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Synergistic Effect of Some Plant Extracts with Selected Antibiotics Against Enteric Pathogens of Turkey Poults

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Abstract | Antibiotic resistance has been considered a major problem for both human health and poultry production. Therefore, this study was conducted to improve the antibiotics activity by combination with natural plant extracts. Turkey poults were assayed for the presence of Enterobacteriaceae species with prevalence 40% (40/100) of the tested samples. Escherichia coli has the highest prevalence rate with 77.5% followed by klebsiella spp and Salmonella spp. with7.5%and 5% respectively.Resistance of the collected Enterobacteriaceae strains was determined by the Kirby-Bauer disk diffusion test against a panel of antibiotics.. The isolates were highly resistance for Amoxicillin clavulanic acid with 71.05% of tested isolates followed by Erythromycin, Chloramphenicol and Doxycycline with 73.68%, 63.16% and 34.21% respectively. In contrast, the isolates demonstrated significant sensitivity to Colistin, with a sensitivity rate of 94.73% while were 60.52% and 52.63% for Gentamicin and Trimethoprim-sulfamethoxazole . the antimicrobial activity of five methanolic plant extracts (garlic, cloves, rosemary, Ficus sycomorus and Ziziphus spina-christi,) were examined toward intermediate resistant strains of Escherichia coli, Salmonella typhimurium and Klebsiella pneumoniae. Among the plant extracts used, cloves showed a broad antimicrobial spectrum three examined strains with inhibition zones between 17-19 mm followed by garlic with inhibition zones between 16-18 mm and rosemary with 8-13mm. Meanwhile Ficus sycomorus and Ziziphus spina-christi have no effect against the three tested strains. Combinations of these extracts and antibiotics (amoxicillin, doxycycline and florophenicol) were applied with Decimal Assay for Additivity method with three ratios of antibiotic: plant extract (9:1,8:2and 7:3) to determine synergistic effects between them and the enhancement of antibiotic activity. The mean zones of inhibition and the minimum inhibitory concentration of plant extracts and of antibiotics and combination between them was determined. Cloves extract display significant antibacterial activity (MIC 64 µg/ml), but it modulated the activity of amoxicillin and doxycycline and florophenicol with MIC 32 µg/ml,32 µg/ml and 16 µg/ml in order, i.e. in combination with amoxicillin, 2fold (8 µg/ml) reduction in the MIC value at the ratios 9:1,8:2and 7:3 of combination against E. coli and 1fold with Salmonella typhimurium and Klebsiella pneumoniae was observed at same ratios. While rosemary deacreased MIC with 2 fold in combination with amoxicillin and doxycycline against E. coli and Salmonella typhimurium but was less effective against Klebsiella pneumoniae. Antimicrobial compounds from medicinal plants had many advantages such as fewer side effects, better patient tolerance, less expensive, acceptance due to long history of use, being renewable in nature and also higher plants represent a potentiall source of novel antibiotic prototypes.

Keywords: Decimal assay for additivity, Antimicrobial, Amoxicillin, Doxycycline, Turkey poults

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INTRODUCTION

Antimicrobial resistance is increasing in several species of *Enterobacteriaceae* (Karlowsky *et al*., 2003) and this has been a major concern with both clinical and commensal bacteria (Kilonzo-Nthenge *et al*., 2013). *Enterobacteriaceae* is distributed widely in nature and in the gastrointestinal tract of humans, other mammals, and birds approved by previous studies (Barham *et al*., 2002; Fluckey *et al*., 2007; Mainali *et al*., 2009).

Turkey farming had several challenges including potential outbreaks of infectious and non-infectious diseases. (Asaduzzaman *et al*., 2017). Infections caused by *Escherichia coli* and *Salmonella* spp. have negative impacts on turkey farming as they lower egg production, reduce hatchability, and increase mortality rates (Kar *et al*., 2017). Avian colibacillosis caused by *E. coli* is responsible for turkey cellulitis, colisepticemia, swollen head syndrome, synovitis, salpingitis, coligranuloma, osteomyelitis, omphalitis, peritonitis, panophthalmitis, and is often deadly for turkeys (De Oliveira *et al*., 2020). *Salmonella* spp. can cause salmonellosis (especially pullorum disease and fowl typhoid) inturkeys (Abdukhalilova *et al*., 2016; Aury *et al*., 2010). *Salmonella* infections reduce hatchability, fertility, growth, and increase mortality rates in poultry (Andino and Hanning, 2015). Additionally, *Klebsiella* species, though naturally present in the digestive mucosa, have been implicated in various infections affecting the excretory and respiratory systems (Martin and Bachman, 2018), with some strains showing increasing virulence in poultry (Hamza *et al*., 2016).

During the last decades, several veterinary antibiotics have been used globally as growth promoters and therapeutic agents in livestock production because of their positive effects (Arikan *et al*., 2007). In Egypt, excessive and incorrect use of antimicrobials in veterinary medicine plays a key role in the spread of antibiotic-resistant bacteria, especially enteric pathogens (*Escherichia coli* and *Salmonella* species), that increasingly hinders the successful treatment of infectious diseases (Andersson, 2003) It is now well-established that antimicrobial resistant bacteria isolated from humans often originate from food animals (Aarestrup, 2000).The transmission of resistance can occur through various mechanisms, with bacteria acquiring antibiotic resistance either through mutations or horizontal gene transfer (Normark and Normark, 2002). The latter has been identified as the primary mechanism for acquired antibiotic resistance in *Enterobacteriaceae*, primarily mediated through conjugation (Barlow, 2009).

This lead to search for alternative antimicrobial agents. Plant-derived antimicrobials present a promising avenue, as they typically do not carry the side effects associated with synthetic drugs and possess significant therapeutic potential against various infectious diseases (Chanda *et al*., 2010; Habbal *et al*., 2011).These natural compounds often exhibit multiple mechanisms of action, making the development of resistance less likely (Cushnie and Lamb, 2011).

Cloves had antibacterial activity against a large number of bacteria such as *Escherichia coli*, *Listeria monocytogenes*, *Salmonella* enterica(Friedman *et al*., 2002), *Campylobacter jejuni, Salmonella* enteritidis, *Escherichia coli* and *Staphylococcus aureus* (Beuchat, 2007; Cressy *et al*., 2003; Kalemba and Kunicka, 2003). The major component of clove oil is usually considered to be eugenol, with β-caryophyllene and lesser amounts of other components such as benzyl alcohol. Bai *et al*. (2023) confirmed that eugenol was the main component of cloves which showed obviously antibacterial activities against *S. aureus* and *E. coli*. The antibacterial mechanism of eugenol against *S. aureus and E.coli* was probably related to the damage of cell wall and membrane, the inhibition on biofilm formation, the oxidative stress-mediated apoptosis and the disruption of DNA synthesis.

Rosemary is used as a spice and a medicinal supplement (El-Demerdash *et al*., 2021a). The major contents are rosmarinic acid, cafeic acid, chlorogenic acid, carnosic acid, luteolin, camphor, riesemanol, carnousol, rosemary-diphenol, rosemary quinone, ureolic acid, glucocytic acid, rosemarcin, borneol, sinwell, alfopinene, and comfan (Barakat *et al*., 2016). Fu *et al*. (2007) found that essential oil of rosemary attached to the surface of bacterial body at lower concentrations. At higher concentrations, essential oil entered the cell wall leading cell wall desquamation and shape distortion. Subsequently, essential oils invaded and damaged the cell membrane, so that the intracellular contents burst from the bacterial body.

Garlic is a bulb-forming herb of the family Alliaceae, cultivated some thousands of years for use as a flavoring agent as well as a medicinal herb (Lewis and Elvin-Lewis, 2003). Several studies, including those of Kim *et al*. (2004) and Lacombe *et al*. (2010) had previously demonstrated the antibacterial potency of EGE against enteropathogens such as *Vibrio parahaemolyticus*, *E. coli*, *Klebsiella spp., Proteus spp., and S. aureus*. Garlic principal phytochemicals that exhibit antibacterial activity are oil-soluble organosulfur compounds that include allicin, ajoenes, and allyl sulfides. The reactive organosulfur compounds form disulfide bonds with free sulfhydryl groups of enzymes and compromise the integrity of the bacterial membrane (Bhatwalkar *et al*., 2021). changes observed in membrane permeability, protein leakage and by scanning electron microscopy suggested that the antimicrobial activity of garlic extracts may be due to destruction of the structural integrity of cell membranes, leading to cell death (Chen *et al*., 2018).

A particularly innovative approach is combination therapy, which explores potential synergism between conventional antibiotics and bioactive plant extracts. This strategy offers multiple advantages, including: expanded antimicrobial spectrum, reduced likelihood of resistant mutant emergence, minimized toxicity and potentially enhanced antimicrobial activity compared to individual treatments (Rakholiya and Chanda, 2012). However, the molecular mechanisms underlying these synergistic interactions remain incompletely understood and warrant further investigation.

Therefore, this study aims to evaluate the interactions between selected plant extracts and antibiotics against *E. coli, Salmonella* and *Klebsiella* isolated from turkey poults, determination of minimum inhibitory concentration for both antibiotics and plant extracts using agar well diffusion method and assess the effects of antibiotic-plant extract combinations. This research aim to develop of more effective and sustainable approaches to managing bacterial infections in poultry production, with potential implications for both animal health and food safety.

MATERIALS AND METHODS

Bacterial Strains

During the period 2023 to 2024, a total of 100 diseased turkey poults were randomly collected from 5 turkey farms in Sharkia governorate, Egypt four hunderad samples (from each bird 4 visceral organ) were collected from the 100 poults for culture and isolation. From these samples, 40 bacterial isolates were obtained and identified, as following: 31 *Escherichia coli* strains, 2 *Salmonella* typhimurium strains, 2 *Proteus mirabilis* strains and 3 *Klebsiella pneumoniae* strains. Three intermediate resistant strains were selected for further testing: *E. coli* O78, *Salmonella* typhimurium, *Klebsiella pneumoniae* spp. pneumonae because most isolates were highly resistant for the tested antibiotics.

Sample Collection and Bacterial Isolation and Identification

Sample size was calculated by sample size calculator according to Survey monkey (https://www.surveymonkey.com). Samples were collected from visceral organ (liver, cecal tonsils, spleen, and gall bladder) of diseased day old to30 days age old poults.

Routine bacteriological examination applied to collected samples for Enterobactreace isolation. Using enrichment broth media for cultivation of samples before plating. The plates incubated for 24 hours at 37°C in aerobic conditions. After incubation, growing colonies were subjected to Gram staining and catalase and oxidase tests to ascertain fermenting Gram-negative bacilli. The oxidase-negative Gram-negative bacilli were subcultured on MacConkey agar for purification and further identification to the species level standard biochemical tests as described by Finegold and Baron (1986) and GnA+B-ID System (Microgen Bioproducts, Ltd,AdmiraltWay, Camberley, SurreyGU15 3DT,U.K.) according to the manufacturer's instructions. Serological typing of *E. coli* isolates by using slide agglutination tests according to Edwards and Ewing (1972) and *Salmonella* serotyping followed the Kauffmann-White Scheme (Kauffmann, 1974).

Antimicrobial Susceptibility Testing

Bacterial isolates were tested in vitro for their susceptibility to 7 antimicrobial agents (Oxoid, Hampshire, UK). This was performed through use of a Kirby–Bauer disc diffusion assay, according to the standards and interpretive criteria described by the Clinical and Laboratory Standards Institute (CLSI, 2011). The following antimicrobial agents were tested: amoxicillin–clavulanic acid, 30 µg; chloramphenicol, 30 µg;; gentamicin ,10 µg; sulfamethoxazole–trimethoprim , 25 µg; erythromycin, 15 µg, colistin, 10 µg and doxycycline, 30 µg. Susceptibility of the isolates to antimicrobial agents was categorized (as susceptible, intermediate or resistant) by measurement of the inhibition zone, according to interpretive criteria that adhered to the CLSI guidelines. The isolates that displayed resistance to ≥ two different antimicrobial classes were categorized as multidrug resistance.

DNA Extraction and Screening of Antimicrobial Resistance Genes

Genomic DNA of bacterial isolates was extracted according to QIAamp DNA mini kit instructions**.** Selected isolates were tested more than once for the presence of genes that were resistant to; erythromycin (*mphA)*, amoxicillin–clavulanic acid (bla_{TEM}), chloramphenicol (*flo*R) and doxycycline *(TetA(A)*. Primer sequences, target genes and polymerase chain reaction (PCR) products are summarised in Table 1.

Table 1: Oligonucleotide primers sequences.

Reference	Length of amplified product	Primer sequence (5'-3')	Gene
Nguyen *et al.*, 2009	403 bp	GTGAGGAGGAGCTTCGCGAG	mphA
		TGCCGCAGGACTCGGAGGTC	
Colom *et al.*, 2003	516 bp	ATCAGCAATAAACCAGC	bla TEM
		CCCCGAAGAACGTTTTC	
Randall *et al.* 2004	570 bp	GGTTCACTCGAACGACGTCA	tetA (A)
		CTGTCCGACAAGTTGCATGA	
Doublet *et al.*, 2003	494 bp	TTTGGWCCGCTMTCRGAC	floR
		SGAGAARAAGACGAAGAAG	

Source: Metabion (Germany).

Several PCR protocols were used to detect the target genes of the isolates Table 2. The PCR products were loaded

onto 1.0% agarose gel (Sigma-Aldrich Co., St. Louis, MO, USA) that was stained with 0.5 µg/mL ethidium bromide (Sigma-Aldrich Co., St. Louis, MO, USA). The amplified DNAs were electrophoresed at 100 V for 60 min on a mini, horizontal electrophoresis unit (Bio-Rad, Hercules, CA, USA). The gel was then visualised and photographed under an ultraviolet transilluminator.

Table 2: Cycling conditions of the different primers during cPCR.

Gene	Primary denaturation	Secondary denaturation	Annealing	Extension	No. of cycles	Final extension
mphA	94°C 5 min.	94°C 30 sec.	58°C 40 sec.	72°C 40 sec.	35	72°C 10 min.
bla_{TEM}	94°C 5 min.	94°C 30 sec.	54°C 40 sec	72°C 45 sec	35	72°C 10 min.
TetA (A)	94°C 5 min.	94°C 30 sec.	50°C 40 sec	72°C 45 sec	35	72°C 10 min.
floR	94°C 5 min.	94°C 30 sec.	54°C 40 sec	72°C 45 sec	35	72°C 10 min.

Plant Materials and Extraction

Plant material: Five plant materials obtained from the Faculty of Agriculture, Zagazig University, Egypt:Garlic (*Allium sativum L.*) bulbs, Rosemary (*Rosmarinus officinalis*) leaves, Clove (*Syzygium aromaticum)* buds, *Ziziphus spina-christi* leaves and *Ficus sycomorus* leaves.

Preparation of the plant extract: Plant samples (Garlic, Rosemary, Clove, *Ziziphus spina-christi* and *Ficus sycomorus*) were cleaned out of other contaminated plants and the fresh plant was collected, air dried away from sunlight then they dried in an oven at 40°C and ground to a fine powder in a mill. Ten grams of the ground material were extracted using 100 mL methanol (80%) by ultrasonic at power 140 watt (40 KHz) at 40°C for 30 min Combined filtrate was evaporated in a rotatory evaporator below 40°C. The residue was freeze-dried. The dried extract was stored at -20°C until further use (Soaad and Ramesa, 2012).

Minimal inhibitory concentration (MIC): The isolated strain matches the 0.5 McFarland standards (1.5×108 CFU µg/ml) and results of antimicrobial agents and herbal extracts showed no visible bacterial growth considered as MIC and interpreted with recommendations of the Clinical Laboratory standards 2018.

Determination of MIC of herbal extract by tube dilution method (Awoyinka *et al*., 2007):Herbal extracts of Garlic, Rosemary, Clove which identified by using serially diluted (2-fold) plant extracts that showed no visible bacterial growth were considered as MIC and interpreted with recommendations of the National Committee for Clinical Laboratory standards (Adams *et al*., 1998; Dorman and Deans, 2000; Lorian, 2005).

Determination of antimicrobial activity of the prepared extract by Agar well diffusion methode: Agar well diffusion method is widely used to evaluate the antimicrobial activity of plants or microbial extracts (Magaldi *et al*., 2004; Valgas *et al*., 2007). Plates were seeded with a 24 h old culture of the bacterial strains which adjusted by saline to be equivalent to McFarland standard 0.5. About 500 micron of each culture spread on Mueller Hinton agar Plates. All culture allow to dry for 3-5 minute. Then, a hole with a diameter of 6 to 8 mm is punched aseptically with a sterile cork borer or a tip on Mueller Hinton agar, and a volume (20–100 mL) of the the five tested natural extract at desired concentration were added for wells antimicrobial extract. The plates were incubated at 4° C for two hrs to allow diffusion of the active compounds in the medium. The standard colistin 10mg (Oxoid, UK) was used as positive control and negative controls were prepared using the same solvent employed to dissolve the plant extract methanol 80%. The inoculated plates were incubated at 37 °C for 24 h. The diameter of the inhibition zones were measured for each plate for each antibacterial species were calculated (Balouiri *et al*., 2016).

Evaluation of the combined activity of antibiotics and extracts using Decimal Assay for Additivity (DAA): Stock solutions of both antimicrobial agents and herbal extract were prepared, each contained 1024 µg/ml. These stocksmixture contained 9 parts of antimicrobial agents and 1part ofherbal extract, 8 parts of antimicrobial agents and 2 parts ofherbal extract and 7 parts of antimicrobial and 3 parts of herbalextract. Double fold serial dilutions for each combination were prepared to detect MIC against the isolated strain. The obtainedconcentrations were ranged from 0.5 to 1024 µg/ml Sanders *et al*., (1993). This method defined as synergism when interactions were greater than additivity such as decreasing MIC of the antibiotic with 1or 2fold comparing in case of antibiotic alone. The antagonism when interactions between them lead to increasing of MIC antibiotic than the antibiotic without adding plant extract (Jackson and Esimone, 2010).

RESULTS AND DISCUSSION

Prevalence of Enterobactereceae Species Isolated from Turkey Poults

The prevalence of *Enterobacteriaceae* species in 100 turkey poults samples collected from various turkey farms was found to be 40% (40/100). The distribution of isolates revealed that *E. coli* had the highest prevalence with 77.5%, followed by *Klebsiella pneumoniae* at 7.5%, *Salmonella* and *Proteus* at 5%. Additionally, *Citrobacter* and *Yersinia* species each accounted for 2.5% of the isolates, as summarized in Table 3. The most frequently identified strains were *E. coli* O78 and *E. coli* O1, which constituted 19.36% of *E. coli* isolates (Table 4). Furthermore, both *Salmonella* isolates

were confirmed as *Salmonella* typhimurium, representing 100% of the *Salmonella* cases identified. As shown in (Figures 1, 2, 3, 4 and 5).

Table 3: Percent of Enterobactreace species among turkey poults age.

Species/ Age	E.coli	Salmonella	Klepsiella	Proteus	Citrobacter	Yersinia	Total
1-10 days	11	1	2	1	1		16(40%)
11-20 days	5	1		1		1	8 (20%)
21-30 days	15		1				40%
Total(40)	31 (77.5%)	2 (5%)	3 (7.5%)	2 (5%)	1 (2.5%)	1 (2.5%)	40

Table 4: Different serotypes of selected *E. coli* isolates and their percentage.

E. coli serotype	n. serotype	percentage
O78	6/31	19.36%
O1	6/31	19.36%
O18	3/31	9.68%
O113	3 /31	9.68%
O119	3/31	9.68%
O103	2/31	6.45%
O111	2/31	6.45%
O25	2/31	6.45%
O26	2/31	6.45%
O104	2/31	6.45%

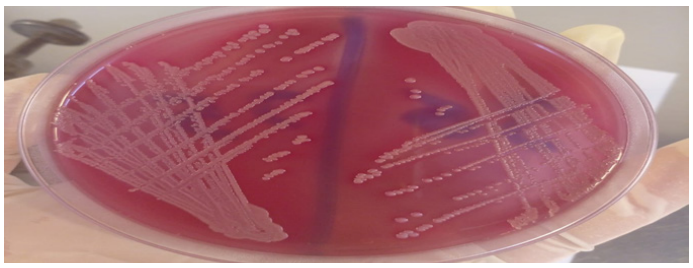

Figure 1: *E. coli* pink colonies on MacConkey agar (lactose fermenter).

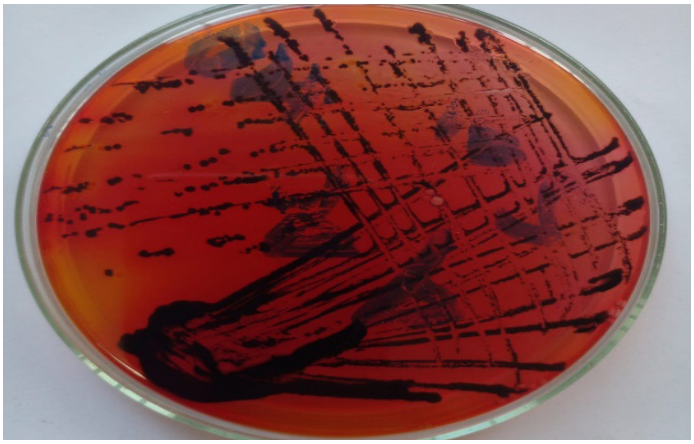

Figure 2: Salmonella on XLD transparent zone of reddish colour with or without black center.

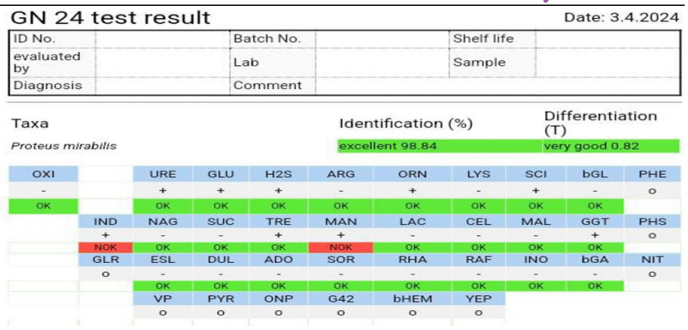

Figure 3: Biotyping results of *proteus miraabilis* isolate from turkey using GN24 system showing very good of *proteus mirabilis* identification(id %: 98.84, T index: 0.82).

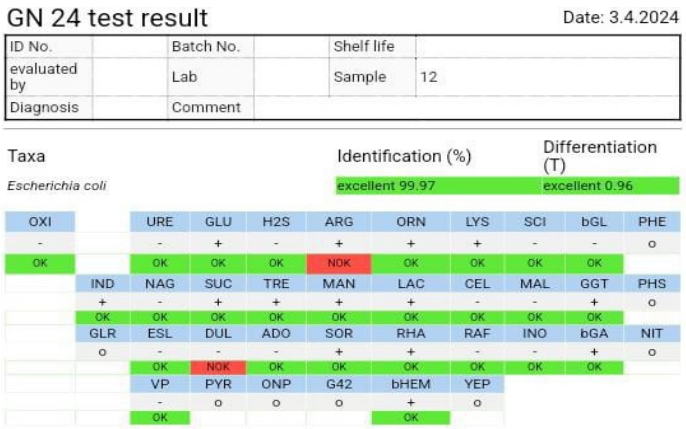

Figure 4: Biotyping results of *E.coli* isolate from turkey poult using GN24 system showing excellent *E.coli* identification(id %: 99.97, T index: 0.96).

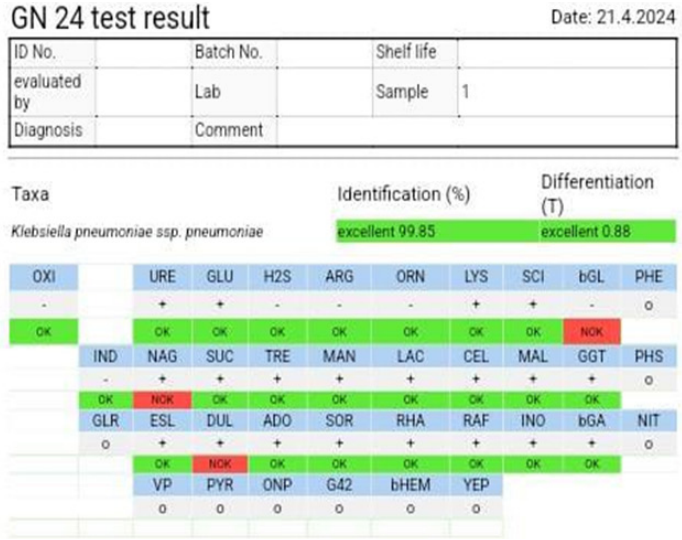

Figure 5: Biotyping results of *klepsiella pneumonia ssp. pneumoniae* isolate from turkey using GN24 system showing excellent of *klepsiella pneumonia ssp. pneumoniae* identification (id %: 99.85, T index: 0.88).

Antimicrobial Susceptibility of Isolates

Antimicrobial susceptibility testing indicated that the highest level of resistance was observed for Amoxicillin clavulanic acid, which exhibited a resistance rate of 71.05%. This was closely followed by Erythromycin at 73.68%,

Chloramphenicol at 63.16% and Doxycycline at 34.21%. In contrast, the isolates demonstrated significant sensitivity to Colistin, with a sensitivity rate of 94.73%, Gentamicin at 60.52%, and Trimethoprim-sulfamethoxazole at 52.63%. Intermediate resistance were noted for Amoxicillin clavulanic acid, Doxycycline, and Gentamicin, with rates of 26.31%, 23.68% and 21.05% respectively. The antibiogram analysis of 31 *E. coli* isolates revealed that 74.19% exhibited high resistance to both Amoxicillin clavulanic acid and Chloramphenicol, while 70.97% were resistant to Erythromycin and 32.25% to Doxycycline. Conversely, the isolates showed high sensitivity to Colistin at 96.77%, Gentamicin at 67.74% and Trimethoprim-sulfamethoxazole at 61.29%, as detailed in Table 5 and (Figures 6 and 7).

Table 5: Antibiogram of 31 *E. coli* isolated from turkey.

	Antibiogram Phenotypic Pattern					
	Resistance				Sensitive	
	Resistant	Intermediate	Total	%*	No	%*
Amoxicillin	23	1	24	77.41	7	22.58
Doxycycline	10	6	16	51.61	15	48.38
Erythromycin	22	1	23	74.19	8	25.8
Chloramphenicol	23	2	25	80.65	6	19.35
Trimethoprim Sulphamethazole	11	1	12	38.7	19	61.29
Gentamycin	2	8	10	32.25	21	67.74
Colistin	0	1	1	3.22	30	96.77

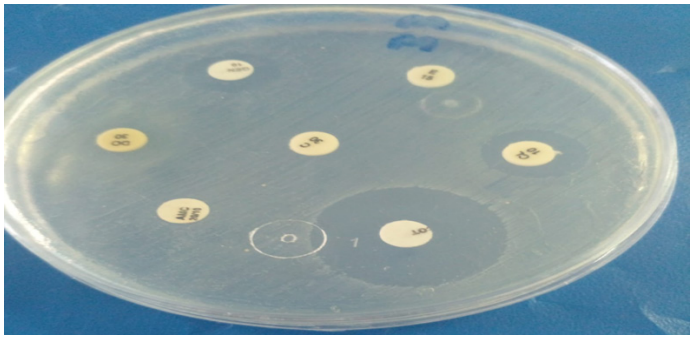

Figure 6: *E. coli* sensitive to COT, CL, GENT resistant to AMC, E, C.

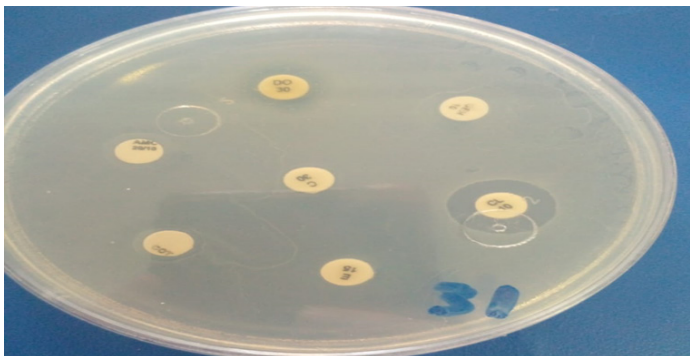

Figure 7: *E. coli* resistant to AMC, DO, E Sensitive to CL,C.

Additionally, two *Salmonella* typhimurium strains were evaluated for their susceptibility to various antimicrobial agents. The antibiogram for *Salmonella* typhimurium demonstrated complete resistance to Amoxicillin combined with clavulanic acid, Doxycycline, and Erythromycin, with a resistance rate of 100%, while showing full sensitivity to Colistin, also at 100%, as presented in Table 6 and Figure 8. Furthermore, three isolates of *Klebsiella pneumoniae* subspecies pneumoniae were tested, with results summarized in (Table 7 and Figure 9).

Table 6: Antibiogram of *Salmonella typhimurium and klebsiella* isolates.

AMA/Sample	AMC	DO	E	C	COT	GENT	CL
Salmonella typhimurium	I	I	R	I	S	S	S
Salmonella typhimurium	R	R	R	R	I	R	S
Klebsilla pneumonia	R	R	R	R	R	R	S
Klebsilla pneumoniae	R	R	R	R	R	S	S
Klebsilla pneumonia	I	I	R	I	I	R	S

R: resistant; **S:** sensitive; **I:** intermediate.

Detection of Resistance Genes Among Resistant Isolates

Six isolates were subjected for detection of resistance genes of Doxycycline (TetA), Erythromycin (MphA),Amoxycillin (blaTEM)and Chloramphenicol (floR). The result show all tested isolates has both genes TetA and Flor with100% while five have blaTEM gene with 83.33% and only three has MphA (50) as shown in (Figures 10 and 11).

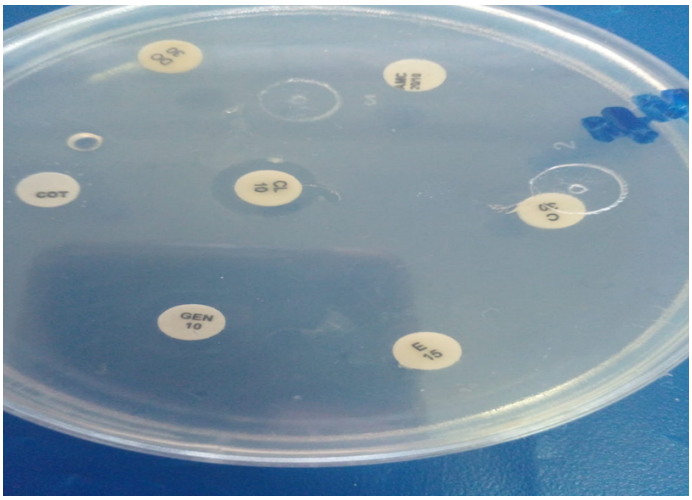

Figure 8: *Salmonella* resistant to AMC,E,DO,C,GENT Senstive to CL.

Antimicrobial Activity of Natural Extracts

The inhibition zones of various natural antimicrobial agents were evaluated against three bacterial strains: *E. coli* O78, *Salmonella* typhimurium, and *Klebsiella pneumoniae* ssp. pneumoniae. Measurements were obtained for the extracts both in isolation and in conjunction with antibiotics.

Table 7: Diameter of I.Z (mm) of antibiotics and extracts as well as combination on *E. coli*.

E. coli	Inhibition zone (mm)						
	Plant alone	Antibiotic alone			Combination		
		Amoxy	Doxy	Flor	Amoxy	Doxy	Flor
Rosemary	8	17	16	16	19	20	16
Cloves	19	17	16	16	26	20	21
Garlic	16	17	16	16	18	18	16
Ficus sycomorus	0	17	16	16	17	16	16
Ziziphus spina-christi	0	17	16	16	17	16	16

Amoxy: amoxycilin; **Doxy:** doxycycline; **Flor:** florofenicol.

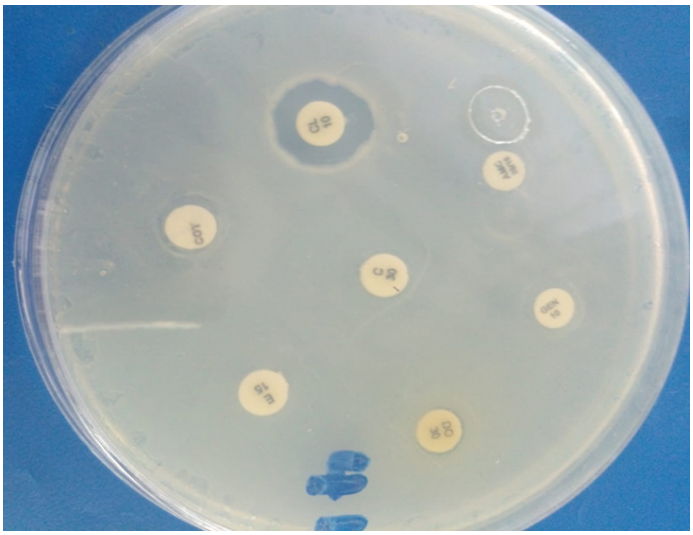

Figure 9: *klebseilla* resistant to all tested antibiotic (COT,C,AMC,E,DO,GEN) and sensitive to CL.

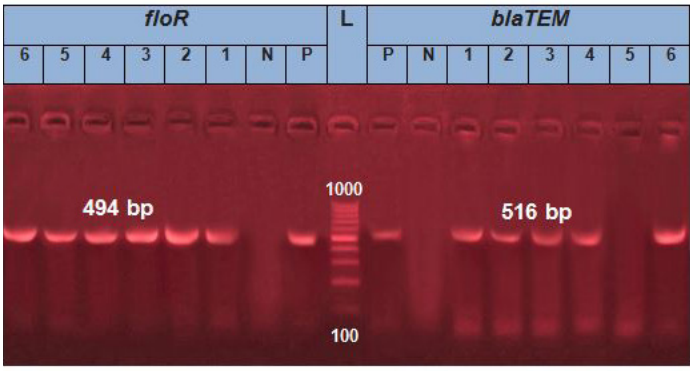

Figure 10: Agarose gel electrophoresis showing the result of PCR for detection of BlaTEM AND floR gene from 6 isolates; **Lane 1,2:** Salmonella typhimurium; **Lane 3:** proteus mirabilis; **Lane 4,5:** E.coli O78; **Lane 6:** Klepsilla pneumonia ssp pneumnoea; **Lane P:** Positive blaTEM,floR control. (Reference strain); **Lane N:** Negative blaTEM,floR control. (Control negative);**Lane L:** DNA molecular weight marker (Gelpilot 100bp ladder); **Lanes 1, 2,3,4,6,:** Positive amplification of 516bp for blaTEM gene of different isolates; **Lanes 1,2,3,4,5 and 6:** Positive amplification of494bp for floRgene of different isolates.

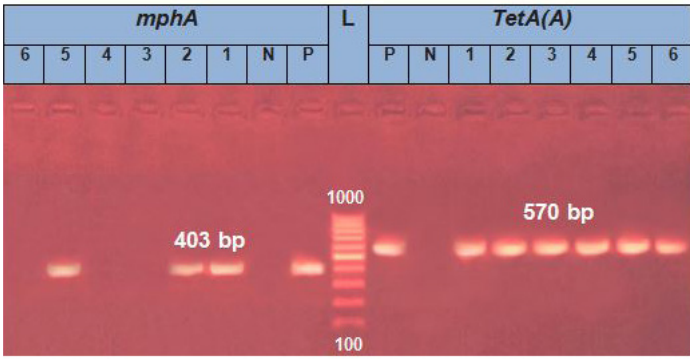

Figure 11: Agarose gel electrophoresis showing the result of PCR for detection of TetA(A) and mphA gene from 6 isolates; **Lane 1,2:** Salmonella typhimurium; **Lane 3:** proteus mirabilis; **Lane 4,5 :**E.coli O78; **Lane 6:** Klepsilla pneumonia ssp pneumnoea; **Lane P:** Positive TetA(A) and mphA control. (Reference strain); **Lane N:** Negative TetA(A) and mphA control. (Control negative); **Lane L:** DNA molecular weight marker (Gelpilot 100bp ladder); **Lanes 1,2,3,4,5 and 6:** Positive amplification of 570bp for TetA(A) gene of different isolates; **Lanes 1,2 and 5:** Positive amplification of 403bp for mphA gene of different isolates.

The methanolic extract of cloves exhibited the most significant inhibitory effect extract with 19 mm, 19 mm, and 17 mm inhibition zones against *E. coli*, *Salmonella* typhimurium, and *Klebsiella pneumoniae*, respectively. While garlic and rosemary methanolic extracts demonstrated inhibition zones of 18 mm and 13 mm against *Salmonella* typhimurium, 16 mm and 8 mm against *E. coli and Klebsiella pneumoniae*, respectively as shown in Figure 12. In contrast, extracts from *Ziziphus spina-christi* and *Ficus sycomorus* showed no inhibitory effects on any of the tested strains.

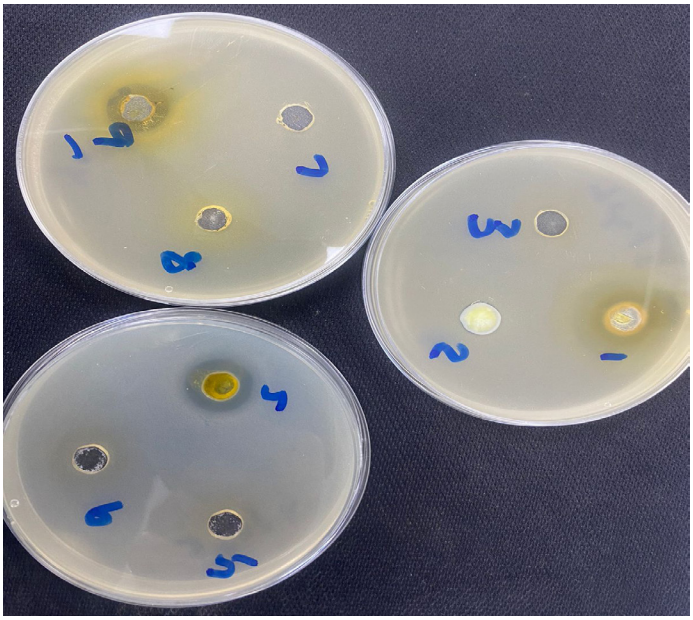

Figure 12: Cloves give inhibition zones as in (1),(4)wells and rosemary as in (9)well against *E. coli* O78.

In terms of antibiotic efficacy, Amoxicillin, Doxycycline, and Florfenicol exhibited inhibition zones ranging from 16 to 17 mm for Amoxicillin and 15 to 16 mm for both Doxycycline and Florfenicol against the three bacterial strains considered as intermediate resistance. However, when these antibiotics were combined with the extracts, particularly cloves, the inhibition zones increased significantly, reaching between 20 and 26 mm with Amoxicillin, 19 to 20 mm with Doxycycline, and 18 to 20 mm with Florfenicol across all 3 strains, which leaded to increase the antimicrobial effect of antibiotic against the bacteria from intermediate to sensitive interpretation reading according to (CLSI, 2011). The combination with rosemary showed enhanced activity only against *E. coli* and *Salmonella*, while *Klebsiella pneumoniae* was less effected by rosemary, yielding inhibition zones of 19 mm with Amoxicillin and 20 mm with Doxycycline, but a diminished effect with Florfenicol.

Table 8: Diameter of I.Z (mm) of antibiotics and extracts as well as combination on *Salmonella.*

Salmonella	Inhibition zone (mm)						
	Plant alone	Antibiotic alone			Combination		
		Amoxy	Doxy	Flor	Amoxy	Doxy	Flor
Rosemary	13	16	15	16	19	20	19
Cloves	19	16	15	16	20	20	20
Garlic	18	16	15	16	18	18	19
Ficus sycomorus	0	16	15	16	16	15	16
Ziziphus spina-christi	0	16	15	16	16	15	16

Amoxy: amoxycilin; **Doxy:** doxycycline; **Flor:**florofenicol.

Table 9: Diameter of I.Z (mm) of antibiotics and extracts as well as combination on *Klebsiella pneumoniae.*

Klebsiella pneumoniae	Inhibition zone (mm)						
	Plant alone	Antibiotic alone			Combination		
		Amoxy	Doxy	Flor	Amoxy	Doxy	Flor
Rosemary	8	16	16	15	18	16	15
Cloves	17	16	16	15	20	19	18
Garlic	18	16	16	15	16	18	16
Ficus sycomorus	0	16	16	15	16	16	15
Ziziphus spina-christi	0	16	16	15	16	16	15

Amoxy: amoxycilin; **Doxy:** doxycycline; **Flor** florofenicol.

The Synergistic Interaction Between Plant Extracts and Antibiotics

The antimicrobial properties of methanol extracts in combination with antibiotics were evaluated on the selected isolates, revealing interactions that can be classified as antagonistic, additive, or synergistic. An additive interaction occurs when the combined effect of the substances is equivalent to the sum of their individual effects. In contrast, antagonism identified when the efficacy of one or both compounds diminishes when used together. Synergism characterized by a combined effect that exceeds the total of the individual effects. The synergistic effect between plant extracts and antibiotics was evaluated by comparing the MIC values of plant extracts alone and the antibiotics alone and both on the selected isolates.

Table 10: Combination activity of antibiotics with extracts using DAA methods on *E. coli.*

E. coliO78						
Plant extracts	Antibiotics	DAA			MIC	Effect
		AB	E	DAA	AB alone	
Rosemary	A)Amoxycillin	9	1	8	32	Synergy
		8	2	8	32	synergy
		7	3	8	32	synergy
		9	1	8	32	synergy
	B)Doxycycline	8	2	8	32	synergy
		7	3	16	32	synergy
Cloves	A)Amoxycillin	9	1	8	32	synergy
		8	2	8	32	synergy
		7	3	8	32	synergy
	B)Doxycycline	9	1	8	32	synergy
		8	2	16	32	synergy
		7	3	16	32	synergy
	C)Florfenicol	9	1	8	16	synergy
		8	2	8	16	synergy
Garlic	A)Amoxycillin	9	1	16	32	synergy
		8	2	16	32	synergy
	B)Doxycycline	9	1	16	32	synergy
		8	2	16	32	synergy

DAA: Decimal Assay for Additivity; **AB:** antibiotic; **E:** extract; **MIC:** Minimal inhibitory concentration.

The findings indicated that amoxicillin (MIC 32 µg/ml) show good result synergy with cloves extract against *E. coli* with. 2fold (8 µg/ml) reduction in the MIC value at the ratios 9:1,8:2and 7:3 of combination but with 1fold reduction (16 µg/ml) with *Salmonella* typhimurium and *Klebsiella pneumoniae* was observed at same ratios. Meanwhile it show better result with rosemary against *Salmonella* typhimurium with 2fold (8 µg/ml) reduction in the MIC value at the three ratios. but synergy effect was intermediate in case of *E. coli* with 2fold (8 µg/ml) reduction in the MIC value at 9:1 and 8:2 ratios. Incase of garlic had only synergy against *E. coli with 1 fold reduction* (16 µg/ml) at 9:1 and 8:2 ratios.

Otherwise doxycycline (32 µg/ml) give good result with cloves and rosemary extracts which was reduced with 2fold

at 9:1 and with 1fold at 8:2 and 7:3 in challenge with *E. coli* and *Salmonella* but only with 1fold at 9:1 against *Klebsiella.* Garlic extract show lesser synergy than other extract with 1fold with the ratios with *Salmonella isolates.*

The MIC of florophenicol (16 µg/ml) was modulated when combined with garlic and cloves with 1 fold reduction (8 µg/ml) when used against E. coli, Salmonella and Klebsiella.in contrast very good synergy obtained when combined with rosemary with 2 fold decrease (4 µg/ml) at Salmonella but no effect obtained with E. coli and Klebsiella as shown in Tables 10, 11 and 12.

Table 11: Combination activity of antibiotics with extracts using DAA methods on *Salmonella.*

Salmonella typhimurium						
Plant extracts	**Antibiotics**	**DAA**			**MIC**	**Effect**
		AB	**E**	**DAA**	**AB alone**	
Rosemary	A)Amoxycillin	9	1	8	32	Synergy
		8	2	8	32	synergy
		7	3	8	32	synergy
		9	1	8	32	synergy
	B)Doxycycline	8	2	8	32	synergy
		7	3	8	32	synergy
	C)Florfenicol	9	1	4	16	synergy
		8	2	8	16	synergy
		7	3	8	16	synergy
Cloves	A)Amoxycillin	9	1	16	32	synergy
		8	2	16	32	synergy
		7	3	16	32	synergy
	B)Doxycycline	9	1	8	32	synergy
		8	2	16	32	synergy
		7	3	16	32	synergy
	C)Florfenicol	9	1	8	16	synergy
Garlic	A)Amoxycillin	9	1	16	32	synergy
	B)Doxycycline	9	1	16	32	synergy
		8	2	16	32	synergy
		7	3	16	32	synergy
	C)Florfenicol	9	1	8	16	synergy
		8	2	8	16	synergy
		7	3	8	16	synergy

DAA: Decimal Assay for Additivity; **AB:** antibiotic; **E:** extract; **MIC:** Minimal inhibitory concentration.

Foodborne infections linked to poultry and poultry products present a significant public health challenge, particularly due to the emergence of antibiotic-resistant bacteria. One of major foodborne infections is Salmonellosis, in 2007 Salmonellosis remained the second most commonly reported human zoonosis in the European Union (EU) in spite of a decrease in incidence over the past 4 years (European Food Safety Authority , 2009a).

The most commonly identified causative agent in the food-poisoning outbreaks in the UK in 2007 was *Salmonella* (EFSA, 2009b). In 2007 around 8.0% of the fattening turkey flocks in the EU tested positive for *Salmonella*, while in 2005 to 2007 1.5% or less of the turkey production flocks in the EU were positive for *Salmonella* Enteritidis or *Salmonella* Typhimurium (EFSA, 2009a). Other member of *Enterobacteriaceae like E. coli* also considered an important pathogen that causes diarrhea and death among humans and animals, and its presence gives an indication of the environmental status in poultry farms (Bo *et al.*, 2018). An increase in the percentage of β-lactamase-producing *E. coli* has been observed among humans and in food samples, which are a potential serious risk to public health because of the considerable number of multidrug-resistant genes (Chong *et al.*, 2011). Studies on prevalence of antibiotic resistance *Enterobacteriaceae* in turkey is important with searching for new aspect for treatment to overcome the emergence of antibiotic resistance.

Table12: Combination activity of antibiotics with extracts using DAA methods on *klebsiella pneumoniae.*

Klebsiella pneumoniae						
Plant extracts	**Antibiotics**	**DAA**			**MIC**	**Effect**
		AB	**E**	**DAA**	**AB alone**	
Rosemary	Amoxycillin	9	1	16	32	Synergy
Cloves	Amoxycillin	9	1	16	32	synergy
		8	2	16	32	synergy
	B) Doxycycline	9	1	16	32	synergy
	C) Florfenicol	9	1	8	16	synergy
		ss				

DAA: Decimal Assay for Additivity; **AB:** antibiotic; **E:** extract; **MIC:** Minimal inhibitory concentration.

In this study, *Enterobacteriaceae* was collected from turkey poults. Overall, there was a significant difference in *Enterobacteriaceae species*, with the most prevalence rate seen in *E. coli* with 75%. This finding aligns with several previous studies, including Giovanardi *et al.* (2013) and Hoepers *et al.* (2018), who reported prevalence rates about 86.7% of 15 necropsied turkey poults isolates in Italy and 84% of 364 different organ samples recovered from brazilian turkey flocks respectively, and Altekruse *et al.* (2002), who noted 89% of 1,104 fecal specimens were *E. coli positive*. Some researchers have reported even higher rates finding, like Tawyabur *et al.* (2020), who collected 30 fecal samples from healthy turkeys and 25 intestinal samples from diseased turkeys that died of enteritis in Egypt. All 55 samples were positive for E. coli (using PCR targeting the malB gene) with prevalence 100%. However, lower rates have also been

reported by Eid and Samir (2019), who recorded 18/120 (15%) of the total examined birds. The high prevalence of *E. coli* is attributed to its natural presence in the gastrointestinal flora of poultry, with specific strains possessing virulence factors enabling pathogenicity (Dho-Moulin and Fairbrother, 1999).

Notably, *E. coli* O78 and O1 were the most frequently isolated serotypes, consistent with global trends reported by multiple researchers (Altekruse *et al*., 2002; D'Incau et al., 2006; Giovanardi et al., 2007; 2011; Vaillancourt and Barnes, 2008; Circella et al., 2009). However recent meta-analyses indicate increasing diversity in pathogenic serotypes affecting poultry (Guabiraba and Schouler, 2015; Paudel *et al*., 2022). Also it is important to know the serotype of an APEC strain because the immune response in poultry primarily is directed against O antigens (Rikihisa *et al*., 2003). Effective inactivated vaccines against various serotypes including O2:K1 and O78:K80 have been produced which provide protection against the homologous serogroups, but no significant cross protection against heterologous serogroups (Trampel and Griffth, 1997).

E. coli strains are highly diverse antigenically, expressing a multitude of colonization factor and coli surface antigens, toxins, and other virulence proteins. To control colibacillosis in poultry under these circumstances, unique and alternative management strategies must be developed. Probiotics (Wang *et al*., 2017), bacteriophages (Kaikabo *et al*., 2017) and various new therapies (innate immune stimulants, growth and QS inhibitors (Peng *et al*., 2018) and antimicrobial peptides (Wang *et al*., 2016) have alldemonstrated promising efficacy in reducing Avian E. coli infections in chickens. However, none of these have yet made it into field applications.

Klebsiella pneumoniae often functions as a primary pathogen in respiratory tract diseases in birds (Jesus and Correia, 1998). In this study *Klebsiella pneumoniae* was isolated at a rate of 7.5%, which is comparable to the 5.8% reported by (Eid and Samir, 2019) collected from 120 freshly dead turkey poults but significantly lesser than (Funmilayo *et al*., 2020) with *Klebsiella* sp had the highest percentage of 26%, recovered from the 258 faecal droppings of turkey. This variation could be due to environmental settings in which the birds are raised and water sources of the birds (Ezekiel *et al*., 2011).

Salmonella has low prevalence rate of 5%, which is parallel with (Snow *et al*., 2010) but is lower than the 12.6% taken 250 paper-lined poults boxes reported by Osman *et al*. (2010) and El Kamshishy (2012). Meanwhile Tawyabur *et al*., (2020) has significant rate with 49.09% of 55 turkey poults sample. Which was significantly higher in diseased (64%; 16/25) than in healthy turkeys (36.67; 11/30). This high prevalence may be due to using PCR targeting the invA gene which is more accurate method for identification. Efficient laboratory methods for isolation, identification and typing of bacteria are essential elements in monitoring and control programs (Salm-Surv, 2003). One of the most common methods is the culture techniques and media that may work best in a particular diagnostic situation depends on a variety of factors, including serovar, source and type of specimens, animal species of origin, experience of the microbiologist, and availability of selective enrichment and selective plating media (OIE, 2014). In particular, culture could recognize viable organisms only, while amplification tests are not dependent on viable or structurally intact cells and the presence of DNA was sufficient to yield a positive result. Thus, the potential for detecting non-viable microorganisms explained the discrepancies between PCR and culture results following antibiotic therapy (Moalic *et al*., 1997).

Regarding antibiotic resistance, this findings revealed high resistance rates in *E. coli* isolates, with 74.19% resistant to Amoxicillin clavulanic acid and chloramphenicol, and 70.97% resistant to Erythromycin. Notably, 96.77% remained sensitive to colistin. The high resistance to Amoxicillin clavulanic acid is likely due to β-lactamase production, with the blaTEM gene detected in 83.33% of isolates. These findings are consistent with Eid and Samir (2019), who reported 88.9% resistance to Amoxicillin clavulanic acid in addition to 94.4%) of isolates were positive for blaTEM gene, and multiple other studies reporting similar patterns (Guerra *et al*., 2003; Hoepers *et al*., 2018; Osman *et al*., 2018).

E. coli isolates were highly resistant to Chloramphenicol, Erythromycin and Doxycycline with 74.19% 70.97% and 32.25% which regarded for presence of resistance genes doxycycline (TetA), erythromycin (MphA) and chloramphenicol (floR) detected with 100% in tested sample. this result is matching with Tawyabur *et al*. (2020) who reported that all *E. coli* isolates were resistant to erythromycin and chloramphenicol while was 52.73% with tetracycline among them tetA was detected in 27 (27/29; 93.1%). Another similar result in (Abd ElGawad *et al*., 2023) in which 5 (100%) isolates were found to have tetA(A) and MphA which correlated with phenotypic susceptibility with oxytetracycline and erythromycin with 90% and 80%. The result was higher than (Guerra *et al*., 2003; Safika *et al*., 2022) with 66% and 85.0% had tetA genes from *E.coli* and *Salmonella* strain respectively.

Antibiogram of *Salmonella typhimurium* revealed high resistance against Amoxicillin+ clavulanic acid, Doxycycline, Erythromicin with 100% and highly sensitive to Colistin with 100%.

It was analogous with Hasman *et al*. (2005) and Beutlich *et al*. (2010) in which all *Salmonella* isolates from turkey were resistant to Amoxicillin+ clavulanic acid and with Hui and Das (2001) with 86.66% of *Salmonella* isolates were resistant to chloramphenicol. In contrast with Shahada *et al*. (2006) who found that all 135 strains were susceptible to chloramphenicol. This result is higher than Diarra *et al*. (2014) who reported 43% of the isolates were resistant to amoxicillin-clavulanic acid accompined by presence of blaTEM. Meanwhile Penha Filho *et al*. (2016) has similar finding in which *S*. Gallinarum was full resistant to chloramphenicol but it was resistant to trimethoprime sulphamethazol with 96% and all isolates were sensitive to Amoxicillin+ clavulanic acid.

Consequently, isolates of *Klebsiella pneumoniae* exhibited resistance to 100% of all antibiotics subjected to testing, with the exception of gentamicin, which demonstrated a resistance rate of 66.6%, while these isolates were found to be 100% susceptible to colistin. Investigators such as Younis *et al*. (2016) in Egypt documented a complete resistance to amoxicillin, whereas Kowalczyk *et al* (2022) in Poland observed a sensitivity of 92.9% to colistin, 88.56% to florfenicol, and 82.6% to amoxicillin clavulanic acid. Among the tested agents, amoxicillin clavulanic acid emerged as the most efficacious against the *Klebsiella* spp. isolates. All strains of *Klebsiella* spp. displayed resistance to amoxicillin. A minor fraction of the strains (3.3%) were also capable of producing extended-spectrum beta-lactamases.

In recent years, there have been concerns about the greater frequencies of antibiotic resistance among bacteria isolated from food animals and from the environment (Jensen *et al*., 2001). The extensive use of antibiotics in human medicine, in animal practice for therapy and as growth promoters in agriculture are considered to be the major reasons for the development of bacterial resistance to antibiotics (Barton, 2000; Sengeløv *et al*., 2003). There is a growing interest in using natural antibacterial compounds such as extracts of spices and herbs for food preservation (Shan *et al*., 2007) . Plant-derived antibacterial compounds may be of value as a novel means for controlling antibiotic resistant zoonotic pathogens which contaminate food animals and their products (Palaniappan and Holley, 2010).

Plants are rich in a wide variety of secondary metabolites, such as tannins, terpenoids, alkaloids, and flavonoids, which have been found in vitro to have antimicrobial properties (Cowan, 1999; Lewis and Ausubel, 2006). This led to Tegos *et al*., (2002) hypothesizing that; Plants produce compounds that can be effective antimicrobials if they find their way into the cell of the pathogen especially across the double membrane barrier of Gram negative bacteria. Production of efflux pump inhibitors by the plant would be one way to ensure delivery of the antimicrobial compound. This hypothesis has been supported by the findings of (Stermitz Lorenz *et al*., 2000; Stermitz, Tawara-Matsuda *et al*., 2000). It has often been reported that Gram negative bacteria are more resistant to essential oils than Gram positive bacteria (Blaszyk and Holley, 1998; Shelef, 1984; Smith-Palmer *et al*., 1998).

Given the increasing antibiotic resistance, our study explored natural alternatives. Clove extract (*Syzygium aromaticum*) was the most effective extract against all selected strains, with inhibition zones of 19mm for both *E. coli* and S*almonel*la, and 17mm for *Klebsiella.* These effects were enhanced when combined with antibiotics. The antimicrobial activity is attributed to the high content of eugenol (70-90%) and tannins (10-19%). The antibacterial mechanism of eugenol against *S. aureus and E.coli* was probably related to the damage of cell wall and membrane, the inhibition on biofilm formation, the oxidative stress-mediated apoptosis and the disruption of DNA synthesis. Several researches were parallel with this finding as (Shaheen *et al*., 2015; Thanissery *et al*., 2014).

Garlic extract (*Allium sativum*) was the second most effective, showing inhibition zones of 16mm for *E. coli* and *Klebsiella*, and 18mm for *Salmonella*, with increased effectiveness in antibiotic combinations. it was comparable with (Alzowahi *et al*., 2013; Sudhir Kumar *et al*., 2012) with inhibition zones varies between 15-20mm and 24 mm against *Escherichia coli* and *Salmonella* spp respectively. The active compound in garlic, allicin, has been well-documented for its antimicrobial properties (Conner, 1993). The reactive organosulfur compounds form disulfide bonds with free sulfhydryl groups of enzymes and compromise the integrity of the bacterial membrane (Bhatwalkar *et al*., 2021). changes observed in membrane permeability, protein leakage and by scanning electron microscopy suggested that the antimicrobial activity of garlic extracts may be due to destruction of the structural integrity of cell membranes, leading to cell death (Chen *et al*., 2018).

Rosemary extract (*Rosmarinus officinalis*) showed variable effectiveness, with inhibition zones of 8mm, 13mm, and 8mm for *E. coli*, *Salmonella*, and *Klebsiella,* respectively. It was identical with (Abd-El Tawab *et al*., 2019; Celiktas *et al*., 2007; Mathlouthi *et al*., 2011). Their activity is linked to compounds such as 1,8-cineole, camphor, and borneol(-Knobloch *et al*., 1989; kordali *et al*., 2005; Lopes-Lutz *et al*., 2008). Fu *et al*. (2007) found that essential oil of rosemary attached to the surface of bacterial body at lower concentrations. At higher concentrations, essential oil entered the cell wall leading cell wall desquamation and shape distortion. Subsequently, essential oils invaded and damaged the cell membrane, so that the intracellular contents burst from the bacterial body.

Ficus sycomorus and *Ziziphus spina-christi* extracts have no effect on tested strains with several concentration 500mg/ml, 250mg/ml, 125mg and 60mg/ml, this finding is correlated with (Agbidye *et al*., 2020). As reported by Moreno *et al*. (2006), the absence of an inhibition zone does not necessarily mean an inactive compound. Compounds less polar diffuse more slowly into the culture medium. The diameter of the inhibition zone is influenced by the rate of diffusion of the antimicrobial agent through the agar, and the hydrophobic nature of most plant extracts prevents uniform diffusion of these substances through agar media (Davidson and Parish, 1989; Rios *et al*., 1988). Other researches have promising antimicrobial effect such as (Al-Mutairi *et al*., 2016; Ghareeb *et al*., 2015; Jebur *et al*., 2020; Olusesan *et al*., 2010). The variability in antimicrobial efficacy among different plant extracts in our study reflects the complex nature of phytochemical-bacterial interactions. Recent research suggests that the effectiveness of plant-derived antimicrobials may be influenced by multiple factors, including the bacterial stress response and adaptive mechanisms (Baptista-Silva *et al*., 2020). Also antibacterial activity of a plant extract might be attributable to the age of the plant used, freshness of plant materials, physical factors (temperature, light water), time of harvesting of plant materials and drying method used before the extraction process (Parekh and Chanda, 2006).

The observed synergistic effects between plant extracts and antibiotics, particularly at specific ratios, support the emerging paradigm of combination therapy as a promising approach to combat antibiotic resistance (Caesar and Cech, 2019). However, the molecular mechanisms underlying these synergistic interactions remain incompletely understood and warrant further investigation. Significant synergistic effects were evaluated between plant extracts and antibiotics then observe the enhancement of antibiotic activity so the ratio chosen to be antiobiotic part is more than natural extract part. Clove and garlic extracts showed synergy with amoxicillin and doxycycline at ratios of 9:1, 7:3, and 8:2 for *E. coli* and *Salmonella,* though effectiveness against *Klebsiella* was limited to a 9:1 ratio. Rosemary showed synergy at 7:3 and 9:1 ratios, excluding *Klebsiella*. The obtain results regarding the limited effectiveness of some plant extracts against *Klebsiella* compared to other pathogens align with the general observation that Gram-negative bacteria often show higher resistance to plant-derived compounds (Gibbons *et al*., 2015). This differential susceptibility highlights the need for targeted approaches in developing alternative antimicrobials. Recent advancements in nanoformulation and delivery systems might offer solutions to enhance the efficacy of plant-derived compounds against resistant pathogens (Wang *et al*., 2021).

The inconsistency in efficacy among different plant extracts raises important questions about the underlying mechanisms of their antimicrobial properties. For instance, while clove extract has demonstrated significant synergy with antibiotics against resistant strains, as evidenced by a 64.2% inhibition rate of tested microorganisms (Nascimento *et al*., 2000), rosemary's variable results suggest that its phytochemical profile may not interact favorably with certain bacterial targets or antibiotic classes. Furthermore, research indicates that specific compounds within these extracts can exhibit distinct modes of action; for example, eugenol from clove and other bioactive constituents might disrupt microbial cell membranes differently than the active components found in rosemary (Jouda *et al*., 2016). Several mechanism could explain the synergy between extract and antibiotic. Compounds within that extract may block the efflux mechanism or alter the process of efflux and in so doing, extend the life of existing antibacterial drugs, allowing these antibiotics to again block the growth of bacteria such as *P. mirabilis* strain, which was completely resistance to chloramphenicol (Chusri *et al*, 2009). Meanwhile specific plant compounds have been reported to induce perturbations in the cell membrane and increase the permeability of antibiotics to bacterial cells (Abreu *et al*., 2012).

These membrane perturbations, coupled with the action of β-lactams on the transpeptidation of the cell membrane, may enhance the inhibitory activity of the antibiotic (Aiyegoro *et al*., 2009). Furthermore, some plant-derived compounds can improve the *in vitro* activity of peptidoglycan inhibiting antibiotics by directly attacking the same site in the cell wall. Similarly, enhanced antimicrobial activity was observed in combinations of clove-ampicillin and clove-tetracycline against *K. pneumonia* and *Proteus* spp. Respectively (Nascimento *et al*., 2000). Abreu *et al*. (2012) reported that carnosic acid isolated from *Rosmarinus officinalis* L. potentiated the activity of erythromycin. That study determined that the increased erythromycin activity was due to an inhibition of the bacterial MDR pumps by carsonic acid.

While it remains imperative that research continues in the area of the development of the use of extracts derived from plant species as synergistic potentiators of medicines that had been previously effective signals a coming of age in the treatment of highly resistant infectious diseases that threaten the global community. By regaining the susceptibility of such pathogens to rigorously tested antibiotics, the fight against pervasive, transmissible, and deadly bacteria may finally shift in favor of the clinical treatment of such illnesses.

CONCLUSIONS AND RECOMMENDATIONS

On the basis of the antibacterial assay of this study *E. coli* was found the more (susceptible to the employed

plant extracts) than *Salmonella* typhimurium and *klebsiella pneumoae*. All plant extracts were evaluated for their MIC against *E. coli*, *Salmonella* typhimurium and *klebsiella pneumoae*, The strongest effect against *E. coli* was recorded when cloves was mixed with amoxicillin and doxycycline at (9:1,8:2and 7:3) while *Salmonella* when cloves and rosemary were mixed with amoxicillin and doxycycline at same ratios . *klebsiella was more affected when* when cloves was mixed with amoxicillin at (9:1,8:2) . florophenicol hasn't significant synergy with any of tested extracts.

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NOVELTY STATEMENT

Remerakable antimicrobial effect of cloves against tested bacteria alone or with antibiotics especially *E. coli*.

AUTHOR'S CONTRIBUTIONS

Prof.Dr. Mohamed E. Enany provide the idea and experimental design, Prof. Dr. Ahmed M. Hamouda make the statics of the experiment and Reem M. Khashaba performing the experiment and write the research .

Conflict of Interest

The authors declare that they have no competing interests.

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