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Full Length Research

Isolation Rates of Specific Pathogens from Meat Carcasses In Nairobi County, and their Susceptibility to Garlic Extract

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Meat is a major source of pathogens and spoilage *microorganisms*. Meat preservation methods commonly used to inhibit growth of pathogensand spoilage *microorganisms* in meat include heat treatment, preservative salts, irradiation, drying, refrigeration and smoking, however, there is little information available on the novel application of garlic extract as meat preservative. The aim of this study was to determine if garlic (*Allium sativum*) has antimicrobial activity against pathogens and spoilage bacteria commonly found to contaminate meat.The antimicrobial activity of garlic ethanol and ethyl acetate extracts were determined against the following bacteria standards and isolates obtained from meat samples; *Bacillus cereus*(ATCC 11778), *Salmonella typhimurium* (ATCC 72225671), *Staphylococcus aureus* (ATCC 25923), *Escherichia coli*(ATCC 25922) and *Campylobacter spp.*isolated from meat. Beef swabs and Chicken rinse-washes were obtained from slaughter and sale points in Nairobi County with the aim of isolating pathogenic *microorganisms*. Sub-culturing was done on selective media and subsequently followed by biochemical- and geno-typing with specific primers for identification. Susceptability of microoorganism to ethyl acetate garlic extract was carried out using agar well diffusion method.There was no significant difference in the total percentage number of *E.coli* from Dagoretti abattoirs (30%), Dagoretti butcheries (23.3%), Kawangware butcheries (23.3%) and Pangani butcheries (30%), (P = 0.919. Df value =3). .However, there was a significant difference in the percentage of *E. coli* at Burma market (27.8%), Kariorkor market (58.3%) and City market (55.6%), (P = 0.0418. Df value =3). There was also a significant difference in the percentage of *Campylobacter species* at Burma market (67.2 %), Kariorkor market (8.2 %) and City market (33.3%), (P = 0.045, Df value =3).. The novel application of garlic ethyl acetate extract in meat preservation has great potential for use for decontaminationand preservation of meat for extending the shelf life of fresh meat and enhancement of safety against pathogens of public health concern.

Keywords: Garlic extracts, antimicrobial activity,bacterial pathogens, spoilage bacteria

INTRODUCTION

Meat is obtained from large and small domestic and wide-range of wild animals as well as seafood (Jay, 2005). Globally, meat production has tripled in the past three decades and could double its present level by 2050 (FAO, 2009). In Kenya,livestock sector contributes 3.3% of the gross domestic product (GDP), mainly from cows, sheep, goats and poultry(Noah and Waithaka,2005). The quality and safety of meat can easily deteriorate when improperly preserved due to spoilage. The most common cause of meat spoilage is the *microorganisms* (Frazier and Westhoff, 2008). Indication of spoilage includes production of ammonia or sulfur smell, and bad odor, due to degradation of proteins, lipids (fats) and carbohydrates caused by bacteria and/or enzymes naturally present in meat (Frazier and Westhoff, 2008). Some *microorganisms* produce toxins on meat making it unfit for human consumption. For example *S. typhi* contains an

endotoxin typical of Gram negative organisms, as well as the Vi antigen which is thought to increase virulence. *B. cereus* causes food poisoning due to the toxins it produces when the bacilli sporulates especially on foods like meat, and meat products (Cheesbrough, 2006).

Meat contamination by *microorganisms* mainly occurs through operations carried out in animal husbandry, processing, preparation, treatment, packaging and transporting and also from the environment (WHO, 2002). At the moment of slaughtering the meat is almost sterile so that the primary contamination concerns in particular is the meat surface. Later the *microorganisms* penetrate into deeper layers of meat. When this primary contamination is reduced, the shelf-life of meat can be significantly prolonged (Ransom *et al*., (2001). Thus it is advantageous to decontaminate the surface of carcasses to increase their shelf-life and to enable the safe distribution of meat. There are a number of methods used for the surface decontamination of meat. The most common methods are use of organic acids such as acetic acid, lactic acid, formic acid and propionic acid which act by decreasing pH, and due to their bactericidal properties, stops growth of bacteria (FDA, 2003). These acids are often used for surface decontamination as they are natural component of meat produced during postmortem glycolysis and thus they are not typical additives (Raftri *et al*., 2009). However, Organic acids may select for the presence of acid-resistant bacteria that may accelerate rates of product spoilage, increase undesirable effects on product appearance, and speed equipment corrosion (Stopforth *et al*., 2007).

Preservation methods used to inhibit pathogen growth on meat include use of salts, irradiation, drying, refrigeration and smoking (Hui, 2001). Salts like sodium chloride dehydrate *microorganisms* but do not retard growth of pathogenic halophiles such as Staphylococcus aureus which grow readily even in 7.5% salt concentration (Talaro, 2006). The use of refrigeration is common in urban areas but is not widely available to rural community due to poverty and lack of electricity. Nitrites /nitrates like Sodium nitrite inhibit the germination of Clostridium botulinum spores. However they react with amines to form nitrosamines that are carcinogenic and cause high blood pressure (WHO, 2002).

Garlic is one of the most commonly used ingredients as a flavor enhancement agent for meat products. Garlic contains very active compounds that are both nutritive and antimicrobial (Njue *et al*.,2017a; Njue *et al*., 2017b ; Njue *et al* 2015c;Njue *et al* 2014d; Njue *et al* 2009e).

It has a wide spectrum of activity; including antibacterial, antiviral, antifungal and antiprotozoal activities (Avato *et al*., 2000; Seong soo *et al*., 2010; Arunkumar &Muthuselvam 2009). It also has beneficial effects on the cardiovascular and immune systems (Harris *et al*., 2001).

During the last decade, the antimicrobial activity of garlic and garlic-derived organo-sulfur compounds was widely investigated against both food spoilage bacteria and food-borne pathogens (Naidu, 2000). Garlic-rich organosulfur compounds and their precursors (allicin, diallyl sulfide and diallyl trisulfide) are believed to play a key role in these biological effects. Aqueous garlic extracts contain primarily S-allyl-l-cysteines derived from γ-glutamyl-S-allyl-l-cysteines (Kodera *et al*.,2002), S-Allyl-l-cysteine and trans-S-1-propenyl-l-cysteine, together with a small amount of S-methyl-l-cysteine, are found in garlic extract such as aged garlic extracts (AGE). These cysteine derivatives are colorless crystals and are odorless and stable in the solid state or aqueous solution under neutral or slight acidic conditions (Kodera et al., 2002.) The proprietary aging process produces an odorless preparation and converts the harsh, unstable organosulfur compounds in garlic (e.g., allicin) into milder and more beneficial compounds including water-soluble, sulfur-containing, antioxidant rich amino acids such as S-allylcysteine (SAC), S-allyl mercaptocysteine (SMAC) and Maillard reaction products. It is worth noting that SAC has a 98% absorption rate into the blood giving it robust bioavailability. SAC is the key compound in AGE and is used to standardize it (Borek, 2000; Kyolic 2010).

The aim of this study was to assess isolation rates of specific pathogens from meat carcasses from Nairobi County, and to determine their susceptibility to garlic extracts.

MATERIALS AND METHODS

Sampling of beed and chicken carcases in abbatoirs and butcheries/selling points in Nairobi

Sampling of beef carcases

Sampling of beef carcasseswas carried out at slaughterhouses in Dagorretti as well as from butcheries in Kawangware and Pangani, Nairobi. From each sample collection site, a total of 30 swab samples were collected from carcases using template plates of 10 cm by 10 cm. The template plates had been sterilized using 70% ethanol and flamed using a blow lamp before using them to mark the swabbing areas. Three different sites of the carcass, thehind limb (brisket), belly and forelimb (flank)areas were swabbed using the non-destructive technique relying on wet and dry swabs as recommended by European Commission decision (EU,2001). Swabbing was done diagonally, horizontally and vertically nine times after which the swabs were put in 10ml of sterile buffered peptone water in universal bottles. All the samples were put in a coolant box with ice cubes and then taken to the laboratory within the next one hour. Incubation was at 37ºC for 24h. to accelerate microbial growth for the next procedure.

Sampling of chicken meat

Sampling of chicken was carried out at Burma, Kariorkor and City markets, Nairobi. A total of 36 samples were collected from each sampling area. At Burma and Kariorkor markets the samples were obtainedfrom carcasses that had been slaughtered on the same day of sampling while at City market, the samples were either refrigerated or frozed for more than 24h. However, most of the butcheries did not have records on duration of refrigeration/freezing for the carcases. From each chicken, areas randomly selected for swabbing comprised of the wing, breast and the drumstick. Each part was wash-rinsed with 200mLof sterile buffered peptone water and then incubated at 37ºC for 18-24h to allow for microbial multiplication.

Reference strains

The following reference strains; Bacillus cereusATCC11778, Salmonella typhimurium ATCC7222567), Staphylococcus aureusATCC25925 and Escherichia coliATCC25922 were used to determine the minimum Inhibitory concentration (MIC) and Minimum Bactericidal concentration (MBC) of Ethyl acetate extract, Acetic acid and Sodium hypochlorite. The reference strains were obtained from Microbiology laboratory, Department of Public Health, Pharmacology and Toxicology, Nairobi, Kenya. There were no Campylobacter spp.reference strains available for this study and therefore campylobacter isolates obtained in this study were used for the assays.

Isolation and biochemical assays for pathogens from carcass swabs

Isolation and biochemical assays forE.coli

A loopful of microbial suspension in buffered peptone water that had been incubated at 37 ºC for 18h was aseptically streaked on MacConkey agar(Oxoid, England, UK) media plates. The streaked plates were incubated at 37ºC for 18h. Pink colonies (lactose fermenters) growing on MacConkay agar were streaked onto Levine - Eosin Methylene Blue(L- EMB) agar (Oxoid, England, UK) media plates for further characterization. All colonies with green metallic sheen and dark centered were characterized using the following biochemical tests: Indole production, acid production, production of acetylcarbinol and utilization of citrate as the sole source of carbon.Characteristic *E. coli* colonies produce Indole from L-tryptophan, acid production rather than alcohol from the MR-VP medium (Oxoid, England, UK), and without the ability to grow in Simmon's citrate agar (Oxoid) due to inability to use citrate as the sole source of carbon.Presumptive *E.coli* were subjected to PCR analysis for further confirmation.

Isolation and biochemical assays forSalmonella species

Nine millilitersof Tetrathionate broth base(Oxoid, England, UK was distributed in culture tubes andeach tube inoculated with 1mL of sample homogenate that had been pre-incubated at 37ºC for 18h. This was followed by addition of 200µL of iodine. The inoculated tubes were then incubated at 37ºC for 24h. After 24h, oneloopful each of Tetrathionate broth (Oxoid, England, UK)th (was streaked on XLD agar(Oxoid, England, UK), incubated at 37ºC for 24 h. Black colonies in XLD plateswere used forbiochemical tests including TSI (Oxoid, England, UKand Urea(Oxoid, England, UK). A portion of a typical S*almonella* colony was picked by a sterilestraight wire loop and inoculated onto TSI and urea by stabbing onto the agar medium. Innoculated TSI andUrea medium were incubated at 37^{o}C for 24h. In TSI salmonella produced alkaline (red) slant and acid(yellow) butt, with or without production hydrogen sulfide (blackening in TSI agar), and gives a negative urea reaction.

Isolation and biochemical assays for B. Cereus

Microbial samples were inoculated on Mannitol–Egg Yolk-Polymyxin (MYP) agar plates (M56) (Oxoid, England, UK) and were incubated at 37ºC for 24h. Typical *B. cereus* colonies had distinctive blue color surrounded by a blue egg yolk precipitate. The colonies were further subjected to the rapid confirmatory staining method as described by Holbrook and Anderson (1980).

Isolation and biochemical assays forS. Aureus

Manittol agar (Oxoid, England, UK) selective medium for *S.aureus* was used. Sample homogenates were streaked on Manittol agar and incubated at 37^{o}C 24h. Characteristic yellow colonies were tested for productionof coagulase and catalase. Coagulase test was done by mixing three parts of rabbit plasma in 4 parts of normal saline and 0.5mL of themixture distributed in sterile culture tubes into which one colony of typical *S. aureus* was inoculated. The catalase test was done by emulsifying a typical *S. aureus* colony using a sterile wooden applicator stick on a drop of 3% hydrogen peroxide on a clean slide. Presence of effervescence indicatedpositive test while abscenceof effervescence was a negative test. For microscopy, gram stain was done and purple colonies characteristic of Staphylococcus was positive result.

Isolation of Campylobacter species

Campylobacter Blood-Free Selective Agar Base, Modified CCDA-PRESTON (Oxoid,

England, UK) and one vial ofCefoperazone selective supplement SR 125(Oxoid, England, UK) were used. Chicken sample homogenate were streaked on CCDA agar (Oxoid) and sealed under wet microaerophilic conditions in plastic jars. They were then incubated at 42oC for 48h. Presumptive Campylobacter isolates that were gram-negative, curved organisms as determined by microscopic examination and were oxidase and catalase positive. PCR assay was used for further confirmation.

DNA Extraction

One day old cultured microbial cellsfrom the isolates and the reference strains were collected from corresponding agar platesusing a sterile loop and suspended in 0.2mL of distilled water in eppidorf tubes. The samples were vortexed, then boiled for 30 minutesin a water bath and cooled, then centrifuged for 5 minutes (Centrifuge 5413, Hinz GMBH, Hamburg). The supernatants were transferedinto eppendorf tubes for DNA analysis using multiplex PCR assays.

PCR IDENTIFICATION

PCR Protocols

PCR reaction was conducted usingthermal cycler (Mj Research, Inl 149, water Town, USA) as follows;Initial denaturation at 94 °C for 10 mins, amplification repeated 35 cycles (denaturation 94°C for 30 sec, Annealing temperature specific for each organismfor 90 sec and Extension 72 °C for 60 sec) and Final extension at 72 °C for 10 mins. The annealing temperatures for the various organisms included; E. Coli, Salmonellaspp.and Bacillus cereus 50 oC while Campylobacter spp. 59 oC

PCR reactants

A single reaction volume for E. coli,S. aureus and Salmonella sppwas 10 µLwhichincluded;
Premix(2x)5.0µL, Primer F (100picanoles/µL) 0.02 µL Primer R (100picamoles/µL) 0.02 µL, Coral load (10x),No distilled Water was addedand DNA sample3.96µL.
For Campylobacter spp, asingle reaction volume was 10 µLthat included; Premix(2x)6.25µL, Primer F (100 picanoles/µL) 0.05 µL, Primer R (100picamoles/ µL) 0.05 µL, Coral load (10x) 1.25µL, distilled water 2.4 µL, and 2.5µLof DNA sample.
On the other hand, a single reaction volume for B. Cereuswas 20 µLand included; Premix(2x)10µL, Primer F (100 picanoles/µL) 0.04 µL, Primer R (100picamoles/µL) 0.04 µL, Coral load(10x) 2.0µL, distilled Water 2.92 µL, and 5.0µL of DNA sample.

Primers used for different isolates

The PCR primers used in this study are listed in Table 1 below.

Gel-electrophoresis and imaging

Gel-electrophoresis was preformed using1.8% agarose gel (Oxoid, England, UK) and stained with ethidium bromide(Oxoid, England, UK). A ladder (markers) was placed in the first well of the gel to mark base pairs (bp) of bands. Isolates that developed a single band that corresponded to reference strains were confirmed to be positive for the straits assayed with specific primers.

Antimicrobial activity of garlic extract testing

Antimicrobial activity of garlic extractwas carried out using agar diffussion method against this study'reference strainsand bacterial isolates**.**

Preparation of garlic extract

The preparation of garlic extractincluded the following steps:One hundred grams of peeled garlic cloves wasweighed on a clean aluminium foil using weighing balance (Mettler pm 4600, Deltarange, Zurich).

Table 1: *PCR primers used in this study for E. coli, Salmonella spp, S. aureus, B. cereus and Campylobacter spp*

Species	Target gene	PCR primers sequences (5'-3')	Product size	References
Escherichia coli	malB promoter	Eco-1 GACCTCGGTTTAGTTCACAGA Eco-2 CACACGCTGACGCTGCCA	585 bp	Candrian *et al.*, (1991)
Salmonella spp.	Inv A gene	Sal-3 TATCGCCACGTTCGGGCAA Sal-3 TCGCACCGTCAAAGGAACC	275 bp	Raln *et al.*, (1992)
Staphylococcus aureus	Nuclease gene	SAL-1 GCGATTGATGGTGATACGGTT SAL-2 CAAGCCTTGACGAACTAAAGC	276bp	Brakstal *et al.*, (1992)
Bacillus cereus	Hemolysin gene	BC-1 CTGTAGCGAATCGTACGTATC BC-2 CAAGCCTTGACGAACTAAAGC	185 bp	Wang *et al.*, (1997)
Campylobacter spp.	C412F	C-1 GGATGACACTTTTCGGAGC C-2 CATTGTAGCACGTGTGTC	812 bp	Giessen *et al.*, (1998)

The weighed material was then blended with an electric blender (Ohms, Internationalfzc,China) and 125mLof 99.9% ethanol(Oxoid,England, UK) was added. The mixture was then homogenized by blending to a pasteand put in a 1000 mL flat bottomed-flask using a glass funnel and then covered with an aluminium foil. This procedure was repeated six times to yield a total weight per volume of 600g of garlic in 750mLethyl acetate, and the total volume was put in one flask. Three such volumes in 1000mLflat bottomed flasks were prepared and a total of 1800g of garlic in 2250mLof ethyl acetate was prepared and was then kept in a dark cabinet for 24h. Shaking was done in the morning and in the evening to homogenize all the flask contents. The contents were filtered using whatman's paper No.1. The resulting filtrate was evaporated using rotary evaporator (Rotor Vapour Pump, Laboratoriums-Technic Ag, Buchi) at 50°C to remove ethyl acetate. This process yielded 812g of extract. The prepared crude extract was divided into two portions; one portion (2 g) was poured into the glass vials to be tested as crude ethanol extract for antimicrobial activity. The extract was then mixed with sterile distilled water at a ratio of 1:1.Garlic ethyl acetate extract was prepared the same way and yielded 710g

Inhibition assay of garlic ethanol extract using agar diffusion method

The antibacterial activity of the garlic ethanol extract was evaluated by agar diffusion method as described by Shobana *et al*., (2009). Bacterial colonies weresuspended in saline to a density of McFarland 0.5 turbidity standard, which corresponded to 1-2x10^{8}CFU/mL (Barry 1973). The adjusted bacterial suspension was swabbed evenly over the entire surface of Mueller Hinton Agar (Oxoid, England, UK) plate using a sterile swab. Using a standard cork borer (11mm), two wells were made at equi-distance on the inoculated Muller Hinton agar (9cm), and the agar plug aseptically removed. Five such sets were made for each test samples. A volume of 100µL of all the garlic ethanol extract was prepared at a concentration ratio of extract to water of 1:1 mg/mL, and was transferred into the corresponding microbial wells with *Bacillus cereus*, *Salmonella typhimurium*, *Staphylococcus aureus*and *Escherichia coli*in duplicates using sterile micropipette tips. Plates were then incubated at 37ºC for 18 hand inhibition zones diameters (mm), expressed in mm according to National Committee for Clinical Laboratory Standards (NCCLS, 2000).
The same volume of 1.5% Acetic acid (100µL), Ampicillin 10µg per well and Ciprofloxacin 5µg per well wereused as positive controlswhile sterile distilled water was the negative control for this study.

Determination of minimum Inhibitory concentration (MIC) and Minimum Bactericidal concentration (MBC) of Ethyl acetate garlic extraction.

One day old microbial cultures of the four reference strains, prepared from the corresponding media. The reference strains were constituted in 4mLnormal saline in culture tubes and adjusted to 0.5McFarlandstandards which was equivalent to 1.2 × 10^8 CFU/mL. Four hundred grams of the extract was dissolved in 2mL of sterile Muellar Hinton Broth ((Oxoid, England, UK) in culture tubes. Serial dilution of 1mL was carried out up to the 5th serial dilution. Preparations were carried out in three sets. One set had the broth and extract alone, the other set had broth, extract and 100µL of bacteial strain.The other set had broth and100µL of bacteial strainonly. This process performed for *B. Cereus* ATCC11778, *S. Typhimurium*ATCC72225671, *S.Aureus*ATCC25925 and *E. Coli*ATCC25922, independently. For the positive controls Acetic acid and Hypochlorite 3% and 1% were used respectively and the

experiment was carried out in a similar manner as for the extracts.The tubes were then incubated at 37°Cfor 18h and turbidity was observed. Tubes without turbidity were considered to have Minimum Inhibitory Concentration (MIC). These tubes were then sub-cultured in duplicates on Muellar Hinton gar. Incubation wasat 37°Cfor 18h and microbial count was done to determine Minimum Bactericidal Concentration (MBC). The concentration of the tubes with no growth or tubes where 99.9% of the *microorganisms* were killed was considered to be the one with MBC(Heijden *et al*. 1999).

Data analysis

Statistical packages used for data analysis were Statistical Package for the Social Sciences (SPSS),Data collected was analyzed by analysis of variance (ANOVA)

RESULTS

The prevalence of bacterial pathogens isolated from beef and chicken carcases

E.coli was isolated from 30% beef samples from Dagoretti abattoirs and Pangani butcheries, while Dagoretti and Kawangware butcheries each had 23.3% prevalence(Table 2).

Table 2: Percentage isolation of pathogens from beef samples

	E. coli	***Salmolla* spp**	***S. aureus***	***B. cereus***	***Campylobacterspp***
Beef swabs (N=30)					
Dagoretti abattoirs	30%	0	0	0	nd
Dagoretti butcheries	23.3%	0	3.3%	0	nd
Kawangware butcheries	23.3%	0	0	3.3%	nd
Pangani butcheries	30%	0	3.3%	0	nd
Chicken carcase samples N=36					
Burma market	27.8%	0	0	0	67.2%
Kariorkor market	58.3%	0	0	25%	8.3%
City market	55.6%	0	0	5.6%	33.3%

N Number of samples; Beef carcases N= 30; Chicken carcases N=36; nd not done

There was no significant difference in the *E.coli* isolated from the different areas (P-value = 0.919). In chicken carcases the prevalence was more than 50% of the samples in two locations Kariakor and city market which had frozen and refrigerated chicken. *S. aureus*was only isolated in very low prevalence of 3.3% in beef carcases only from Dagoretti and Pangani Butcheries. *B. Cereus*was isolated in beef carcases at Kawangware butcheries (3.3%), while in chicken carcases the prevalence of B. Cereus was highest (25%) at Kariokor market although city market only 5% was isolated at city market. *Salmonella* was not isolated from beef or chicken carcases from any of the study sites. In chicken carcases, *Campylobacter* spp. were highest at the Burma market (67% of samples), but 33% was observed in Kariakor market. *Campylobacter spp* were not assayed in beef carcarses (Table 2).

Antimicrobial activity of garlic extract on *Bacillus cereus*, *Salmonella typhimurium*,*Staphylococcus aureus*and *Escherichia coli*

.Antimicrobial activity of garlic ethanol extract and ethyl acetate extract was tested against *B.cereus*, *S. typhimurium*, *S. aureus*and *E. coli*isolated from beef and chicken carcases through diffusion assays with acetic acid as a control (Table 3). The measurement and interpretation of halo diameter (mm) was done according to National Committee for Clinical Laboratory Standards (NCCLS, 2000) (Table 3). Analysis of the comparison of antimicrobial activity of garlic ethanol extract and acetic acid showed that there was no significance difference in the inhibition zones of garlic extract and acetic acid

Table 3: Comparison of antimicrobial activity of garlic ethanol extract and acetic acid

Test Organism	Source of Isolates	Average inhibition zones in millimetres	
		Garlic ethanol Extract(Test substance)	Acetic Acid (Control)
E. coli	Burma market	20.6	22.0
	City market	21.5	21.0
	Dagoretti	20.9	22
	Kariokor	17.7	20.3
B. cereus	Burma	27.6	31.1
	City market	26.3	25.3
Campylobacter spp	Burma	18.6	19.6
	City market	19.2	21.5
	Kariokor market	17.7	20.3

Table 4: Antimicrobial agents and zones of inhibition in millimetres

Antimicrobial agents	**Disk content**	**Zone diameter (mm)**			**MIC/ml**		
		S	**I**	**R**	**S**	**I**	**R**
Ampicillin	10µg	≥ 17	14-16	≤13	≤8	16	≥ 32
Ciprofloxacin	5µg	≥ 21	16-20	≤15	≤1	2	≥ 4

S=Susceptability, I= Intermediate and R= Resistance

(Table3. Comparison of the inhibition of *microorganisms* by garlic extract and standard druga showed that the inhibition zones were within the acceptable range of drugs, ampicilin ≥ 17 and Ciprofloxacin ≥ 21 (Table 4). Ethyl acetate extract, being less toxic than ethanol was preferred as a the reagent for extraction for all MIC and MBC.

Minimum Inhibitory Concentration and Minimum Bactericidal concentration of Ethyl acetate garlic extract

The results of this work indicates that the MIC g/mL and MBC mg/mL of ethyl acetate extract against standard *microorganisms* was as follows *E.coli* 100mg/ml (MIC), 100mg/mL (MBC); *S. typhimurium,*200mg/mL (MIC), 200mg/mL (MBC); *S. aureus,* 200mg/mL (MIC), 200mg/mL (MBC); *B.cereus*, 200mg/mL (MIC), 200mg/ml (MBC). There was a significant difference in the concentration of extract used on *E. coli* p< 0.005.*E. coli* required100mg/mL (MIC), 100mg/mL (MBC); which was far less compared with concentrations for all *microorganisms* which was 200mg/mL (MIC), 200mg/mL (MBC).

For the controls Acetic acid the MIC mg/mL and MBC mg/mL of ethyl acetate extract against standard *microorganisms* was as follows *E.coli* 0.1875 %/mL (MIC), 0.375 %/mL (MBC); S. Typhimurium,0.75% /mL (MIC), 1.5 %/mL (MBC); *S. aureus,* 0.375 %/mL (MIC), 0.75%/mL (MBC); *B.cereus*, 1.5% /mL (MIC), 3.0 %/mL (MBC).

For Sodium Hypochlorite the MIC mg/ml and MBC mg/ml of ethyl acetate extract against standard *microorganisms* was as follows *E.coli* 0.0625 %/mL (MIC), 0.125 %/mL (MBC); S. typhimurium,0.25% /mL (MIC), 0.5 %/mL (MBC); *S. aureus,* 0.125 %/mL (MIC), 0.25%/mL (MBC); *B.cereus*, 0.5% /mL (MIC), 1 %/mL (MBC).

DISCUSSION

Garlic (*Allium sativum* L.), is among the oldest cultivated plants, and is used both as a food and for medicinal applications. The value of garlic extracts and combinations lie in their ability to provide essential phytochemicals that have antibacterial activity against pathogens. They are able to provide phytochemicals for hypercholesterolemia, diabetes type 2, hypertension, cataract and disturbances of the gastrointestinal tract (e.g. colic pain, flatulent colic and dyspepsia) (Sivam 2001).Table 7a indicates isolation of pathogens from beef swabs. There was no significant difference in the total percentage number of *E.coli* from Dagoretti abattoirs (30%), Dagoretti butcheries (23.3%), Kawangware butcheries (23.3%), and Pangani butcheries (30%). Transport did not affect the total microbial load.

There was a significant difference in the percentage of *E. coli* at Burma market (27.8%), Kariorkor market (58.3%) and City market (55.6%) (Table 2). . There was also a significant difference in the percentage of *Campylobacter species* at Burma market (67.2 %), Kariorkor market (8.2 %) and City market (33.3 %). It is well established that glucose, lactic acid, and certain amino acids followed by nucleotides, urea and water-soluble proteins are catabolized by almost all the bacteria of the meat microflora (Nychas *et al*., 2007) as a source of energy. Concentration of these compounds

can affect the type, the rate of spoilage and, moreover, seems to be the principal precursor(s) of those microbial metabolite(s) that are perceived as spoilage (Skandamis & Nychas, 2002; Tsigarida & Nychas, 2001). This could have led to microbial increase Table 2. *E.coli*being a lactose fermentor may be could not have enough metabolites for multiplication. Water is required by micro-organisms, so reducing the water available below the optimum level (below 95 percent), moisture will be lost from the surface and this will increase microbial load, and thus spoilage. May be the moisture level of water was low to allow any significance to be observed (Table 2).

Garlic extracts are active against *microorganisms* that are resistant to antibiotics. The emergence of multi-drug resistant strains of Gram negative (*Pseudomonas*, *Klebsiella*, *Entero-bacter*, *Acinetobacter*, *Salmonella* species, etc) and Gram positive (*Staphylococcus*, *Enterococcus*, *Strepto-coccus* species, etc) bacteria is troubling for human and animals. The emergence of epidemic methicillin resistant *Staphylococcus aureus* (MRSA) resistant to mupirocin has led to the suggestion that the use of mupirocin should be controlled more strictly, especially as there is a lack of alternative agents. Consequently, garlic is an alternative agent for the treatment of MRSA and in a great demand (Sharma *et al*., 2005).

The results of this study indicate that the garlic ethanolic and ethyl acetate extracts had the same antibacterial activity (Table 3) and it is in accordance to (*Njue et al*., 2014).. Garlic can be used as a potent inhibitor of food pathogens. Use of garlic ethyl acetate extract would increase the shelf life and decrease the possibilities of food poisoning and spoilage in processed foods. These results are also comparable to those of author (Sasaki, 1999) who had reported an increased Antibacterial activity of garlic extract on *E. coli* O157:H7. Garlic extract inhibits the growth of Gram positive and Gram negative bacteria, such as *Staphylococcus, Streptococcus, Micrococcus*, *Enterobacter*, *Escherichia*, *Klebsiella*, *Lactobacillus*, *Pseudomonas*, *Shigella, Salmonella, Proteus*, and *Helicobacter pylori* (Tsao, 2001). Its antibacterial activity is mainly due to the presence of allicin produced by the enzymatic activity of allinase on alliin. However, the use of a water-based extract of allicin stabilizes the allicin molecule due to the hydrogen bonding of water to the reactive oxygen atom in allicin or there may be water soluble components in crushed garlic that destabilize the molecule (Lawson, 1996).

The antibacterial activity of garlic is reported to be due to the action of allicin or diallyl thiosulphinic acid or diallyl disulphide (Avato *et al*., 2000). The role of allicin in warding off infection may be particularly valuable in light of the growing bacterial resistance to antibiotics. It is unlikely that bacteria would develop resistance to allicin because this would require modifying the very enzymes that make their activity possible (Tsao *et al*., 2003).

The factors responsible for the high susceptibility of both gram positive and gram negative to organic solvents may be attributed to the secondary metabolites of garlic, including γ-glutamyl peptides, scordinins, steroids, terpenoids, flavonoids and other phenols, which may be responsible for the range of the antibacterial effects reported for garlic extracts (Sivam, 2001). These metabolites work antagonistically and as a result *microorganisms* can never develop resistance depending on the method of extraction. In case of *B. cereus* endospores do not form normally during active growth and cell division. Rather, their differentiation begins when a population of vegetative cells passes out of the exponential phase of cell growth which occurs usually as a result of nutrient depletion (Todar, 2005). This explains why it was most susceptible in all the extracts.

CONCLUSION

In summary it can be stated that Good Hygienic Practices in meat processing requires efforts by both management and staff. It is the duty of the plant management to procure investments in goodquality premises and equipment and in continuous plant and equipment maintenance. For the meat plant staff it is an obligation to observe during all meat processing operations relevant hygienic rules. Such efforts will result in good storage life of attractive meat products with desirable appearance, flavour and taste. Ethyl acetate garlic extract can prolong shelf life of meat.

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