Determine the antimicrobial activity of methanolic extract of Tulsi against the antibiotic resistant bacteria isolated from poultry meat

Thesis Submitted to Vidyasagar University
for the Partial Fulfillment of the Degree of
Bachelor of Medical Laboratory Technology (BMLT)

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Ref. No. MCC/TIC-UG(CER)/07/23-832-GrA

Dated 26th July, 2023

CERTIFICATE

This is to certify that the project report entitled '**Determine the antimicrobial activity of methanolic extract of Tulsi against the antibiotic resistant bacteria isolated from poultry meat**' submitted by Sujay Nayak, Roll-1597766 No-200355; Sujoy Ghosh, Roll-1597766 No-200356; Suman Brahma, Roll-1597766 No-200357; Suman Chakraborty, Roll-1597766 No-200358; Suman Panigrahi, Roll-1597766 No-200360; Suman Patra, Roll-1597766 No-200361; Sumit Ghosh, Roll-1597766 No-200362; Sumona Mandal, Roll-1597766 No-200363 to the Midnapore City College, Midnapore, West Bengal, India during the year of 2023 in partial fulfillment for the award of the degree of Bachelor of Medical Laboratory Technology (BMLT) is a bona fide record of project work carried out by him/her under my/our supervision. The contents of this report, in full or in parts, have not been submitted to any other Institution or University for the award of any degree.

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Declaration

I do hereby declare that the present Bachelor thesis entitled '**Determine the antimicrobial activity of methanolic extract of Tulsi against the antibiotic resistant bacteria isolated from poultry meat**' embodies the original research work carried out by me in the Department of Paramedical and Allied Health Sciences, Midnapore City College, Paschim Medinipur, West Bengal, India under the supervision of Mr. Subhadeep Mondal, Assistant Professor, Paramedical & Allied Health Science, Midnapore City College, Kuturiya, Bhadutala, Pin-721129. No part thereof has been submitted for any degree or diploma in any University.

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Dedicated to my beloved Parents and Teachers

Acknowledgement

I would first like to acknowledge Dr. Pradip Ghosh, Hon'ble Founder Director, Midnapore City College, Paschim Medinipur for providing me the opportunity to study and complete my thesis work in this college. I am gratefully indebted to him for his very valuable comments on this thesis.

I would like to thank my thesis advisor Mr. Subhadeep Mondal of the Paramedical & Allied Health Science at Midnapore City College. The door to Prof. Mondal office was always open whenever I ran into a trouble spot or had a question about my research or writing. He consistently allowed this paper to be my own work, but steered me in the right the direction whenever he/she thought I needed it.

I would also like to thank the other Faculties (Dr. Kuntal Ghosh, Dr. Santanu Kar Mahapatra, Miss. Shilpa Sarkhel, Mr. Sourya Sekhar Mitra, Mrs. Monalisha Karmakar, and Mr. Surja Kanta Dey) and research scholars (Tridip Kumar Das, Smita Ghosh and Priyanka Kar) for their support to carry out this research project. Without their passionate participation and input, the validation survey could not have been successfully conducted.

Finally, I must express my very profound gratitude to my parents for providing me with unfailing support and continuous encouragement throughout my years of study and through the process of researching and writing this thesis. This accomplishment would not have been possible without them.

Thank you.

Abstract

The present study aimed to estimate the load of antibiotic resistant bacterial community exist in the local poultry meat. For this aim, initially 71 bacteria were isolated from the poultry meat purchased from the different poultry slaughterhouse of local market, Midnapore town. After performing antibiotic sensitivity test, among the initial bacterial isolates, 7 were found to be antibiotic resistant. Generation of multidrug resistant bacteria is one of the critical challenges of the 21st century. Mother Nature has provided us with all the remedies, which we need to protect humanity from the life-threatening spread of multidrug resistant pathogens. One of the plants that has benefits as traditional medicine is Tulsi (*Ocimum sanctum*) and its leaves can use as an antimicrobial agent from the ancient period. The MIC values of methanolic extract of Tulsi was observed as 30, 20, 40, 70, 30, 40, and 50 mg/ml from the Isolates 1 to 7, respectively. The total polyphenols content of the Tulsi was 218.28±5.9 mg GAE/g while the flavonoid content 61.32±1.9 mg QE/g. This phytochemicals content is the prime factors behind the antimicrobial nature of Tulsi. The current study extends our counter strategies to deal gradually raising antibiotic resistant bacterial community.

Keywords: Antibiotic resistant bacteria; Poultry meat; Tulsi; MIC, Phytochemicals.

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Chapter 1: Introduction

1. Introduction

The increased demand for animal protein according to the steady growth of the world population has resulted in the expansion of the population of poultry chickens and poultry meat production. Poultry meat is one of the best sources of proteins, vitamins and minerals, which are essential nutrients required for proper growth and maintenance (Orpin et al., 2018). The increased use of antimicrobials at the poultry farms to maintain chicken's health may have accompanied increased antimicrobial drug-resistant bacteria (Van Boeckel et al., 2019; Dewulf et al., 2020). As a result, the increased antimicrobial resistance (AMR) can be a burden for human and animal health, since AMR can be transferred between humans, animals, and their environment through horizontal gene transfer by transformation or conjugation (Pollock et al., 2020). In addition, the prevalence of the pathogens with multidrug resistance (MDR) is raising serious concerns for human and animal health and welfare through the limitation of antimicrobials available to treat diseases. These issues can be also important for food safety and security, as well as global trade (Avraam et al., 2021). The production of poultry meat is globally expanding due to economical and nutritional values. Therefore, the inhibition of increased antimicrobial-resistant bacteria in poultry meat and its byproducts is one of the main concerns for farmers, food policymakers, stakeholders, and consumers, and is highlighted as there is an increased demand for the consumption of the safe meat products. The development of AMR is a typical evolutionary process in microbes, and the increasing use of antibiotics has led to the emergence of AMR, which limits the treatment of various bacterial infections (Bae et al., 2022). The use of antimicrobials in the livestock sector for over 60 years has resulted in the dissemination and co-selection of antimicrobial-resistant bacteria in food-producing animals (Vanderhaeghen and Dewulf, 2017; Ceccarelli et al., 2020). Consequently, the transmission of antimicrobial-resistant traits or bacteria between humans and livestock, and the associated environment, has been frequently present (Zwirzitz et al., 2020). Currently, the therapeutic use of antimicrobials is allowed to treat diseased animals, whereas the use of antimicrobials for the growth promotion and prophylactic purposes is strictly prohibited in most developed countries (Caekebeke et al., 2020). However, antimicrobials are still used for this purpose in many developing countries due to the lack of legislative systems and veterinary

infrastructure (Dewulf et al., 2020). Davis et al. (2018) isolated antibiotic resistant (ampicillin, ampicillin-sulbactam, cefazolin, tetracycline, gentamycin) *Escherichia coli* from

turkey meat products. Saud et al. (2019) showed that the 32.7 % antibiotic resistant (amoxicillin, tetracycline, cotrimoxazole and nalidixic acid) *Escherichia coli*, *Proteus* spp. And *Staphylococcus aureus* among the 70 bacterial isolates from raw poultry meat in Nepal. Besides, they compared antibiotic resistant bacterial profile found in poultry meat with the buffalo meat and observed that poultry meat contains higher percentage of AMR bacteria. Schwaiger et al., (2012) 500 coliforms bacteria [*Escherichia coli* (n=677), *Enterobacter* spp. (n=167), *Citrobacter* spp. (n=83), *Serratia* spp. (n=116), *Klebsiella* spp. (n=125), and *Salmonella* spp. (n=89)], among them specifically *E. coli* strains were often resistant to antibiotics: penicillins, streptomycin, spectinomycin, doxycycline and sulfamethoxazole/trimethoprim. Kim et al. (2020) isolated 87.9% multidrug (nalidixic acid, ampicillin, tetracycline) resistant *E. coli* 719 *E. coli* strains were isolated from 1,107 raw poultry (chicken and duck) meat samples purchased from nationwide retail stores in Korea between 2017 and 2019 and they also found that multidrug resistant bacterial population higher in chicken compared to duck meat.

Ocimum sanctum, commonly known as tulsi or holy basil, has been used since ages in Ayurveda due to its healing property. It is also known as "queen of herbs" and is one of the holiest herbs in India with Kingdom – Plantae, Subkingdom – Tracheobionta, Superdivision – Spermatophyta, Division – Magnoliophyta, Class – Magnoliopsida, Subclass – Asteridae, Order – Lamiales, Family – *Lamiaceae*, Genus – *Ocimum*, and Species – *O. sanctum* (Kumar et al., 2018). *Ocimum* is a genus of about 35 species of aromatic annual and perennial herbs and shrubs. Some species includes *O. basilicum* or Thai basil; *O. campechianum* or Amazonian basil; *O. gratissimum* or African Basil; *O. tenuiflorum* or *O. sanctum* or Tulsi or Holy Basil; *O. citriodorum* or Lemon Basil (Subramanian et al., 2014). Tulsi is used in treatment of a number of diseases like mental illness, cough and fever, gut diseases, bone and joint problems, eye diseases and other optic problems, ringworm, insect bite, snake bite and scorpion bite and malaria (Londhe et al., 2015). Tulsi has antimicrobial activities against many pathogens and can be used as mouth wash agent, for wound healing, and preservation of food stuff. Tulsi is antibacterial, antiviral, antifungal, antiprotozoal, antimalarial, and can be used also for killing mosquitoes (Verma, 2016).

It has anti-oxidants and can be used as anti-cataract agent, anti-inflammatory agent, as well as protects from chemicals and radiations, good for the liver and nerves and heart,

anticancerous agent, protects the immune system, central nervous system and memory its antiasthma and thyroid, and solves fertility issues (Yamani et al., 2016). Natural compounds like terpenoid, alkaloids, glycosides, tannins, flavonoids, etc and essential oils apart from carbohydrates, proteins, lipids are present in Tulsi. Herbal medicine imparts an integral role in the treatment and management of diseases (Dixit et al., 2021). Eswar et al., 2016 found that at a 6% ethanolic extract of Tulsi (*Ocimum sanctum*) was efficient in preventing the growth of periodontal pathogen in human dental plaque: *Actinobacillus actinomycetemcomitans*. Yamani et al. (2016) reported that the essential oil obtained from Tulsi (*Ocimum tenuiflorum*) was found to be effective at a concentration of 4.5 and 2.25% completely inhibited the growth of *Staphylococcus aureus* (including methicillin resistant *S. aureus*) and *Escherichia coli*, while the same concentrations only partly inhibited the growth of *Pseudomonas aeruginosa*.

Based on this background present study aimed to isolate antibiotic resistant bacteria from the local poultry meat, biochemical characterization of the isolated bacterial pathogens and determined the efficiency of methanolic extract of Tulsi (*Ocimum sanctum*) against these pathogens.

Chapter 2: Literature Review

2. Literature Review

Ceccarelli et al., 2020 reported that the overuse of antimicrobials in the livestock sector for over 60 years has resulted in the dissemination and co-selection of antimicrobial-resistant bacteria in food-producing animals.

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Subramanian et al., 2014 documented the details of taxonomic status of the Tulsi (*Ocimum sanctum*) and different species of Tulsi grow in different parts of the world.

Londhe et al., 2015 documented the pharmaceutical potential of Tulsi in the treatment of a number of diseases like mental illness, cough and fever, gut diseases, bone and joint problems, eye diseases and other optic problems, ringworm, insect bite, snake bite and scorpion bite and malaria.

Verma, 2016 documented the medicinal and insecticidal properties of Tulsi. Tulsi has antimicrobial activities against many pathogens and can be used as mouth wash agent, for wound healing, and preservation of food stuff. Tulsi is antibacterial, antiviral, antifungal, antiprotozoal, antimalarial, and can be used also for killing mosquitoes.

Yamani et al., 2016 pharmaceutical properties of Tulsi. It has anti-oxidantive, anti-cataract agent, anti-inflammatory agent, as well as protects from chemicals and radiations, good for the liver and nerves and heart, anticancerous agent, protects the immune system, central nervous system and memory its antiasthma and thyroid, and solves fertility issues.

Dixit et al., 2021 reported the chemical ingredients responsible for medicinal properties of Tulsi. Natural compounds like terpenoid, alkaloids, glycosides, tannins, flavonoids, etc and essential oils apart from carbohydrates, proteins, lipids are present in Tulsi. Herbal medicine imparts an integral role in the treatment and management of diseases.

Eswar et al., 2016 found that at a 6% ethanolic extract of Tulsi (*Ocimum sanctum*) was efficient in preventing the growth of periodontal pathogen in human dental plaque: *Actinobacillus actinomycetemcomitans*.

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Chapter 3: Aim and Objectives

3. Aim and Objectives:

Aim: Isolation of antibiotic resistant bacteria from the poultry meat and determined the antimicrobial efficiency of methanolic extract of Tulsi against these antibiotic resistant bacteria.

Objectives:

I. Isolation of antibiotic resistant bacteria from the poultry meat.

II. Biochemical characterization of the isolated bacterial pathogen.

III. Determine the methanolic extract of Tulsi against the isolated bacterial pathogens.

Chapter 4: Materials and Methods

4. Materials and Methods:

4.1. Materials: Nutrient broth, Agar, Antibiotic disc, Muller-Hinton agar, Hydrogen peroxide, Tryptone, Simmons citrate agar, Methyl red, Barrit's reagent A, Barrit's reagent B, Crystal violet, Gram's iodine, Sodium nitrite, Aluminum chloride, Sodium carbonate, Safranin, Tetra-methyl-p-phenylenediamine dihydrochloride, Malachite green, Gelatin, Soluble starch, Gelatin, Triple-sugar iron agar, Kovac's reagent, Urea, Blood agar, Folin-ciocalteu reagent, Casein digest, Phenolphthalein (Himedia, India); and Ethanol, Methanol (Merck, India).

4.2. Collection of poultry meat samples and isolation of bacteria: From different poultry-slaughterhouses in the Midnapore town, West Bengal, India, 250 g of poultry meat were collected and aseptically transfer 1 g of poultry meat in sterilized nutrient broth media and kept for 24 h incubation at 37°C. After incubation, 100 µl of cultured broth spread on the nutrient agar plate and kept for 24 h incubation at 37°C. After analysis of the colony morphology, each colony was pure cultured in nutrient broth medium and kept for 24 h incubation at 37°C (Yamani et al., 2016). After incubation all the pure cultures were kept in refrigerator for future studies.

4.3. Isolation of antibiotic resistant bacteria from poultry-meat

In order to isolate antibiotic resistant bacteria, serially diluted intestinal excreta of slaughterhouse animal were plated on the nutrient agar and kept for 24 hours at 37°C. After incubation, the pure colonies were inoculated into nutrient broth and again incubated at 37°C for 18 to 24 h to reach the final cell density of approximately 10^5 to 10^6 cfu/ml. Antibiotic resistance of the isolates were determined by Kirby-Bauer disk diffusion susceptibility test (Hudzicki, 2009). Muller-Hinton agar plates were prepared and a sterile cotton swab was dipped into the standardized suspension. The culture was spread evenly over the entire surface of the Muller-Hinton agar plates and the plates were allowed to dry before applying antimicrobial discs. The following standard drug discs were used: Norfloxacin (NOR) disk of 10 µg; Ciproflaxicillin (CIP) disk of 5 µg; Doxycycline (DC) of 30 µg, Amoxicillin (AMX) disk of 10 µg, Erythromycin (ERYC) disk of 15 µg, Ampicillin (AMP) disk of 10 µg, Ceftriaxone (CRO) disk of 30 µg, Chloramphenicol (CHL) disk of 30 µg, Penicillin (PEN) of 10 µg, Novobiocin (NB) disk of 30 µg, Cloxacillin (CLOXA) disc of 1 µg, and Gentamicin (GEN) disk of 10 µg, which were commonly used and clinically important antibiotics in

Indian healthcare facilities (NCDC: India, 2016). After incubation at 37°C for 18 to 24 h, inhibition zones were measured and scored as susceptible, intermediate, or resistant based on the guidelines developed from the Clinical and Laboratory Standards Institute of US (CLSI, 2023). The *E. coli* ATCC 25922, and *Shigella flexneri* ATCC 12022 were used as reference strains for antibiotic disk control.

4.4. Biochemical characterization of antibiotic resistant bacterial pathogens

Biochemical tests like IMViC test, Catalase test, Oxidase test, Triple sugar iron agar test, Hemolysis test, Protease activity, Starch utilization test, Coagulase test, Urease test, and staining like Gram staining, and Endospore staining were executed according to the standard protocol (Cappuccino and Sherman, 2019; Mahon and Lehman, 2022).

4.5. Preparation of methanolic extract of Tulsi leaves

25 g leaves paste of Tulsi (sterilized by 1% sodium hypochlorite) was added in sterile conical flask with 50 mL of methanol, and kept for 24 h in shaker at 120 rpm. After 24 h, the flask content was filtered using Whattman no.1 filter paper and then concentrated by evaporation (Yamani et al., 2016).

4.6. Estimation of phytochemicals content of methanolic extract of Tulsi leaves:

4.6.1. Estimation of total flavonoid content

The total flavonoid content of each methanolic plant extract was determined according to the Hor et al. (2019) with slight modifications. Based on this method, each methanolic plant extract (1.0 ml) was mixed with 4 ml of distilled water and subsequently with 0.30 ml of $NaNO_2$ solution (10%). After 5 min, 0.30 ml $AlCl_3$ solution (10%) was added followed by addition 2.0 ml of NaOH solution (1%) to the mixture. Immediately the mixture was thoroughly mixed and absorbance was measured at 510 nm versus blank. Standard curve of quercetin was prepared by using quercetin (1-12 mg/mL).

4.6.2. Estimation of total phenolic content

The total phenolic content of each methanolic plant extract was performed with Folin-Ciocaltaeu assay (Hor et al., 2019). 1 mL of each methanolic plant extract was mixed with 1 mL of Folin Ciocalteu's phenol reagent. After 5 minutes, 10 mL of 7% sodium carbonate solution was added to the mixture followed by the addition of 13 mL of deionized distilled

water and mixed thoroughly. The mixture was kept in the dark for 90 minutes at 23°C, after which the absorbance was read at 760 nm. The total phenolic content was determined from extrapolation of calibration curve which was made by preparing gallic acid solution.

4.7. Antibacterial activity of the methanolic extract of Tulsi

The antibacterial activities of methanolic extract of Tulsi examined against the selected antibiotic resistant isolates. To evaluate the antibacterial properties, different concentrations of Tulsi (10 to 100 mg/ml) were added separately to 10 ml of Mueller–Hinton broth medium pre-inoculated with respective bacteria (final inoculum concentration of 10^5 cfu/ml) and incubated at 37°C under shaking condition for 24 h. The inhibitory effect was accessed by measuring the turbidity of the medium at 620 nm. MIC value was evaluated on the basis of dose dependent inhibitory effect (Rakshit et al., 2020).

4.8. Statistical analysis

The experimental data was represented as mean ± standard error of mean of three replicates. For graphical representation and analysis Microsoft Excel 2016 was used.

Chapter 5: Results and Discussion

5. Results and Discussion:

5.1. Isolation of bacteria from poultry meat and determined their antimicrobial resistance

Initially, total 71 bacteria were isolated from the poultry meat, that were later used to perform antibiotic sensitivity test. By performing antibiotic sensitivity test, it was observed that seven isolates were antibiotic resistant (Table 1).

Table 1: The antibiotic resistant bacteria isolated from the local poultry meat.

Isolates	Antibiotic resistance profile
Isolate 1	AMX, PEN, ERYL, GEN, CRO
Isolate 2	AMX, PEN, CLOXA, CHL
Isolate 3	PEN, CLOXA, AMX, ERYL, CHL, NB
Isolate 4	PEN, CIP, NB, CHL, GEN, CLOXA, ERYL
Isolate 5	DC, AMX, PEN, NOR, CLOXA
Isolate 6	PEN, CLOXA, AMX, CRO, DC, PEN
Isolate 7	ERYL, GEN, CHL, CRO, CHL, CLOXA

5.2. Biochemical characterization of antibiotic resistant bacterial pathogens

Table 2: Biochemical and staining properties of antibiotic resistant bacterial pathogens.

Biochemical characters		Isolate 1	Isolate 2	Isolate 3	Isolate 4	Isolate 5	Isolate 6	Isolate 7
IMVIC test	Indole	+	_	_	+	_	+	_
	Methyl red	+	+	-	-	-	+	-
	Voges proskauer	_	-	+	-	-	-	-
	Citrate	+	+	+	_	+	-	+
Hemolysis	α-hemolysis	+						
	β-hemolysis				+			+
	γ-hemolysis		+	+		+	+	
Oxidase		+	+	+	+	+	+	+
Catalase		+	_	+	+	+	+	_
Gram-staining		_	_	_	+	_	_	_
Endospore staining		_	_	_	_	_	_	_
Triple sugar iron agar		yellow, black	yellow	yellow	yellow, red	red, black	yellow	Yellow, red
Coagulase		_	_	_	+	_	_	+
Protease		-	-	+	-	+	+	-
Urease		-	+	+	_	+	-	-

5.3. Phytochemicals content of methanolic extract of Tulsi

The pharmacological property of any medicinal plants is dependent on their various phytochemicals content (Yu et al., 2021). The total polyphenols content of the Tulsi was 218.28±5.9 mg GAE/g while the flavonoid content 61.32±1.9 mg QE/g. Our result was consistent with the Chaudhary et al. (2020). Polyphenols content of a plant absorb, quench and neutralize free radicals, act as reducing agents, metal chelators and can efficiently protect biological systems from degeneration under high oxidative stress (Sharifi-Rad et al. 2020). Flavonoids, a class of polyphenolics with free radical scavenging properties are known to inhibit hydrolytic and oxidative enzymes, reduce blood glucose and lipids, exhibit anti-inflammatory effect and enhance immunity in human beings (Al-Ishaq et al., 2019).

5.4. Antibacterial activity of the methanolic extract of Tulsi

In this study it was observed that the methanolic extract of Tulsi showed antibacterial activity against all the multidrug resistant isolates. The MIC values of 30, 20, 40, 70, 30, 40, and 50 mg/ml were found from isolates 1 to 7, respectively. The leaves of *Ocimum* are the rich source of various compounds including β-caryophyllene, derivatives of eugenol, vanillin, rosmarinic acid, ursolic acid, gallic acid and vanillic acid (Chaudhary et al., 2020). Polyphenols and flavonoids are the major essential components present in the various form of tulsi extract and responsible for different pharmacological activities such as anticancer, antioxidant, antimicrobial, anti-inflammation, etc. Further, the antimicrobial activity is amplified by the presence of unsaturated fatty acids such as linolic acid and linoleic acid which manipulate the membrane integrity of microbial. The molecular mechanism for such activity is mediated through destabilization of the microbial membrane by interfering with the electron transport chain and oxidative phosphorylation process (Pradhan et al., 2022).

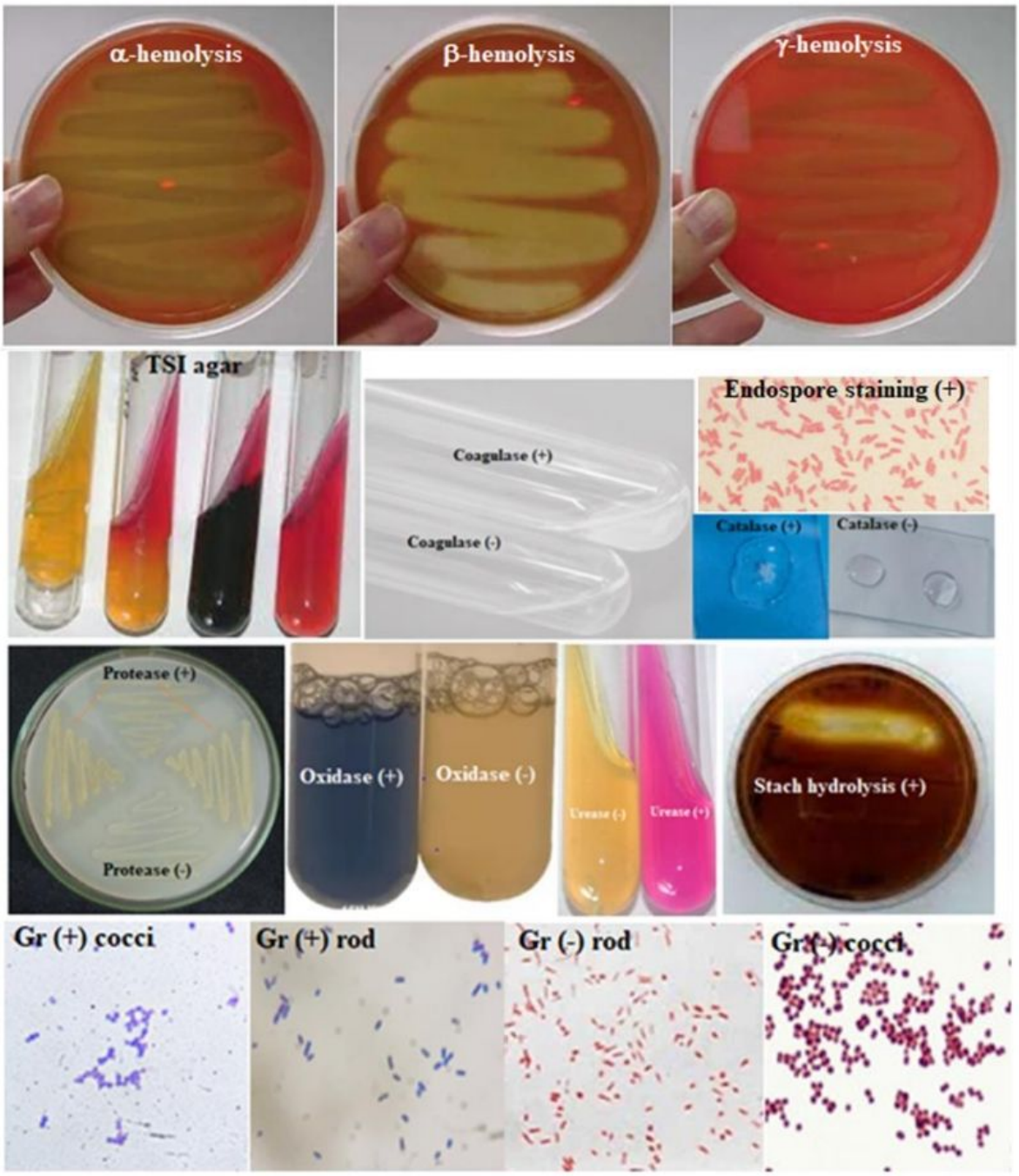

Fig. 1- Biochemical and staining properties of isolated antibiotic resistant bacteria.

Chapter 6: Conclusion

6. Conclusion

Gradual antibiotic resistance by various pathogenic bacteria are one of the prime challenges of the pharmaceutical industries. The present study approved the antimicrobial potentiality of Tulsi against the isolated antibiotic resistant bacterial pathogens. Among the isolated multidrug resistant bacteria, it was also observed that majority of these are Gram negative and aerobic in nature. Our results offer a potential alternative strategy for the control of antibiotic resistant bacterial pathogens.

Chapter 7: Future Scope

7. Future Scope

Generation of antibiotic resistant bacteria is one the major health concern of the current human society. Moreover, application of natural product as a drug of choice against the such pathogens is beneficial to the human health, since chemicals have various types of side effects. Our work influences the identification and isolation of active ingredients from Tulsi and choosing other ancient medicinal plants effective against the different forms of antibiotic resistant pathogens.

Chapter 8: References

8. References

Al-Ishaq, R. K., Abotaleb, M., Kubatka, P., Kajo, K., & Büsselberg, D. (2019). Flavonoids and their anti-diabetic effects: Cellular mechanisms and effects to improve blood sugar levels. Biomolecules, 9(9), 430.

Avraam, C., Lambrou, A. S., Jiang, W., & Siddiqui, S. (2021). Antimicrobial resistance and livestock trade for low- and middle-income countries: Regional analysis of global coordination policies. Frontiers in Sustainable Food Systems, 5, 650315.

Bae, D., Macoy, D. M., Ahmad, W., Peseth, S., Kim, B., Chon, J. W., & Kim, M. G. (2022). Distribution and characterization of antimicrobial resistant pathogens in a pig farm, slaughterhouse, meat processing plant, and in retail stores. Microorganisms, 10(11), 2252.

Caekebeke, N., Jonquiere, F. J., Ringenier, M., Tobias, T. J., Postma, M., Van den Hoogen, A., & Dewulf, J. (2020). Comparing farm biosecurity and antimicrobial use in high-antimicrobial-consuming broiler and pig farms in the Belgian–Dutch border region. Frontiers in veterinary science, 7, 558455.

Cappuccino, J. G., & Sherman, N. (2019). Microbiology, Laboratory manual. 12 th edition, Person education. INC, New Delhi, 282-283.

Ceccarelli, D., Hesp, A., Van Der Goot, J., Joosten, P., Sarrazin, S., Wagenaar, J. A., & EFFORT consortium, O. B. O. T. (2020). Antimicrobial resistance prevalence in commensal Escherichia coli from broilers, fattening turkeys, fattening pigs and veal calves in European countries and association with antimicrobial usage at country level. Journal of Medical Microbiology, 69(4), 537-547.

Chaudhary, A., Sharma, S., Mittal, A., Gupta, S., & Dua, A. (2020). Phytochemical and antioxidant profiling of *Ocimum sanctum*. Journal of Food Science and technology, 57(10), 3852-3863.

Clinical and Laboratory Standards Institute (CLSI):US; 2023. Performance Standards for Antimicrobial Susceptibility Testing, 33rd ed. ISBN Number: 978-1-68440-171-0.

Davis, G. S., Waits, K., Nordstrom, L., Grande, H., Weaver, B., Papp, K., & Price, L. B. (2018). Antibiotic-resistant Escherichia coli from retail poultry meat with different antibiotic use claims. BMC microbiology, 18, 1-7.

Dewulf, J.; Sternberg-Lewerin, S.; & Ryan, M. (2020). Tackling antimicrobial resistance in the food and livestock sector. In Challenges to Tackling Antimicrobial Resistance; Anderson, M., Cecchini, M., Mossialos, E., Eds.; Cambridge University Press: Cambridge, UK. pp. 99–123.

Dixit, A., Gulati, B., Sharma, G., Bhatia, G., Priya, R., & Bhattacharya, S. (2021). Evaluation of phytochemical and antimicrobial activity of *Ocimum* spp. Integr Food Nutr Metab. 2021; 8: 1-4. doi: 10.15761/IFNM, 1000299.

Eswar, P., Devaraj, C. G., & Agarwal, P. (2016). Anti-microbial activity of Tulsi {*Ocimum sanctum* (Linn.)} extract on a periodontal pathogen in human dental plaque: an *in vitro* study. Journal of clinical and diagnostic research: JCDR, 10(3), ZC53.

Hor, P. K., Ray, M., Pal, S., Ghosh, K., Soren, J. P., Maiti, S., & Mondal, K. C. (2019). Some functional properties of khambir, an ethnic fermented cereal-based food of Western Himalayas. Frontiers in Microbiology, 10, 730.

Hudzicki, J. (2009). Kirby-Bauer disk diffusion susceptibility test protocol. American society for microbiology, 15, 55-63.

Kim, S., Kim, H., Kim, Y., Kim, M., Kwak, H., & Ryu, S. (2020). Antimicrobial resistance of Escherichia coli from retail poultry meats in Korea. Journal of Food Protection, 83(10), 1673-1678.

Kumar, V., Chakraborty, A., Kaur, M., Pandey, S., & Jena, M. K. (2018). Comparative study on antimicrobial activity of tulsi (*Ocimum sanctum*) and neem (*Azadirachta indica*) methanol extract. Asian Journal of Pharmaceutical and Clinical Research, 514-517.

Londhe, A. M., Kulkarni, A. S., & Lawand, R. V. (2015). In-vitro comparative study of antibacterial and antifungal activities: A case study of *Ocimum kilimandscharicum*, *Ocimum tenuiflorum* and *Ocimum gratissimum*. International Journal of Pharmacognosy and Phytochemical Research, 7(1), 104-110.

Mahon, C. R., & Lehman, D. C. (2022). Textbook of diagnostic microbiology-e-book. Elsevier Health Sciences.

National Centre for Disease Control (NCDC): India, 2016. National Treatment Guidelines for Antimicrobial Use in Infectious Diseases.

Pollock, J., Muwonge, A., Hutchings, M. R., Mainda, G., Bronsvoort, B. M., Gally, D. L., & Corbishley, A. (2020). Resistance to change: AMR gene dynamics on a commercial pig farm with high antimicrobial usage. Scientific reports, 10(1), 1708.

Pradhan, D., Biswasroy, P., Haldar, J., Cheruvanachari, P., Dubey, D., Rai, V. K., & Ghosh, G. (2022). A comprehensive review on phytochemistry, molecular pharmacology, clinical and translational outfit of *Ocimum sanctum* L. South African Journal of Botany, 150, 342-360.

Rakshit, S., Mondal, S., Pal, K., Jana, A., Soren, J. P., Barman, P., & Halder, S. K. (2020). Extraction of chitin from *Litopenaeus vannamei* shell and its subsequent characterization: an approach of waste valorization through microbial bioprocessing. Bioprocess and biosystems engineering, 44(9), 1943-1956.

Saud, B., Paudel, G., Khichaju, S., Bajracharya, D., Dhungana, G., Awasthi, M. S., & Shrestha, V. (2019). Multidrug-resistant bacteria from raw meat of buffalo and chicken, Nepal. Veterinary medicine international, 2019.

Schwaiger, K., Huther, S., Hölzel, C., Kämpf, P., & Bauer, J. (2012). Prevalence of antibiotic-resistant enterobacteriaceae isolated from chicken and pork meat purchased at the slaughterhouse and at retail in Bavaria, Germany. International journal of food microbiology, 154(3), 206-211.

Sharifi-Rad, M., Anil Kumar, N. V., Zucca, P., Varoni, E. M., Dini, L., Panzarini, E., & Sharifi-Rad, J. (2020). Lifestyle, oxidative stress, and antioxidants: Back and forth in the pathophysiology of chronic diseases. Frontiers in physiology, 11, 694.

Subramanian, G., Tewari, B. B., & Gomathinayagam, R. (2014). Studies of antimicrobial properties of different leaf extract of tulsi (*Ocimum tenuiflorum*) against human pathogens. Am Int J Contemp Res, 4(8), 149-57.

Tasneem, S., Mangi, S., Qazi, M. A., & Kanhar, N. A. (2015). Antibacterial effect of different solvent extracts of *Ocimum sanctum* (Tulsi plant) leaves against multidrug resistant pathogens.

Van Boeckel, T. P., Pires, J., Silvester, R., Zhao, C., Song, J., Criscuolo, N. G., & Laxminarayan, R. (2019). Global trends in antimicrobial resistance in animals in low-and middle-income countries. Science, 365(6459), eaaw1944.

Vanderhaeghen, W., & Dewulf, J. (2017). Antimicrobial use and resistance in animals and human beings. The Lancet Planetary Health, 1(8), e307-e308.

Verma, S. (2016). Chemical constituents and pharmacological action of *Ocimum sanctum* (Indian holy basil-Tulsi). The Journal of Phytopharmacology, 5(5), 205-207.

Yamani, H. A., Pang, E. C., Mantri, N., & Deighton, M. A. (2016). Antimicrobial activity of Tulsi (*Ocimum tenuiflorum*) essential oil and their major constituents against three species of bacteria. Frontiers in microbiology, 7, 681.

Yu, M., Gouvinhas, I., Rocha, J., & Barros, A. I. (2021). Phytochemical and antioxidant analysis of medicinal and food plants towards bioactive food and pharmaceutical resources. Scientific reports, 11(1), 10041.

Yusuf, M. A., Hamid, T. A., & Hussain, I. (2012). Isolation and identification of bacteria associated with balangu (roasted meat product) sold in Bauchi. Nigeria. Journal of Pharmacy, 2(6), 38-48.

Zwirzitz, B., Wetzels, S. U., Dixon, E. D., Stessl, B., Zaiser, A., Rabanser, I., & Selberherr, E. (2020). The sources and transmission routes of microbial populations throughout a meat processing facility. npj Biofilms and Microbiomes, 6(1), 26.