1 cm	0.0	0.0	0.0	0.0
	Lysol®	0.0	0.0	0.0	0.0	0.0
11Piii *(Listeria murrayi)*	Omnicide®	2.4 cm	2.2 cm	2.0 cm	1.9 cm	1.7 cm
	Sodium Hypochlorite	1.5 cm	0.7 cm	0.0	0.0	0.0
	Bromosept 50®	2.0 cm	1.9 cm	1.9 cm	1.4 cm	1.2 cm
	Lavik®	1.5 cm	1.2 cm	1.2 cm	0.7 cm	0.0
	Dettol®	2.4 cm	2.4 cm	2.0 cm	1.8 cm	1.0 cm
	Kleenol®	1.6 cm	1.4 cm	1.0 cm	0.0	0.0
	Lysol®	0.0	0.0	0.0	0.0	0.0

Appendix 8: Disinfectant sensitivity testing on Katani field samples (farms)

Sample code	DISINFECTANT TYPE	DILUTIONS				
		1	2	3	4	5
I (C +P)	Omnicide®	2.0 cm	1.5 cm	1.4 cm	1.4 cm	1.1 cm
	Sodium Hypochlorite	2.3 cm	2.1 cm	1.1 cm	1.0 cm	0.0
	Bromosept 50®	1.1 cm	1.1 cm	1.0 cm	1.0 cm	1.0 cm
	Lavik®	0.3 cm	0.5 cm	0.5 cm	0.0	0.0
	Dettol®	1.5/20 cm	0.9/ 1.7 cm	1 /1.6 cm	0.5 cm	0.5 cm
	Kleenol®	1.2 cm	0.5 cm	0.5 cm	0.0	0.0
	Lysol®	0.0	0.0	0.0	0.0	0.0
J (C+P)	Omnicide®	1.7 cm	1.5 cm	1.3 cm	1.2 cm	0.5 cm
	Sodium Hypochlorite	1.5 cm	1.2 cm	1.0 cm	0.7 cm	0.0
	Bromosept 50®	1.2 cm	1.1 cm	1.1 cm	1.1 cm	1.0 cm
	Lavik®	1.5 cm	1.4 cm	1.3 cm	0.0	0.0
	Dettol®	2.5 cm	2.0 cm	1.6 cm	1.2 cm	0.0
	Kleenol®	0.7 cm	0.5 cm	0.5 cm	0.0	0.0
	Lysol®	0.0	0.0	0.0	0.0	0.0
K (c+p)	Omnicide®	2.0 cm	1.6 cm	1.5 cm	1.2 cm	1.1 cm
	Sodium Hypochlorite	2.1 cm	1.5 cm	1.2 cm	1.0 cm	0.7 cm
	Bromosept 50®	1.3 cm	1.3 cm	1.2 cm	1.0 cm	1.0 cm
	Lavik®	1.7 cm	1.6 cm	1.3 cm	1.1 cm	1.0 cm
	Dettol®	2.9 cm	2.7 cm	2.5 cm	2.3 cm	1.5 cm
	Kleenol®	1.1 cm	0.5 cm	0.0	0.0	0.0
	Lysol®	0.0	0.0	0.0	0.0	0.0
L (C+P)	Omnicide®	1.5 /1.9 cm	0.5 cm	1.0 cm	0.5 /0.9 cm	0.5 cm

	Sodium Hypochlorite	0.9 /1.2 cm	0.8 cm	0.5 cm	0.0	0.0
	Bromosept 50®	2.1 cm	2.1 cm	1.5 cm	1.4 cm	1.2 /0.6 cm
	Lavik®	1.2 cm	1.3 /1.5 cm	0.0	0.0	0.0
	Dettol®	2.3 cm	2.0 cm	0.8 cm	0.5 cm	0.8 cm
	Kleenol®	0.7 /0.9 cm	0.5 cm	0.0	0.0	0.0
	Lysol®	0.0	0.0	0.0	0.0	O.0
M(C+P)	Omnicide®	1.5 cm	0.0	0.0	0.0	0.0
	Sodium Hypochlorite®	1.0 / 1.7 cm	0.0	0.0	0.0	0.0
	Bromosept 50®	2.8 cm	2.0 cm	1.5 /2.0 cm	1.5 cm	1.3 cm
	Lavik®	1.5 /2.2 cm	1.2 /1.9 cm	1.1 /1.9 cm	0.9 /1.6 cm	0.5 cm
	Dettol®	0.0	0.0	0.0	0.0	0.0
	Kleenol®	0.8 cm	0.0	0.0	0.0	0.0
	Lysol®	0.0	0.0	0.0	0.0	0.0
N (C+P)	Omnicide®	1.3 /2.2 cm	1.1 /1.7 cm	0.9 /1.8 cm	0.0	0.9 cm
	Sodium Hypochlorite	0.5 cm	0.0	0.0	0.0	0.0
	Bromosept 50®	1.4 /2.3 cm	1.5 /2.3 cm	1.2 /1.7 cm	1.0 /1.5 cm	0.5 /1.4 cm
	Lavik®	1.2 /1.2 cm	1.0 /1.3 cm	0.0	0.0 cm	0.0
	Dettol®	1.4 cm	1.1 cm	0.0 cm	0.0 cm	0.0 cm
	Kleenol®	1.7 cm	0.5 cm	0.0	0.0	0.0
	Lysol®	0.0	0.0	0.0	0.0	0.0

Appendix 9: Disinfectant sensitivity testing on Ruai field samples (farms)

Sample code	DISINFECTANTS	DILUTIONS				
		1	2	3	4	5
A (C+P)	Omnicide®	1.3 cm	1.2 cm	0.9 cm	0.6 cm	0.0 cm
	Sodium Hypochlorite	1.0 cm	0.7 cm	0.0	0.0	0.0
	Bromosept 50®	1.0 cm	1.0 cm	0.9 cm	0.9 cm	0.7 cm
	Lavik®	1.0 cm	0.7 cm	0.0	0.0	0.0
	Dettol®	1.2 cm	1.2 cm	0.4 cm	0.0	0.0
	Kleenol®	0.6 cm	0.0	0.0	0.0	0.0
	Lysol®	0.0	0.0	0.0	0.0	0.0
B (C +P)	Omnicide®	1.0 cm	0.9 cm	0.8 cm	0.6 cm	0.4 cm
	Sodium Hypochlorite	0.9 cm	0.7 cm	0.0	0.0	0.0
	Bromosept 50®	1.3 cm	1.1 cm	1.0 cm	1.0 cm	0.4 cm
	Lavik®	0.9 cm	0.9 cm	0.7 cm	0.0	0.0
	Dettol®	1.0 cm	1.0 cm	0.7 cm	0.0	0.0
	Kleenol®	0.0	0.0	0.0	0.0	0.0
	Lysol®	0.0	0.0	0.0	0.0	0.0
C (C+P)	Omnicide®	1.0 cm	0.7 cm	0.0	0.0	0.0
	Sodium Hypochlorite	0.8 cm	0.0	0.0	0.0	0.0
	Bromosept 50®	1.2 cm	1.2 cm	0.9 cm	0.7 cm	0.0
	Lavik®	0.9 cm	0.0	0.0	0.0	0.0
	Dettol®	0.3 cm	0.0	0.0	0.0	0.0
	Kleenol®	0.0	0.0	0.0	0.0	0.0
	Lysol®	0.0	0.0	0.0	0.0	0.0
D (C+P)	Omnicide®	1.2 cm	0.8 cm	0.6 cm	0.4 cm	0.0 cm
	Sodium Hypochlorite	1.0 cm	0.7 cm	0.0	0.0	0.0

	Bromosept 50®	1.3 cm	1.0 cm	1.0 cm	1.0 cm	1.0 cm
	Lavik®	1.2 cm	0.7 cm	0.7 cm	0.0	0.0
	Dettol®	1.3 cm	1.2 cm	0.7 cm	0.6 cm	0.0
	Kleenol®	0.0	0.0	0.0	0.0	0.0
	Lysol®	0.0	0.0	0.0	0.0	0.0
E (C+P)	Omnicide®	1.3 cm	0.9 cm	0.8 cm	0.3 cm	0.0
	Sodium Hypochlorite	0.7 cm	0.7 cm	0.7 cm	0.0	0.0
	Bromosept 50®	1.2 cm	0.9 cm	0.9 cm	0.7 cm	0.3 cm
	Lavik®	1.2 cm	1.0 cm	1.0 cm	0.9 cm	0.6 cm
	Dettol®	1.2 cm	1.0 cm	0.6 cm	0.0	0.0
	Kleenol®	0.3 /1.3 cm	1.0 cm	0.0	0.0	0.0
	Lysol®	0.0	0.0	0.0	0.0	0.0
F (C+P)	Omnicide®	1.1 cm	1.0 cm	0.5 cm	0.0	0.0
	Sodium Hypochlorite	0.7 cm	0.2 cm	0.0	0.0	0.0
	Bromosept 50®	2.5 cm	1.3 cm	1.2 cm	0.5 cm	0.2 cm
	Lavik®	0.0	0.0	0.0	0.0	0.0
	Dettol®	1.3 cm	1.1 cm	0.0	0.0	0.0
	Kleenol®	0.0	0.0	0.0	0.0	0.0
	Lysol®	0.0	0.0	0.0	0.0	0.0
G (C+P)	Omnicide®	1.1 cm	1.0 cm	0.7 cm	0.2 cm	0.2 cm
	Sodium Hypochlorite	1.0 cm	0.9 cm	0.6 cm	0.0	0.0
	Bromosept 50®	1.4 cm	1.0 cm	1.2 cm	1.2 cm	1.0 cm
	Lavik®	1.2 cm	0.9 cm	0.7 cm	0.0	0.0
	Dettol®	1.3 cm	1.3 cm	1.0 cm	0.0	0.0
	Kleenol®	0.7 cm	0.3 cm	0.0	0.0	0.0
	Lysol®	0.0	0.0	0.0	0.0	0.0
H (C+P)	Omnicide®	1.3 cm	1.2 cm	1.0 cm	0.0	0.0
	Sodium Hypochlorite	1.6 cm	1.0 cm	0.8 cm	0.0	0.0

	Bromosept 50®	1.5 cm	1.1 cm	1.0 cm	1.5 cm	1.0 cm
	Lavik®	1.6 cm	0.0	0.0	0.0	0.0
	Dettol®	1.2 cm	1.6 cm	0.0	0.0	0.0
	Kleenol®	0.0	0.0	0.0	0.0	0.0
	Lysol®	0.0	0.0	0.0	0.0	0.0

Appendix 10: Isolates from Ruai samples

Sample code	Isolates per bird
A (C+P) Duck	*Escherichia coli* *Streptococcus spp.* *Staphylococcus aureus* Unidentified G ve cocci
B(C+P) Duck	*Escherichia coli* *Staphylococcus aureus*
C(C+P) Duck	*Escherichia coli* *Streptococcus spp.* *Pseudomonas spp.*
D(C+P) Duck	*Escherichia coli* *Streptococcus spp* *Klebsiella spp.*
E(C+P) Duck	*Escherichia coli* *Streptococcus spp* *Pseudomonas spp.* *Staphylococcus aureus*
F(C+P)	*Escherichia coli* *Streptococcus spp* *Pseudomonas spp.* *Staphylococcus aureus* *Staphylococcus epidermidis* Unidentified gram positive rods
G(C+) Chicken	*Escherichia coli* *Streptococcus spp* *Staphylococcus epidermidis*
H(C+P) Chicken	*Staphylococcus aureus* *Streptococcus spp* *Listeria seeligeri* Unidentified G ve cocci

Legends:

C - Cloacal swab

P - Oro – pharyngeal swab

Appendix 11: Isolates from Katani samples.

Sample code	Isolates per bird
I (C+P)	*Proteus spp.* *Escherichia coli* *Streptococcus spp* *Staphylococcus aureus* Unidentified G+ve cocci
J (C+P)	*Proteus spp.* *Escherichia coli* *Staphylococcus aureus* Unidentified G+ve cocci
K(C+P)	*Bacillus spp.* Unidentified yeast _ like cells *Escherichia coli* *Staphylococcus aureus* *Streptococcus spp* Unidentified G+ve cocci
L(C+P)	*Escherichia coli* *Staphylococcus aureus* *Streptococcus spp* *Bacillus spp.* *Pseudomonas spp.* Unidentified G+ve cocci Unidentified G_ve cocci
M(C+P)	*Escherichia coli* *Staphylococcus aureus* Unidentified G+ve cocci Unidentified G_ve cocci
N(C+P)	*Bacillus spp.* Unidentified G+ve cocci

Legend:

C: Cloacal swab
P: pharyngeal swab