Antibacterial Effect of *Allium sativum* (Garlic) and *Zingiber officinale* (Ginger) Extracts against Antibiotic Resistant Organisms Isolated from Chicken Abattoir

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Abstract: Gradually human race is progressing to the era of using plant derived medication such as phytomedicines to treat, prevent infectious diseases and solve problem of resistant strains. Several plants possess some biomolecules that could be antioxidant, anti-inflammatory, antibacterial, plant extract that performs preventive and therapeutic roles through modulation of biological activities. However, *Allium sativum* and *Zingiber officinale* plants can be employed for this purpose. This study was carried out to investigate the *in vitro* antibacterial activity of aqueous extract of garlic cloves and ginger rhizomes on resistant isolates from chicken abattoir. Isolation of resistant organism from chicken dressing water and slaughter equipment was done using spread plate and streaking methods. The recovered isolates were exposed to a panel of eight antibiotics using Kirby-Bauer disc diffusion technique. The identity of the resistant strains was done using standard biochemical and molecular techniques. Disc and agar well diffusion method were used to test antimicrobial efficacy of aqueous extracts of garlic cloves and ginger rhizomes resistant isolates. *E. coli* (90%) was the predominant organism isolated while *Vibrio* spp *(50%), Salmonella* spp *(50%), Shewanella* spp *(30%), Klebsiella* spp *(20%), Aeromonas* spp *(20%), and Providencia* spp *(10%)* with high percentage of multiple antibiotic resistance of 87.5% above. Maximum zone of inhibition at 0.1 concentrations is 8mm-18mm for ginger and 10mm-16mm for garlic on resistant organism tested. In conclusion, ginger and garlic have active metabolites that possess antimicrobials capacity against resistant organisms associated with chicken slaughtering and its dressing water. Therefore, it is advisable to include ginger and garlic to cooking of chicken to serve as both spices to cooking chicken and therapeutic to infections caused by resistant strains associated with chicken.

Key words: *Allium sativum, Zingiber officinale,* resistant organisms**,** zone of inhibition

INTRODUCTION

Improvements of machinery used by bacteria to inhibit the effect of antibiotics are resulting to antibiotic resistance (Seema, 2015). The resistance could be natural, acquired, genetic, Furthermore, the developments of resistance could be as a result of spontaneous mutation in genes, acquisition of plasmid or transposon (Jalal and Nasroallah, 2014). However, antibiotic resistance is an immense pandemonium to global health, particularly in low and middle income country (Yadufashije, *et al.,* 2020).

Medicinal plants had been as ancient as human more than thousand years and it's uses is significant, to cure various infectious and non-infectious diseases worldwide (Block 2010).

In addition, ginger is from Zingiberaceae family, the Zingiberaceous plants have natural strong aromatic and medicinal properties (Yadufashije, *et al.,* 2020). It is available and accessible at low cost for everyone to use (Foster, 2011), and possess numerous phenolic compounds (active metabolites) such as; Paradol, gingerols, Zingerone and shogaols, that are rich in antimicrobial effects against numerous resistant microbes (bacteria, fungi even viruses) with very low toxicity (Okiki, *et al.,* 2015). It antimicrobials competence as a prophylactic and therapeutic plant to some infections such as; colds, fever, menstrual pain, joints pain, Nausea, bloat, Indigestion, chest diseases, cough, sore throat, skin, kidney and bladder infections, constipation and other digestive problems and dysentery and intestinal inflammations had been reported by researchers (Arshad *et al.*, 2014; Okiki, *et al.*, 2015; Mahmoud Rafieian-Kopaei, *et al.*, 2016).

Garlic (*Allium sativum*) belongs to the family Alliaceae. Onion, shallot, and leek are close member of that family (Huzaifa*et al.,* 2014).

Garlic has been used as medicine from centuries in many continents. It is also claimed to help prevent heart diseases including atherosclerosis, high cholesterol, high blood pressure, and improve immune system also prevent development of cancerious cells (Ponmurugan and Shyamkumar, 2012)

MATERIALS AND METHODS

Description of Study Area

Chicken abattoir is located in Olu-ode market Osogbo, Osun state, South western part of Nigeria. Osogbo covers an area of approximately 14,875 square kilometers which lies between longitude 4^0 33'59.99" and latitude 7^0 45' 59.99" its total population is approximately 3,416,959, it is about1100 meters above sea level.

Collection of chicken abattoir Samples

Sample collection was done between August 2020 and December 2020. Ten (10) dressing water samples were collected in to separate labeled sterile universal sampling bottles from ten different individuals assisting buyers to slaughter and dress chicken been purchased. sterile swabs were used to swabbed the cutting slab and butchering knives aseptically and put inside sterile tryptic soy broth (Reza *et al*.,2014; Shrestha *et al*., 2017). All samples collected from the market were immediately transported on ice pack to Osun state University microbiological laboratory for further analyses.

Sample Inoculation

The method used by Mpundu *et al.*, 2019 with modification was adopted. 1ml of the chicken dressing water sample was aseptically taken into a sterile test tube containing 9ml sterile Ringer solution and serially diluted up to 10^{10}, 100 µl of the diluted samples were spread plated on Nutrient agar, MacConkey and Thiosulfate citrate bile salts agar plates, incubated at 37°C for 24 hours. A loopful of the sample was taken from each flavor bottle (containing the swab that has already been immersed in Tryptic Soy Broth) and was streaked on Nutrient agar, MacConkey agar and Thiosulfate citrate bile salts agar plates and were incubated at 37°C for 24 hours. After inoculation, colony count, morphological and biochemical characterization were performed on the isolates.

Biochemical Identification of the isolated bacteria from chicken abattoir

Isolates were subjected to morphological and biochemical tests according to the procedures recommended in the Bergey's Manual of Determinative Bacteriology, 9th edition. Biochemical tests done using standard methods include; Gram stain, motility, carbohydrate utilization, starch hydrolysis, oxidase, catalase, indole production, citrate utilization, nitrate reduction, lysine decarboxylation, gas and hydrogen sulphide production (Yadufashije, *et al.*, 2020).

Antibiotics Sensitivity Testing on isolated bacteria from chicken abattoir:

The antibiotic susceptibility testing of the isolates were carried out using Kirby-Bauer disc diffusion technique, to determine the antibiotics sensitivity of the isolates, and their MAR index was calculated by the ratio of number of antibiotics ineffective. The antibiotics used includes; Colistin CT (10 µg), Aztreonam ATM (30 µg), Azithromycin AZM (15 µg), Cefepime FEP (30 µg), Tygercycline TGC (15 µg), Doripenem DOR (10 µg), Kanamycin K (30 µg) and Oxacillin OX (10µg) which were all obtained from Oxoid UK. Using a sterile inoculating loop, a colony of organisms was taken and inoculated inside buffer. The culture was standardized to obtain turbidity that is optically comparable to 0.5 McFarland standards as described by Wolde *et al.,* 2018 with modification; sterile swab stick was dipped into the standardized broth culture. The swab stick was rotated several times and presses firmly on the wall of the tube to remove excess inoculums from the swab. The dry surface of the prepared sterile Mueller Hinton agar was inoculated by streaking the swab over the entire agar surface.

Later, antibiotic discs were fixed on the media using disc dispenser and incubated at 37°C for 24 h. The zone of inhibition was measured in mm and interpreted using EUCAST break point version 11. Resistance to ≥1 antibacterial agent in ≥3 classes of antibiotics was used as an indicator of multidrug resistance.

MAR index of each isolate = No. of antibiotic against which isolate is resistant /Total no. of antibiotic used for testing.

Antimicrobials testing of Ginger and Garlic on resistant bacteria isolated from chicken abattoir

Preparation of Crude Extracts and discs

Fresh garlic cloves and ginger rhizomes were well peeled and rinsed well; 100 g were blended in 100 mL sterile distilled water separately. The mixture was crushed finely using a clean sterile blender. The resulting paste was centrifuged at 4000 rmp for 30 minutes and the supernatant was then sterilized by a filter (0.45 µm pore size, Millipore) (Mostafa *et al*., 2014). Subtraction of final weight precipitated from the weight of the original peeled garlic bulbs and garlic rhizomes, the final concentration of their aqueous solution was determined to be 55%; 48% (w/v) respectively and stocked in 1.5 mL micro test tubes at -20°C until used.

Discs of 6 mm in diameter were punched out using Whatman No. 1 filter paper with the aid of a paper punch and placed in MacCartney bottles. The discs were then sterilized by autoclaving at 121° C for 15 min after which they were allowed to cool (Giriraju and Yunus 2013; Yadufashije, *et al*., 2020). Stock solutions of the garlic and ginger crude aqueous extracts therefore, had a concentration of 0.1, two more concentrations of 0.05 and 0.025 were prepared, while 10µl of each concentrated solutions were dispensed aseptically on separate sterile discs. The discs were allowed to absorb the solution and kept for further analysis (Ponmurugan *et al*., 2012)

Agar Diffusion Assay of ginger and garlic extract on resistant isolates

Molten Mueller Hinton agar (20 ml) was seeded with 0.2 ml of broth culture of 0.5 McFarland turbidity standards (1×10^8 bacterial/ml inoculum) of the test microorganisms in sterile Petri dishes, which were rotated slowly to ensure their uniform distribution. Petri dishes were left to solidify and sterile borer was used to make hole of 8mm in diameter on the agar, 100µl of the crude plant extract was put in each hole as described by n Wallock-Richards *et al*., 2014; Agnieszka *et al.,* 2021. Petri dishes were allowed to stand for about 30 min at room temperature to allow for proper diffusion of the extracts. The plates were then incubated at 37°C for 24 hr. The zone of inhibition (in mm) were measured and recorded.

Disk Diffusion Assay of ginger and garlic extract on resistant isolates

Bacteria cell suspensions were adjusted to 0.5 McFarland turbidity standards (1×10^8 bacterial/ml inoculum). Each bacterial suspension was swabbed on Mueller-Hinton agar plates, and the plates were then allowed to dry for 5minutes. The sterile filter paper disks (Whatman No. 1, diameter 6mm) were soaked in 10µl of each aqueous extract concentration separately. The extract-soaked filter paper disks were then placed on the inoculated Mueller-Hinton agar plates. Tigercycline (15µg) disk was used as the positive control, and sterile distil water-soaked filter paper disk was used as the negative control for the two assay method. Mahmoud *et al*., 2016. Plates were incubated for 24hr at 37°C. After incubation, the zones of inhibition were recorded as the diameter of the growth-free zones in mm.

Molecular Studies of Multi Drug Resistant Isolates

DNA Extraction

Bacteria chromosomal DNA was extracted from all resistant presumptive isolates using Zymo Research Genomic DNA™- Tissue MiniPrep Kit following the manufacturer's

instructions. The quality and quantity of the extracted DNA was determined using a UV-Vis ThermoScientific^{TM}Nanodrop Lite Spectrophometer (model S-22, Boeco, Germany). The DNA samples were stored at -80^{o} C for future use.

16SrRNA gene detection and Gel Electrophoresis of resistant isolates

The PCR reaction in a 25µL volume comprises of 1.5µL of $MgCl_2$ (15mM), 0.5µL of 1mM dNTP. 2.5µL of Taq buffer (5×), 0.5µL of each primer (2.5pM each) 16SrRNA Forward; AGAGTTGATCCTGGCTCAG; 16SrRNA Reverse; GGTTACCTTGTTACGACTT; described by Ni*et al.*,(2015), 0.2µL of Taq DNA polymerase and 5µL of the DNA sample. 14.8µL PCR water was used to bring the final reaction volume to 25µL.Moura*det al.*, (2019). The thermocycler cycling conditions were 1 cycle of denaturation at 94^{o}C for 5min, then 30 cycles of denaturation at 94^{o}C for 1 min, elongation at 72^{o}C for 1 min and final elongation at 72^{o}C for 7 minutes and allowed to cool at a temperature of 4^{o}C. The amplified DNA fragments were run alongside a 100 bp ladder on 2% agarose gels containing ethidium bromide and visualized under UV light.

Sequencing of resistant isolates

Sequencing was carried out in Inqaba Biotechnical Industry Ltd and EXOSAP protocol was used for purification. Sequencing was done with applied biosystemsBigDye Terminator v 3.1 cycle sequencing kit (catalogue no 43374455) and the procedure was followed according to manufacturer's instructions. The oligonucleotide sequences were blasted using NCBI.

RESULTS

The study was performed to screen detected possible antimicrobial activity of ginger and garlic aqeous extracts against resistant bacteria isolated from chicken abarttoir.

E. coli (90%), *Vibrio (50%), Salmonella (50%), Shewanella (30%), Klebsiella (20%), Aeromonas (20%), and Providencia (10%)* were recovered as shown in figure 1, Table 1 revealed high level of resistance to the class of antibiotic used; Oxacillin, (97% resistant, 0% intermediate and 3% susceptible); Doripenem, (97% resistant, 3% intermediate and 0% susceptibile);Aztreonam, (93.9% resistant, 3% intermediate and 3% susceptibile);Azithromycin,(93.9% resistant, 3% intermediate and 3% susceptibile); Cefepime,(90.9% resistant, 6.1% intermediate and 3% susceptibile); Colistin,(84.8% resistant, 3% intermediate and 12.1% susceptibile);Kanamycin,(81.8% resistant, 15.2% intermediate and 3% susceptibile);,Tigercycline(39.4% resistant, 12.1% intermediate and 48.5% susceptibile), with high percentage of multiple antibiotic resistance of 87.5% above as represented in figure 2.

The present study showed the potential antimicrobial activity of the ginger and garlic extract against the resistant organisms. Tables 3and 4 indicate that maximum zone of inhibition at 0.1ml concentration is as follows: *E. coli* (18mm for ginger and 16mm for garlic) *Vibrio spp*(10mm for ginger and 13mm for garlic) *Salmonella* spp (15mm for ginger 13mm for garlic) *Shewanella* spp (15 mm for ginger and 12 mm for garlic) *Klebsiella* spp(10mm for ginger and 11mm for garlic) *Aeromonas caviae* (13mm for ginger and 15mm for garlic) *Providencia alcalifaciens*(15mm for ginger and 12mm for garlic) *Aeromonas taiwanensis*(15mm for ginger and 10mm for garlic).

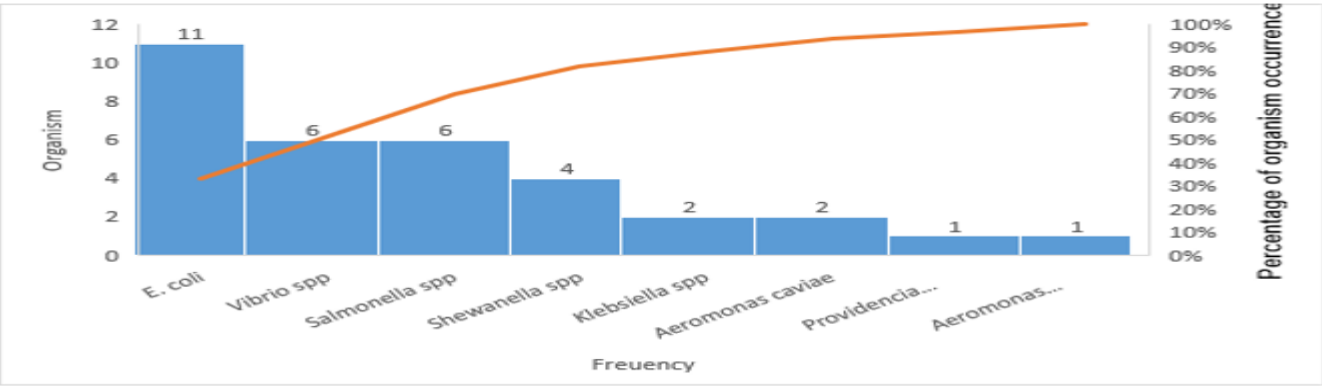

Figure 1: The frequency of isolates recovered from chicken abattoir

Table 1: Antibiotic Sensitivity Testing of Isolates recovered from Chicken Abattoir

Antibiotic family	Antibiotics	*E. coli* (n=11)	*Vibrio* spp (n=6)	*Salmonella* spp (n=6)	*Shewanella* spp (n=4)	*Klebsiella* spp (n=2)	*Aeromonas caviae* (n=2)	*Providencia alcalifaciens* (n=1)	*Aeromonas taiwanences* (n=1)
Tetracycline	TGC	**R**(5), **I**(1), **S**(5)	**R**(1), **I**(1), **S**(4)	**R**(2), **I**(1), **S**(3)	**R**(0), **I**(1), **S**(3)	**R**(2), **I**(0), **S**(0)	**R**(1), **I**(0), **S**(1)	**R**(1), **I**(0), **S**(0)	**R**(1), **I**(0), **S**(0)
Penicillin	OX	**R**(10), **I**(0), **S**(1)	**R**(6), **I**(0), **S**(0)	**R**(6), **I**(0), **S**(0)	**R**(4), **I**(0), **S**(0)	**R**(2), **I**(0), **S**(0)	**R**(2), **I**(0), **S**(0)	**R**(1), **I**(0), **S**(0)	**R**(1), **I**(0), **S**(0)
Polymyxin	CT	**R**(10), **I**(0), **S**(1)	**R**(5), **I**(0), **S**(1)	**R**(5), **I**(1), **S**(0)	**R**(3), **I**(0), **S**(1)	**R**(2), **I**(0), **S**(0)	**R**(1), **I**(0), **S**(1)	**R**(1), **I**(0), **S**(0)	**R**(1), **I**(0), **S**(0)
Carbapenem	DOR	**R**(10), **I**(1), **S**(0)	**R**(6), **I**(0), **S**(0)	**R**(6), **I**(0), **S**(0)	**R**(4), **I**(0), **S**(0)	**R**(2), **I**(0), **S**(0)	**R**(2), **I**(0), **S**(0)	**R**(1), **I**(0), **S**(0)	**R**(1), **I**(0), **S**(0)
Macrolide	AZM	**R**(11), **I**(0), **S**(0)	**R**(5), **I**(1), **S**(0)	**R**(6), **I**(0), **S**(0)	**R**(4), **I**(0), **S**(0)	**R**(2), **I**(0), **S**(0)	**R**(1), **I**(0), **S**(1)	**R**(1), **I**(0), **S**(0)	**R**(1), **I**(0), **S**(0)
Aminoglycoside	K	**R**(9), **I**(2), **S**(0)	**R**(5), **I**(1), **S**(0)	**R**(4), **I**(1), **S**(1)	**R**(4), **I**(0), **S**(0)	**R**(2), **I**(0), **S**(0)	**R**(1), **I**(1), **S**(0)	**R**(1), **I**(0), **S**(0)	**R**(1), **I**(0), **S**(0)
Monobactam	ATM	**R**(10), **I**(1), **S**(0)	**R**(6), **I**(0), **S**(0)	**R**(6), **I**(0), **S**(0)	**R**(4), **I**(0), **S**(0)	**R**(1), **I**(0), **S**(1)	**R**(2), **I**(0), **S**(0)	**R**(1), **I**(0), **S**(0)	**R**(1), **I**(0), **S**(0)
Cephalosporin	FEP	**R**(10), **I**(1), **S**(0)	**R**(6), **I**(0), **S**(0)	**R**(6), **I**(0), **S**(0)	**R**(4), **I**(0), **S**(0)	**R**(0), **I**(1), **S**(1)	**R**(2), **I**(0), **S**(0)	**R**(1), **I**(0), **S**(0)	**R**(1), **I**(0), **S**(0)

Key: I= Intermediate, R= Resistant, S= Susceptible, TGC= Tigercycline, OX= Oxacillin, CT= Colistin, DOR= Doripenem, AZM= Azithromycin, K= Kanamycin, ATM= Aztreonam, FEP= Cefepime,

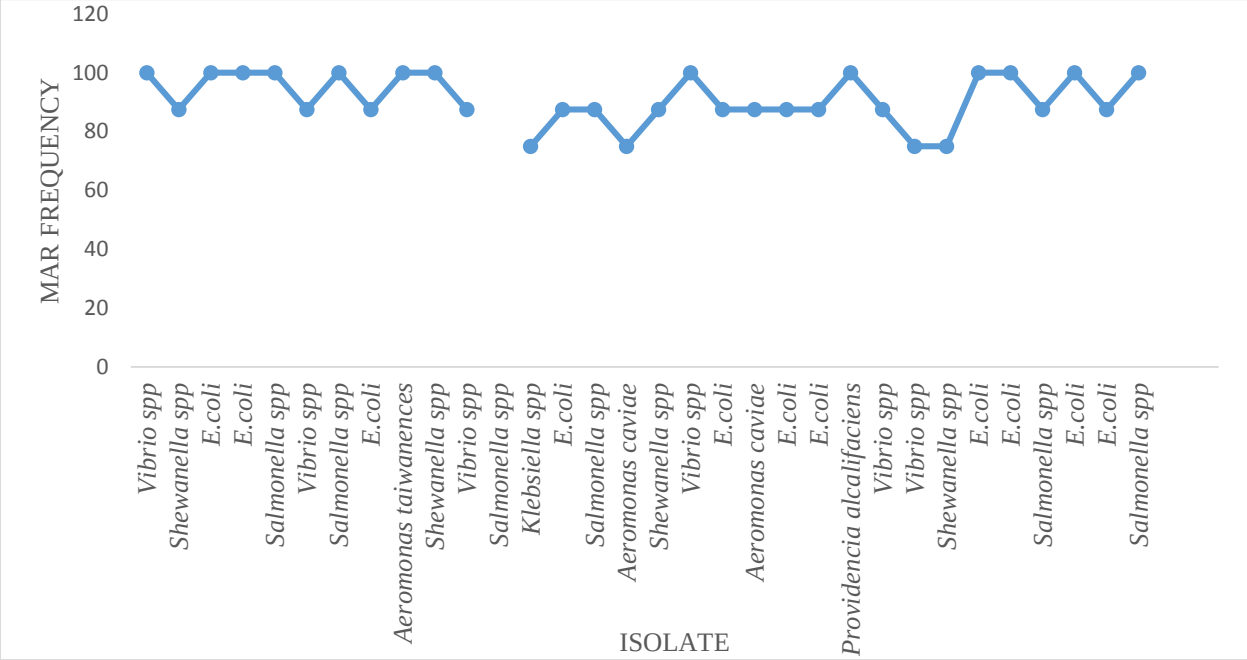

Figure 2: Percentage Multiple Antibiotic Resistant (MAR) INDEX of isolates recovered from chicken abattoirs

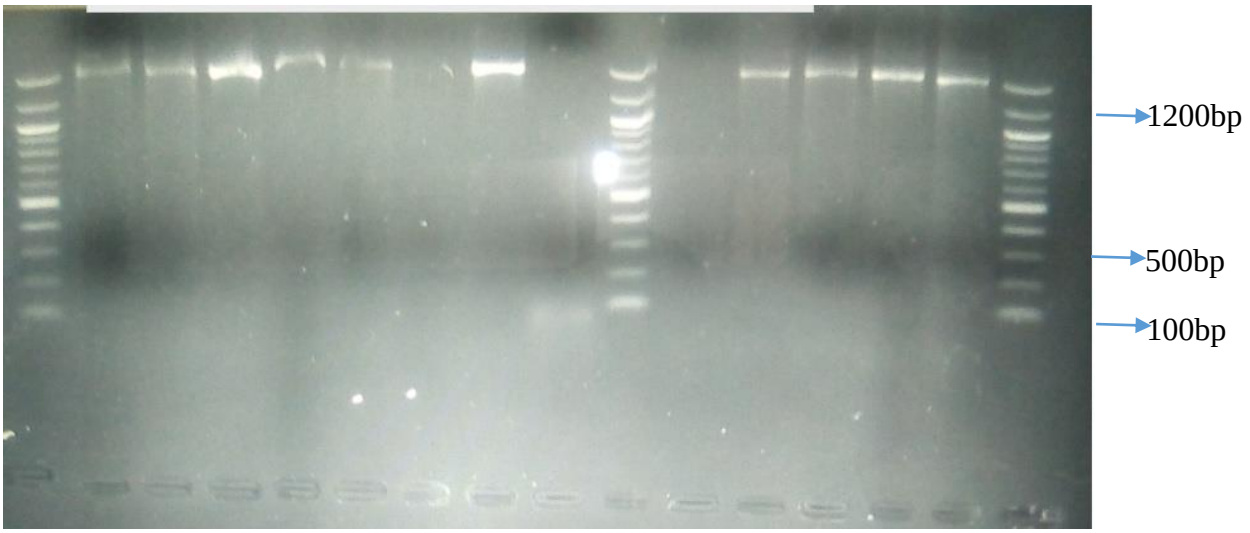

Figure 3: Agarose gel of 16SrRNA gene fragments amplified from the multidrug resistant bacteria isolates showing bands at 1200bp.
M: maker, Lane 1-13: Resistant bacteria isolated from chicken dressing water and slaughter equipment.

Table 2: Antibacterial activity of ginger on sequenced resistant organisms from chicken abattoir

Identified Organisms	**Range of zone of inhibition in diameter (mm) at different concentration**			
	0.025	0.05	0.1	Positive (Tigercycline 15µg)
E.coli (n=11)	9-12	11-15	16-18	23-26
Vibrio spp (n=6)	0	0	9-10	19-22
Salmonella spp (n=6)	8-10	11-12	12-15	22-25
Shewanella spp (n=4)	8-9	10-12	14-15	22-23
Klebsiella spp (n=2)	0	0	8-10	16-19
Aeromonas caviae (n=2)	10-12	11-12	13	21-22
Providencia alcalifaciens (n=1)	7	12	15	19
Aeromonas taiwanensis (n=1)	11	14	15	20

Table 3: Antibacterial activity of garlic on sequenced resistant organisms from chicken abattoir

Identified Organisms	**Range of zone of inhibition in diameter (mm) at different concentration**			
	0.025	0.05	0.1	Positive (Tigercycline 15µg)
E.coli (n=11)	9-11	11-13	12-16	23-25
Vibrio spp (n=6)	0	7-9	9-13	20-24
Salmonella spp (n=6)	8-9	8-10	10-13	21-25
Shewanella spp (n=4)	8-9	10	10-12	22-24
Klebsiella spp (n=2)	0	8-9	9-11	16-17
Aeromonas caviae (n=2)	10	11-12	13-15	20-22
Providencia alcalifaciens (n=1)	9	11	12	18
Aeromonas taiwanensis(n=1)	7	8	10	20

DISCUSSION

This study emphasized on isolation of resistant bacteria recovered from chicken abattoir and the antimicrobial effect of ginger and garlic on the isolated resistant strains. Different bacteria were isolated from chicken dressing water and slaughter equipment which include: *E. coli* (90%), *Vibrio* spp *(50%), Salmonella spp (50%), Shewanella* spp *(30%), Klebsiella* spp *(20%), Aeromonas* spp *(20%), and Providencia* spp *(10%)* in decreasing order, which is similar to studies from Mpundu, *et al*., (2019) that reported that the water used for dressing chickens is probably the major cause of high levels of cross-contamination with entero-pathogenic bacteria. *E. coli* a major contaminant of poultry meat which is presented in this study is also in agreement with report from Kabour, (2011).

Also, isolates recovered from the dressing water and swabs were all resistant to; Oxacillin, Colistin, Doripenem, Azithromycin, Aztreonam, Kanamycin, Cefepime and sensitive to Tigercyline. However, multi drug resistant bacteria were observed in both the chicken dressing water as well as slaughter slab swab and this is similar to studies from Shrestha *et al*., (2017).

In this study the antimicrobial efficacy screening result of resistant bacteria with water extract of ginger and garlic, showed higher zone of inhibition with ginger extract to garlic extract which similar to results of Mahmoud *et al*., 2016 showed inhibitory effects of 10% ginger extract against *Staphylococcus aureus* (ATCC:25923), *Pseudomonas aeruginosa* (ATCC: 27853) and *Listeria monocytogenesis* (ATCC: 27853). Sebiomo *et al*., 2011 reported large sizes of zones growth inhibition produced by ginger extracts against the two bacterial *S. aureus* and *S. pyogene*. Bellik, Y. (2014) reported high antimicrobials activity of essential oil and oleoresin of *Zingiber officinale* on *S. aureus, Bacillus subtilis, E. coli, Pseudomonas aeruginosa, Proteus vulgaris*, moderate activity on *Klebsiella pneumniae ,Candida albicans* and no effect on *Aspergillus niger*.

However, ginger and garlic extracts were effective against antibiotic resistant bacteria, which may be because they contain some active constituents or compounds such as; allicin and other hydrophobic compounds, gingerols, Zingerone and others that affect the growth of bacteria by inhibiting the DNA/RNA and proteins synthesis. Auta*et al.*, 2011 showed that ethanolic extract of ginger (*Zingiber officinalae*) with 20 mg/ml had stronger effect on *Pseudomonas aeruginosa* than *Escherichia coli*, Okiki*et al*., 2015 conducted a study on Soybean oil extract of ginger with zone of inhibition (11.67±1.53mm) against *Salmonella spp*. Allicin prevents biofilm formation by inhibiting early bacterial adhesion and also the secretion of virulence factors by regulating quorum sensing as reported by Lihua*et al*., 2013; Xu *et al*., 2019. The *in-vitro* studies conducted by Ranjbar-Omid *et al.*, 2015 revealed the capabilities of purified garlic active compound allicin in inhibiting the biofilm formation and urease activity of *Proteus mirabilis*

Fresh local ginger and garlic used in this study shown antibacterial effects on resistant bacteria tested which is in support with Strika*et al*., 2016 that reported strongest antimicrobial activity in fresh homemade garlic against isolates tested including *Candida albicans*. The Gram-negative diarrheagenic pathogens from the stool samples isolated by Matthew *et al.*, 2007 were highly sensitive to garlic. Wallock-Richards *et al*., 2014 reported inhibitory activity of allicin-containing garlic extracts against *Burkholderia species*. Banerjee and Maulik, 2002; Zhang *et al*., 2013; Okiki *et al.*, 2015; Yadufashije*et al*., 2020 reported the efficacy of antibacterial activities of ginger extract against bacteria. Tagoe D and Gbadago F. (2009) reported garlic showing stronger antimicrobials activity on Salmonella, *Shigella* and *Bacillus*.

The limitation of this study is that both fresh local ginger and garlic were not combined and

compare the result of their combination with when used separately on resistant bacteria recovered from chicken abattoir.

CONCLUSION

This study had shown that garlic and ginger has great antimicrobial activities against the tested resistant bacteria associated with chicken abattoir. In line with the observations from this study, garlic and ginger aqueous extracts has proven to contain bio therapeutic properties which can be used as a supplement in food and meat preparations in order to serve as preventive and therapeutic food supplements to human in the treatment of many microbial diseases.

Conflict of interest

The authors declared that no competing interest

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