Antibacterial activity of multidrug resistance *Escherichia coli* by some natural products

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ABSTRACT

Multidrug resistance *Escherichia coli* have one of most common resistance in the world. A total of 50 blood samples from Mansoura University Hospitals were collected, isolated, and identified *E. coli*, by molecular and chemical characterization using the Vitek AST automated technique. The results demonstrated antibiotic sensitivity results showed resistance against ciprofloxacin and moxifloxacin 100%. Ampicillin 100% Ampicillin/Sulbactam (91.93%), Aztreonam (96.93), Cefazolin (72.45%), Ceftazidime (100%), Ceftriaxone (94.68%), Cefepime (96%), Ciprofloxacin (100%), Gentamicn (73%), Moxifloxacin (100%), Nitrofurantoin 83.33%, Piperacillin/Tazobactam (100%), Tobramycin (97.53%), Trimethoprim/Sulfamethoxazoleol (70%), Ticarcillin (100%), Ticarcillin/Cavulanic acid (100%). Laboratory cultures of some fluoroquinolone groups, such as ciprofloxacin, levofloxacin, ofloxacin and norfloxacin, were also found to be resistant to *E. coli* bacteria. Two strains of *Escherichia coli* were used, and the antibacterial activity of varying concentrations (25, 50, 75, and 100) of aqueous garlic extract and peppermint was tested against the two strains. The findings revealed that although mint had no effect at all, garlic had a complete effect in all concentrations.

Keywords: MDR, Mint, Resistant *E. Coli*, Anti-bacterial, Garlic aqueous extract.

1. INTRODUCTION

Over the past decade, the intensive use of quinolones in human and veterinary medicines as well as in food animals has led to the rapid emergence of quinolones resistance (Haque *et al.*, 2016). Fluoroquinolones (FQ) are effective antibacterial medications used to treat a wide range of nosocomial and community diseases. FQ resistance rates vary substantially by sex, age, type of urinary infection, and geographic region, although it has recently been revealed that *E. coli* isolated from community-acquired UTIs are becoming more resistant to FQ, with up to 29% of women carrying this strain (Hossain *et al.*, 2016). Since the 1980s, when fluoroquinolones were first used in clinical treatments, this class of antibiotics has been frequently chosen to treat infections caused by *Escherichia coli*, *Salmonella*, and other Enterobacteriaceae in both humans and animals. This is due to the fluoroquinolones'

oral administration benefit and bactericidal activity against Gram-negative bacteria (Kim *et al*., 2009). The rate of quinolone resistance differs widely from one country to another. According to the Center for Disease Dynamics, Economics and Policy the percentage of fluoroquinolone resistant *E. coli* among invasive isolates was 29% in United States of America, ranged from 7% to 46% in European countries, and reached>70% in some countries as India (78%) and Pakistan (72%) (Jeong *et al*., 2005). One of the most prevalent causes of various typical bacterial infections in both humans and animals continues to be Escherichia coli. Enteritis, uTIs, septicemia, and other clinical infections such infant meningitis are frequently brought on by *E. coli* (Briales *et al*., 2012). Chromosome mutations that change DNA gyrase, the medicines' biological target, or decrease intracellular drug concentration are two examples of fluoroquinolone resistance mechanisms in bacteria. Both chromosomal and plasmid-mediated mechanisms are involved in bacterial resistance to quinolones and fluoroquinolones (van der Zee *et al*., 2016). *Escherichia coli* are one of the microorganisms most frequently studied worldwide. they are Gram-negative bacilli, facultative anaerobic, rod-shaped bacterium, which are most often found in the gastrointestinal tract as a normal colonizer of warm blood organisms (mainly in mammals, but are also present in birds, reptiles and fish) *(Ehwarieme et al., 2021). Escherichia coli* can emerge from primary infection sites like percutaneous vascular systems asymptomatically. Bacteremia can develop into sepsis when bacteria in the blood result in a significant systemic response that leads to sepsis syndrome, severe sepsis (sepsis-induced malfunction of at least one organ or system), or septic shock. Septicemia accounted for 1.3% of all fatalities in the United States in 2000, making it the tenth most common cause of death (Aslam *et al*., 2020). Many societies have employed garlic (Allium sativum L.) to combat infectious diseases because it is effective against gram-positive bacteria. (Adler *et al.*, 2002). It is advised to use garlic to cure bacteria that are resistant to many drugs. (Dini *et al.*, 2011). **The goals of this research** are to evaluate the antibiotic resistance phenotype of *E. coli* that tested the effect of garlic and mint extract against multidrug-resistant *E. coli*.

2. MATERIALS AND METHODS

2.1. Population:
Collection of samples

About 50 blood samples from patients (Approval No. H18RE34) who received care while hospitalised and whose data was thoroughly evaluated and subsequently studied were used for the testing. Samples were taken between October 2019 and February 2021 from Mansoura University Hospitals in Egypt. Blood was used to gather the samples. Samples were taken before the administration of antibiotics. Under sterile conditions, blood samples (5–10 ml) were drawn and inoculated into a blood culture container.

2.2. Automated Broth Dilution AST Systems

Vitek AST Automated System VITEK® 2 COMPACT (bioMérieux) Marcy l'Etoile, France Vitek 2 measures the turbidity changes over time (growth curve) and compare a growth control well with wells of other drug concentrations. MicroScan WalkAway, Vitek, and Vitek 2) is a broth microdilution method that utilizes a standard 96 microwell panel. These microwells contain serial dilutions of dehydrated antimicrobial agents. Results are obtained after about 18 hours by turbidimetric readings of overnight conventional panels or after about 7 hours by fluorometric readings

of rapid panels. Fluorometric analysis depends on the degradation of fluorogenic substrates by viable bacteria. The phoenix system detects the susceptibility results by gravity-based inoculation process. Monitoring of the growth depends on a redox indicator system which provide results in 8 to 12 hours.

2.3. Bacterial DNA extraction

Rapid DNA extraction was performed using methods of (**Salah *et al.*, 2019**). Where 10 samples were isolated as in Figure (2).

2.4. Sequencing of the 16SrRNA gene of the isolates

The thermal cycling conditions were 96 °C for 10 seconds, 50 °C for 5 seconds, and 60 °C for 4 seconds for the sequencing reactions in the 9700 thermal cycler with a volume of 20 L. (25 cycles). Afterwards, the cycle sequencing reaction was isolated from the extra dye terminators and primers using the DyeExTM 2.0 Spin Kit (Qiagen PN 63204). The resulting sequences were examined using the Finch TV (version 1.4.0) programme, and the phylogenetic tree was constructed using the closest published type strain sequences created using the Seaview programme.

2.5. Prepare aqueous garlic and mint extract

Fresh garlic obtained from the local market were used to make aqueous garlic extract. Garlic weighing 100 grams have been cleaned. Then, a 60-sec immersion in 70% (volume/volume) ethanol was performed as a method to sterilize the surface (Kalyan, 2000). Garlic was homogenized using a sterile mortar and pestle after the ethanol had been vaporized in a sterile laminar flow chamber. The extract was filtered with 0.45-µm membrane, after centrifugation at 6000 rpm for 10 m. This extract contains 100% concentration of the original substance. Finally, 25, 50, 75 and 100% of the concentrated garlic extract was produced by diluting 100% of it with sterile distilled water (Durairaj *et al.,* 2009). The mint from the local market was extracted in the same way as before.

2.6. Evaluation of the antibacterial activity of garlic and mint extract

Using a well-agar diffusion method two tested bacterial strains of *E. coli* were cultured on MacKonky Agar, then incubated at 37 °C for 8 h. The bacterial culture was spread on the surface of the nutrient agar plate, and a cork borer was used to puncture wells with a diameter of 6 mm. 100 µL of the following concentrations were added into each well: standard ciprofloxacin 5 mg/mL (a reference antibiotic) in sterile distilled water 25, 50, 75, and sterile 100% garlic and mint extract.

2.7.MIC to garli

Two strains of *E. coli* bacteria were tested for their response to various garlic doses. 100, 75, 50, 25, 12.5, 6.25 and 3.13.

3. RESULTS AND DISCUSSION

3.1. Identification of *Escherichia coli* by Vitek

In the current investigation, 50 blood samples in total were gathered. Results of a Vitek sample test are displayed. All samples (n = 50) contained *Escherichia coli*. Furthermore, all samples (n = 50) had a probability of 93% to 99% for *Escherichia coli* identification by Vitek.

3.2. Antibiotic susceptibility profile:

According to the results shown in Table (1), the isolated *E. Coli* is resistant to the following drugs: ciprofloxacin (100%), gentamicin (73%), moxifloxacin (100%), tobramycin (97.53%), piperacillin/tazobactam (100%), trimethoprim/sulfame.70%, ticarcillin (100%), and ticarcillin/cavulanic acid (100%). It is also resistant to ampicillin (10 The resistance ratios for amikacin, ertapenem, imipenem, meropenem, nitrofurantoin, and tigecycline are (10.52, 14.45, 15.3, 17, 17, 27.05, and 1.07, respectively), despite E. coli being less resistant to these drugs. A full allergy to colistin in the interim. The findings in Table (2) show that every isolate is a multidrug-resistant bacterium. 72% of the 50 blood samples had ESBL-*E. coli* in them.

3.3. Sensitivity test

Sensitivity test was performed in the lab on a few fluoroquinolone drugs and the resistance of bacteria to the antibiotic class was seen as in Figure (1).

Table 1. Bacterial identification of the two isolates.

Isolates	Identification	Identified Accession number	Highest homology	Indenty %
1	*Escherichia coli* Strain A1	MW719074	*Escherichia coli* strain GenBank: MT448673.1	100
2	*Escherichia coli* Strain A2	MW719253	*Escherichia coli* strain GenBank: MT735392.1	99.9

Table 2. Antibacterial activity of Garlic extracts against *E. coli* strains**.**

TREATMENTS	CONC.	ZONE OF INHIBITION MM(MEANS ± S.EROR.)	
		E. coli MW719074	*E. coli* MW719253
CIPROFLOXACIN	5mg	0	0
	25%	10.3 ± 0.1552	9.1 ± 0.2445
GARLIC EXTRACTS	50%	15 ± 0.8221	14 ± 0.365
	75%	17 ± 0.4226	16 ± 0.287
	100%	20 ± 0.3225	19 ± 0.726

Fig 1. Testing of a few fluoroquinolone drugs' sensitivities in lab.

3.4. DNA extraction

In this study, 10 samples were isolated and DNA was extracted as in Figure (2).

3.5. Molecular identification of the selected isolates.

The selected isolates can be identified thanks to the study of the 16S rRNA gene sequence (Figure, 3). Two samples were isolated and there results were in Figure (4).

3.6. Antimicrobial activity of hydrolyzed garlic and mint

The results in Tables (2 and 3) and Figures (5 and 6) demonstrated the potential antibacterial activity of 25, 50, 75, and 100% of garlic aqueous extract against *Escherichia coli* MW719074 and MW719253, as well as ciprofloxacin 5 mg (used as a reference). According to the results. While mint has no effect at all, garlic extracts do have an antibacterial effect against two types of bacteria, and the activity increases as the concentration of aqueous garlic extracts increases.

The results demonstrate in figure 7 represent 25% garlic extract was minimam inhibitory concentration (MIC) of two *E. coli*

Table 3. Antibacterial activity of Mint against *E. coli* strain

TREATMENTS	CONC.	ZONE OF INHIBITION MM(MEANS ± S.EROR.)	
		E. coli MW719074	*E. coli* MW719253
CIPROFLOXACIN	5mg	0	0
	25%	0	0
MINT EXTRACTS	50%	0	0
	75%	0	0
	100%	0	0

Table 4. Antibacterial activity of different concentrations of garlic against *E. coli* strain(MIC)

TREATMENTS	CONC.	ZONE OF INHIBITION MM(MEANS ± S.EROR.)	
		E. coli MW719074	*E. coli* MW719253
CIPROFLOXACIN	5mg	0	0
	3.13%	0	0
GARLIC EXTRACTS	6.25%	0	0
	12.5%	0	0
	25%	10.3 ± 0.1552	9.1 ± 0.2445
	50%	15 ± 0.8221	14 ± 0.365
	75%	17 ± 0.4226	16 ± 0.287
	100%	20 ± 0.3225	19 ± 0.726

Fig 2. 10 DNA extraction samples were isolated (1,2,3,4,5,6,7,8,9,10)

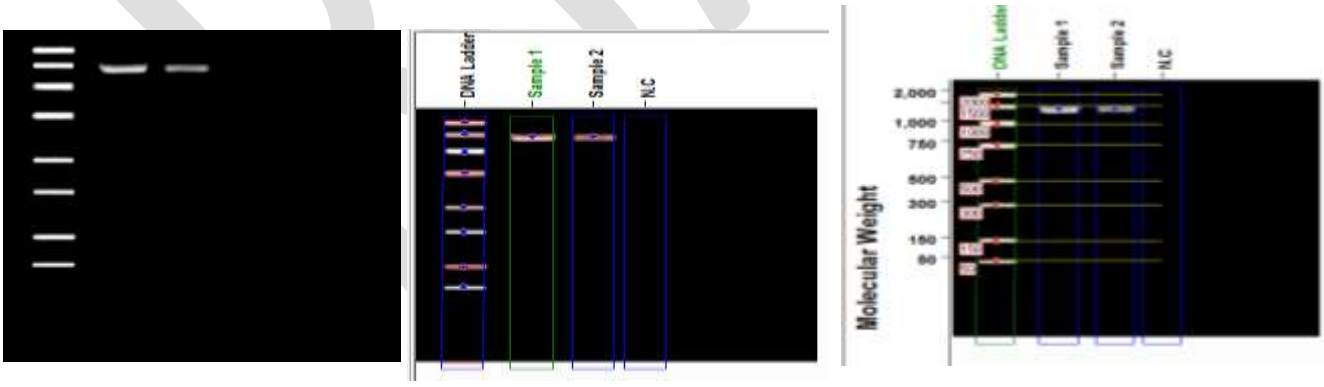

Fig 3. Specific genomic products for 16SrRNA bacterial strains with ≈ 1500bp. and Computerized and molecular weight detection for Specific genomic products for 16SrRNA with ≈ 1500 bp.

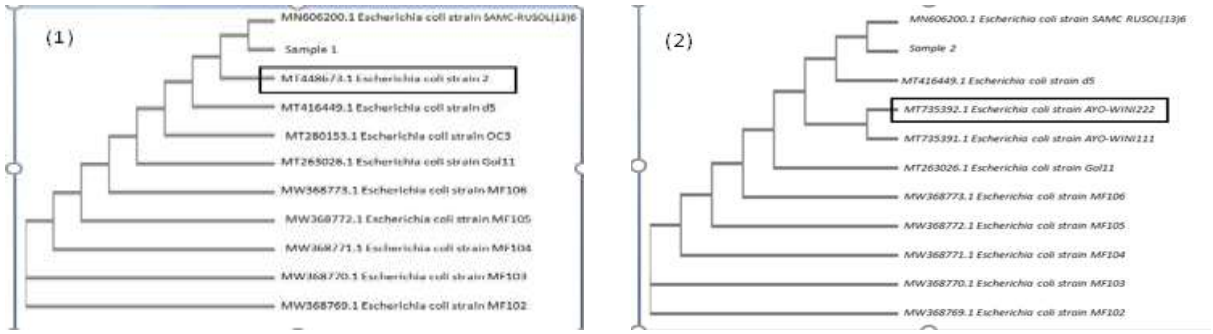

Fig 4. Phyllogenetic tree for the two isolates.

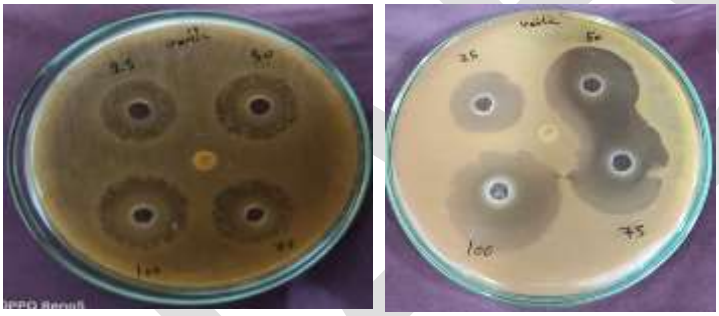

Fig 5. Activity of garlic against two strains of *Escherichia coli*.

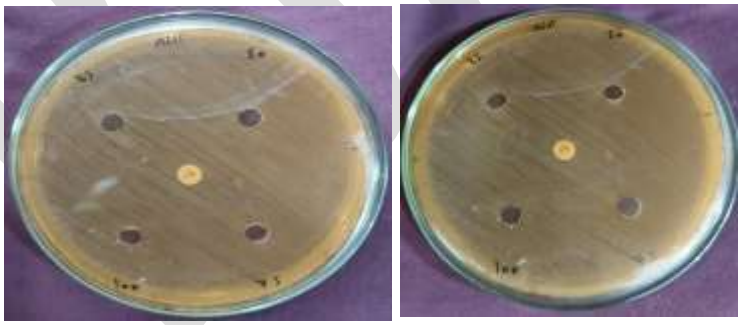

Fig 6. Activity of mint against two strains of *Escherichia coli*.

3.7. Discussion

All isolated samples identified MDR *E. coli*. Antibiotics are all over the place, which could explain the MDR. The causes that are most likely to contribute to the spread of antibiotic resistance include globalization, excessive antibiotic use in aquaculture and animal husbandry, the use of numerous broad-spectrum medications, and a lack of effective antimicrobial management (Vivas *et al.,* 2019). The results showed that *E. coli* are resistant to a number of antibiotics, including ampicillin, ampicillin/sulbactam, aztreonam, cefazolin, ceftazidime, ceftriaxone, cefepime, ciprofloxacin, gentamicin, moxifloxacin, nitrofurantoin, piperacillin/tazobactam, tobramycin, trimethoprim (Aabed *et al.,* 2021). *E. coli* isolated from healthy children in China has extreme resistance to Ampicillin (54.0%), Arimethoprim/Sulphurmethoxazole (47.5%) and Tetracycline (58.9%) (Zhao *et al.,* 2021). This research results showed that *E. coli* bacteria are resistant to a number of antibiotics, including ampicillin, ampicillin/sulbactam, aztreonam, cefazolin, ceftazidime, ceftriaxone, cefepime, ciprofloxacin, gentamicin, moxifloxacin, nitrofurantoin, beprazobactamide. 100% of the evaluated ESBL-*E. coli* bacteria were ampicillin resistant. (Abalkhail *et al.,* 2022). In this research 100% of the ESBL-*E* rating. *coli* resistant to ampicillin I agree with this. Major antibiotic-resistant *E. coli* strains are becoming more prevalent in almost all of Europe. (Chang *et al.,* 2015). This research agreement with (Abalkhail *et al.,* 2022), all *E. coli* isolates are MDR and that 78% of *E. coli* samples are ESBL *E. coli*. 15 antimicrobial medications, including Ceftriaxone, Cefotaxime, Ceftazidime, Cefpime, Cefuroxime, Cephalothin, Cefoxitin, Ampicillin, Amoxicillin-clavulanate, Aztreonam, Trimethoprim-sulfamethoxazole, Gentamicin, and Tigecycline, showed high resistance in ESBL-*E. coli*. *E. coli* with ESBLs present in Iran, Pakistan, and 40% (Hashemizadeh *et al*., 2018; Ali *et al*., 2016). This study proved aqueous garlic extract has antibacterial properties in different doses against *E. coli* MW719074 and MW719253. Garlic and ginger are natural herbs that have powerful antibacterial properties against pathogens that are resistant to several drugs and can be utilised to prevent the development of drug-resistant microbial illnesses (Karuppiah and Rajaram, *et al*., 2012). It was agreed that garlic affected all its concentrations, and the bacteria were affected by mint extract, and the results showed that mint did not affect all its concentrations at all. Many researches on suggested that allicin in garlic is the most vital compounds that makes antibacterial properties and restrictions the speed of RNA synthesis; the preliminary task of allicin is tricking RNA (**Deresse, 2010**). Allicin containing garlic extracts dominate inhibitory and bactericidal activities against the *Burkholderia cepacia* (**Wallock-Richards *et al.*, 2014**). Ethyl acetate, Ethanol, Chloroform, Hexane, Petroleum ether and Methanol, extracts of the Garlic leaves and bulbs were possessing highest antibacterial activity against pathogenic bacteria (**Prasad *et al.*, 2018**). Ultrasonicated garlic extract (garlic nanoparticles) displayed highest effective against *Escherichia coli*, *Streptococcus mutans*, *Staphylococcus aureus*, and *Poryphyromonas gingivalis* (**Gabriel *et al.*, 2022**).Garlic aqueous extract was to damage bacteria and inhibit from working of the protein generating RNA synthesis (**Mozaffari *et al.*, 2014**). The high antimicrobial activities of many bacterial types by garlic are related to the garlic contents such as allicin, ajoenes, and allyl sulfides (**Bhatwalkar *et al.*, 2021**).

The possibility of interbreeding different species of the genus Mentha results in the fact that their taxonomy is complicated, and

thus their chemical composition is heterogeneous. Therefore, the chemical composition and biological properties of individual hybrids can significantly vary from one another, depending on many factors, both environmental and genetic. The heterogeneous botanical systematics of the constantly emerging new mint cultivars creates problems with their proper identification, and thus with the appropriate determination of their chemical profile and possible use for therapeutic purposes. The active constituents of *Mentha* species leaves include flavonoids (eriocitrin, hesperidin, diosmin, luteolin and their glycosides), phenolic acids (derivatives of caffeic acid, e.g. rosmarinic acid), terpenoids, and volatile compounds (Fecka and Turek, 2007; Mahendran and Rahman, 2020; Nilo et al**.,** 2017).

4. Conclusion

This investigation demonstrated that all *E. coli* isolates were resistant to many drugs, including ampicillin, ampicillin/sulbactam, azitreonam, cefazolin, ceftazidime, moxifloxacin, gentamicin, moxifloxacin, nitrofurantoin, piperacillin/tazobactam, tobramycin, trimethoprim/sulfamethoxazole, ticarcillin, ciprofloxacin. Aqueous garlic extract has antibacterial activity o against *E. coli* MW719074, and *E. coli* MW719253. It must be Egyptian food and drug authority (EFDA) launch an electronic awareness campaign, which aimed to raise the awareness in community towards the necessity to avoid taking the antibiotic without a prescription, and warn of complications of excessive use of antibiotics. In addition, stressing the need to adhere to prescribed doses of antibiotics. While mint did not affect this strain of bacteria.

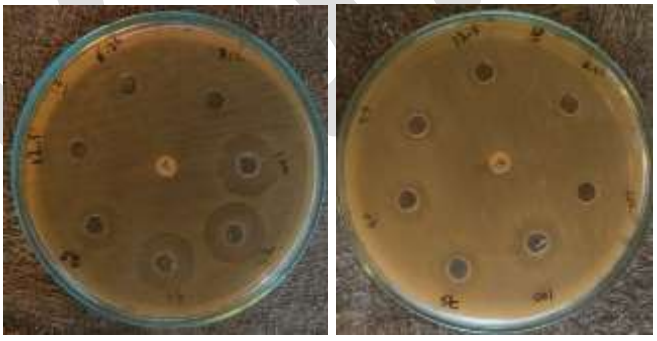

Fig 7. Activity of different concentrations of garlic against two strains of *Escherichia coli*

5. REFERENCES

Aabed, K., Moubayed, N., Alzahrani, S. (2012): Antimicrobial resistance patterns among different *Escherichia coli* isolates in the Kingdom of Saudi Arabia. Saudi J Biol Sci. 2012 Jul;28(7):3776-3782. doi: 10.1016/j.sjbs.2021.03.047. Epub 2021 Mar 23. PMID: 34220231; PMCID: PMC8241624.

Abalkhail, A., AlYami, A.S., Alrashedi, S.F., Almushayqih, K.M., Alslamah, T. Alsalamah, Y.A., Elbehiry, A. (2022): The Prevalence of Multidrug-Resistant Escherichia coli Producing ESBL among Male and Female Patients with Urinary Tract Infections in Riyadh Region, Saudi

Adler, B.B., Beuchat, L.R. (2002): Death of *Salmonella*, *Escherichia* coli 0157:H7, and Listeria monocytogenes in garlic butter as affected by storage temperature. J Food Prot; 65:1976–1980.

Begum, Y.A., Talukder, K.A. Azmi, I.J., Shahnaij, M., Sheikh, A., Sharmin, S., Svennerholm, A.M., Qadri, F. (2016): Resistance pattern and molecular characterization of enterotoxigenic *Escherichia coli* (ETEC) strains isolated in Bangladesh. PLoS One, 11: e0157415.

Bhatwalkar, S.B.; Mondal, R.; Krishna, S.B.N.; Adam, J.K.; Govender, P.; Anupam, R. Antibacterial Properties of Organosulfur Compounds of Garlic (Allium sativum). Front. Microbiol. 2021, 12, 1–20

Briales, A., Rodriguez-Martinez, J.M., Velasco, C., de Alba, P.D., Rodriguez-Bano, J., Martinez-Martinez, L., Pascual, A. (2012): Prevalence of plasmid-mediated quinolone resistance determinants qnr and aac (6')-Ib-cr in *Escherichia coli* and *Klebsiella pneumoniae* producing extended-spectrum ß-lactamases in Spain. Int J Antimicrob Agents; 39:431–4.

Chang, H.H., Cohen, T., Grad, Y.H., Hanage, W.P., O'Brien, T.F., Lipsitch, M. (2015) proliferation of multiple-drug resistance in bacterial pathogens. *Microbiol Mol Biol Rev.*79(1):101–16.

Chibuzor, M.N., Mirabeau, Y.T., Chijioke, A.N. (2021): High prevalence of qnrA and qnrB genes among fuoroquinolone-resistant *Escherichia coli* isolates from a tertiary hospital in Southern Nigeria Bull Natl Res Cent 45:26.

Conley, Z. C., Bodine, T. J., Chou, A., Zechiedrich, L. (2018): Wicked: the untold story of ciprofloxacin. PLOS Pathog ;14: e1006805.

Deresse, D. (2010). Antibacterial effect of garlic (Allium sativum) on Staphylococcu aureus: An in vitro study. Asian J Med Sci, 2(2), 62-5.

Dini, C., Fabbri, A., Geraci, A. (2011): The potential role of garlic (Allium sativum) against the multi-drug resistant tuberculosis pandemic: a review. Ann Ist Super Sanita; 47:465–473

Aslam, A., Gajdács, M., Zin, C.S., Ab Rahman, N.S., Ahmed, S.I., Zafar, M.Z., Jamshed, S. (2020): Evidence of the practice of self-medication with antibiotics among the lay public in low- and middle-income countries: a scoping review. Antibiotics,a; 9:597.

Durairaj, S., Sangeetha, S., Lakshmanaperumalsamy, P. (2009): In vitro antibacterial activity and stability of garlic extract at dič erent pH and temperature. Electron J Biol; 5:5-10.

Ehwarieme, D. A., Whiliki, O.O., Ejukonemu, F.E. (2021): Occurrence of plasmid mediated fuoroquinolone resistance genes amongst enteric bacteria isolated from human and animal sources

in Delta State, Nigeria. AIMS Microbiol 7:75–95.

Faife, S. L., Zimba, T., Sekyere, J.O. Govinden, U., Chenia, H.Y., Simonsen, G. S., Sundsfjord, A., Essack, S.Y. (2020): β-lactam and fluoroquinolone resistance in Enterobacteriaceae from imported and locally-produced chicken in Mozambique J Infect Dev Ctries May 31;14(5):471-478.

Gabriel, T., Vestine, A., Kim, K. D., Kwon, S. J., Sivanesan, I., & Chun, S. C. (2022). Antibacterial Activity of Nanoparticles of Garlic (Allium sativum) Extract against Different Bacteria Such as Streptococcus mutans and Poryphormonas gingivalis. Applied Sciences, 12(7), 3491.

Haque, A., Tateda, K., Ishii, Y., Huda, Q., Islam, A., Miah, R.A. (2016): Quinolone Resistant Enterobacteriaceae and *Pseudomonas* in Environ- mental Water in Dhaka, Bangladesh Bangladesh Med Res Counc Bull; 42: 28-32.

Hashemizadeh, Z., Kalantar-Neyestanaki, D., Mansouri, S. (2018): Clonal relationships, antimicrobial susceptibilities, and molecular characterization of extended-spectrum beta-lactamase-producing Escherichia coli isolates from urinary tract infections and fecal samples in Southeast Iran. Rev. Da Soc. Bras. De Med. Trop. 51, 44–51. [CrossRef]

Hashemizadeh, Z., Samane, M. Davood, K., Adekanmbi, A. O., Usidamen, S., Akinlabi, O.C. (2019): Prevalence of plasmidmediated quinolone resistance and ESBL genes in *Escherichia coli* isolated from urinary tract infections and fecal samples in Southeast Iran. Gen Rep 17:100487.

Hooper, D. C. and Jacoby, G. A. (2016): Topoisomerase inhibitors: fluoroquinolone mechanisms of action and resistance. Cold Spring Harb Perspect Med; 6: a025320.

Hopkins, K. L., Davies, R. H., Threlfall, E.J. (2005): Mechanisms of quinolone resistance in *Escherichia coli* and *Salmonella*: recent developments. Int J Antimicrob Agents, 25(5):358-373.

Hossain, M. S., Khatun, R., Solayman, M., Aktar, B., Ahmed, A.A. (2016): Antibiotic Susceptibility Pattern of Clinical Isolates of *Escherichia coli* at a Tertiary Care Hospital KYAMC Journal Vol. 7 No. 1.

Jeong, J. Y., Yoon, H. J., Kim, E. S., Lee, Y., Choi, S.H., Kim, N.J., Woo, J. H., Kim, Y.S. (2005): Detection of qnr in clinical isolates of *Escherichia coli* from Korea. Antimicrob Agents Chemother; 49:2522-4.

Kalyan, K. D. (2000): An introduction to plant tissue culture. 1st ed. CalcuĴ a: New Central Book Agency (P) Ltd; p. 37-9. 7.

Karuppiah, P., Rajaram, S. (2012): Antibacterial effect of Allium sativum cloves and Zingiber officinale rhizomes against multiple-drug resistant clinical pathogens. Asian Pac J Trop Biomed. Aug;2(8):597-601. doi: 10.1016/S2221-1691(12)60104-X. PMID: 23569978; PMCID: PMC3609356.

Kim, E. S., Jeong, J.Y.; Choi, S.H.; Lee, S., Kim, S., Kim, M., Woo, J.H., Kim, Y. S. (2009): Plasmidmediated fluoroquinolone efflux pump gene, qepA, in *Escherichia coli* clinical isolates in Korea. Diagn Microbiol Infect Dis b; 65:335-8.

Lev, O., Rachel, Khodurskya, F., James, R., Johnsonb., Hiroshi, H., Arkady, K. (2019): Analysis of mutational patterns in quinolone resistance-determining regions of GyrA and ParC of clinical isolates International Journal of Antimicrobial Agents 53 ;318–324

Mozaffari Nejad AS, Shabani S, Bayat M, Hosseini SE. Antibacterial Effect of Garlic Aqueous Extract on Staphylococcus aureus in Hamburger. Jundishapur J Microbiol. 2014 Nov;7(11):e13134. doi:

10.5812/jjm.13134. Epub 2014 Nov 1. PMID: 25774277; PMCID: PMC4332239.

Prasad, R., Mohan, M., & Ugandhar, T. (2018). Studies on antimicrobial activity of garlic extract against. Int J Current Adv Res, 7(1), 1-2.

Van der Zee, A., Roorda, L., Bosman, G., Ossewaarde, J.M. (2016): Molecular diagnosis of urinary tract infections by semi-quantitative detection of uropathogens in a routine clinical hospital setting. PloS One; 11: e0150755.

Vivas, R., Barbosa, A.A.T., Dolabela, S.S., Jain, S. (2019): Multidrug-Resistant Bacteria and Alternative Methods to Control Them: An Overview. Microb Drug Resist. 2019 Jul/Aug;25(6):890-908. doi: 10.1089/mdr.2018.0319. Epub Feb 27. PMID: 30811275.

Wallock-Richards D, Doherty CJ, Doherty L, Clarke DJ, Place M, Govan JR, Campopiano DJ. Garlic revisited: antimicrobial activity of allicin-containing garlic extracts against Burkholderia cepacia complex. PLoS One. 2014 Dec 1;9(12):e112726. doi: 10.1371/journal.pone.0112726. PMID: 25438250; PMCID: PMC4249831.

Yang, H. Y., Nam, Y.S., Lee, H.J. (2014): Prevalence of plasmid-mediated quinolone resistance genes among ciprofloxacin-non susceptible *Escherichia coli* and *Klebsiella pneumoniae* isolated from blood cultures in Korea. Can J Infect Dis Med Microbiol ;25(3):163-169.

Zhao, Q., Shen, Y., Chen, G., Luo, Y., Cui, S., & Tian, Y. (2021). Prevalence and Molecular Characterization of Fluoroquinolone-Resistant Escherichia coli in Healthy Children. *Frontiers in Cellular and Infection Microbiology*, 1269.