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Futuristic Biotechnology (FBT) is a bi-annual journal that publishes broad-spectrum publications with close connection to experimental activity in Biological and Biotechnology fields. FBT is intended for exploring the molecular mechanisms that support key biological processes in the fields of biochemistry, cellular biosciences, molecular biology, plant biotechnology, genetic engineering, nanotechnology, and bioinformatics. Furthermore, it also covers topics related to immunology, antibody production, protein purification studies, primer synthesis, DNA sequencing, production of transgenic animal models, insect resistant crop varieties and edible and ornamental plant varieties.

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ACKNOWLEDGEMENT

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Inaugural Message from Editor-in-Chief

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There could not possibly be a better or more appropriate time to introduce the journal Futuristic Biotechnology (FBT) given the extraordinary advancements made over the past few decades in science and medicine. We have had the good fortune to watch as numerous scientific and medical advancements have been combined to push the boundaries of disease detection and treatment that can affect any part of the body. As a result, numerous cutting-edge therapeutic approaches are currently being developed to address diseases for which there are few effective treatments.

Although at first glance these therapeutic approaches seem disparate, closer examination reveals that they all rely on a "shared signalling platform" that is closely linked through biological pathways and has a major impact on patient clinical outcomes. It is evident that cellular and genetic targets have a straightforward effect on numerous disease entities. For many disorders, the conversion of these pathways into useful clinical treatments can be fruitful. FBT was designed with this notion in mind. FBT is an open access, peer-reviewed international journal that publishes broad-spectrum researches with close connection to experimental activity related to biology and biotechnology. FBT is intended for exploring the molecular mechanisms that support key biological processes in biochemistry, cellular biosciences, molecular biology, plant biotechnology, genetic engineering, nanotechnology, regenerative medicine and bioinformatics. Furthermore, it also covers topics related to immunology, antibody production, protein purification studies, primer synthesis, DNA sequencing, production of transgenic animal models, insect resistant crop varieties and edible and ornamental plant varieties.

FBT will give the scientific and medical communities an international venue to translate basic and clinical research investigations into clinical therapeutics, as well as to report on prognostics, new therapeutic approaches, and the development of biomarkers. As original research papers, review articles, clinical studies, expert opinion, systematic reviews, meta-analysis, commentaries, and letters to the editor, FBT will provide a variety of platforms for authors to share their ideas and discoveries.

In the end, FBT will encourage a thorough, impartial, and helpful appraisal of every paper submitted. All authors will be able to track the development of their papers throughout the phases of evaluation, copyediting, and publication because articles will be processed in a very timely manner. With the debut of FBT, we are incredibly enthusiastic and certain that the journal will significantly fill a gap in the reporting of recent scientific and medical advancements. FBT will act as a global platform for the highest level of scholarship in the scientific and clinical sectors to convert ground-breaking "bench to bedside" innovations into effective treatment procedures. On behalf of the Editorial Team and the Editorial Board, welcome to *FBT*!

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Review Article

In-vitro Propagation of Pharmacologically Important Medicinally Plants Producing Bioactive Compounds

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ABSTRACT

In-vitro plant propagation is one of the promising strategies for sustainable production of medicinal plants. Plant tissue culture involves *in-vitro* propagation of plant parts under controlled conditions. It is a very useful technique for fundamental and applied research and has many commercial uses. Medicinal plants have been used to treat many diseases since decades. They are enriched with pharmacologically important bio-active compounds. These bio-active compounds can be used to treat different human ailments. Because of over-exploitation of these medicinal plants, there is a need to sustain their production. The most commonly used medicinal plants are Aconite, Alfa Alfa , Aloe Vera, Borage, Comfrey, Ephedra, Germander, Ginkgo biloba, Neem, Licorice, Isapghul, Sassafras, Senna, St. John's wort ,Neem ,Tulsi. The current review gives insights into important compounds produced by popular medicinal plants. Some of the important bioactive compounds are taxol, methane, acetic acid, carotenoids, flavonoids, carnitine, choline, dithiolthiones, phytosterols, phytoestrogens, glucosinolates, polyphenols, and taurine. The review also focuses on *in-vitro* propagation of medicinally important plants to ensure their availability irrespective of seasonal and geographical constraints.

INTRODUCTION

Medicinal plants are important in evolution of human civilization. Humans have relied on nature for their fundamental necessities throughout history, including medicines, shelters, food, fragrances, clothing, flavours, fertiliser, and ways of transportation. Medicinal plants continue to play an important role in the healthcare system for large swaths of the worldwide population, particularly in areas where herbal medicine has a long history of use. The discovery and acceptance of these plants' medicinal and economic advantages is growing in both developed and emerging countries [1]. There are around half a million plants on the planet, the most of which have yet to be researched for their therapeutic properties. Medicinal plants have a bright future ahead of them, and their untapped medical potential might be essential in the treatment of current and future studies [2]. Medicinal herbs have shown to be the only effective treatment for a variety of fatal diseases, including cancer and diseases caused by viral infection, such as hepatitis and AIDS [3]. These Plants are no longer just important in health care, but they are also finest source of safe future medication. Despite the fact that we now have a lot of contemporary medications at our disposal, it is quite critical to find and cultivate novel helpful agents [4]. It is believed that just one-third of all known human illnesses have an appropriate treatment. As a result, the war against illnesses must continue unabated. Because of the modest side effects and synergistic action of the combination of components, traditional plant medicines continue to have a major role in the modern pharmaceutical industry [5]. Medicinal herbs have played an important part in the formation of human civilization, including religions and other rites. Many

contemporary medications, such as aspirin, are derived indirectly from medicinal plants. Garlic, for example, is a food crop with therapeutic [6]. The study of therapeutic plants aids in the understanding of plant toxicity and the protection of humans and animals from natural toxins. Plants produce secondary metabolites, which are responsible for their therapeutic benefits [7]. These plants play significant role in a variety of sectors, including sufficient chemicals, cosmetics, medicines plus medications. In the search for bioactive chemicals, traditional healers frequently employ modern research including bioassay-guided fractionation of medicinal plants. As a result, numerous novels medically significant molecules have been isolated [8]. Due to the committed efforts of researchers, a great number of powerful medicines, healing indications, and many new pharmacologically dynamic components discovered from herbal medicines. In 1826, E. Merck of Germany began mass- producing morphine on an industrial scale, marking the beginning of the commercialization of plant-derived medicines [9].

Importance of Medicinal Plants

Plants are necessary for life to exist. Medicine is built on the foundation of plants. Traditional medicinal plants are the source of a number of significant medicines that are still in use today. Medicinal plants plays an important role in the creation novel drugs [10]. Phytoconstituents research has recently provided effective therapies for diseases that the synthetic drug industry has been unable to address [11]. In the search for novel medicines, ethnobotany and ethnopharmacology have emerged as major sources of information, leading to various sources and classes of chemicals [12]. Pharmaco chemistry is a more sophisticated component of the pharmaceutical sciences, and research on structure-activity correlations and their influence on the creation of new medicines have made it one of the most important and therefore noteworthy accomplishments [13]. If there were no plants on the world, it is difficult to imagine how the human race would survive. Plants have been essential to humans from the beginning of humanity. Medicinal plants are a popular source of medicine. In ancient medicinal systems like Ayurvedic, Unani, and Chinese traditional medicine, herbs have been used to cure ailments as well as to restore and strengthen body systems. A beneficial interaction with the body's chemistry was always the aim when employing plants [14].

Safety and Toxicity of Medicinal Plants

Medicinal plant types that are widely utilized for the cure of definite illnesses have been found. Many medicines are derived from biologically active plant compounds, and the active chemicals present in them are responsible for their therapeutic properties [15]. The primary change among utilizing medical plant or chemical medication is most traditionally qualified doctors lack proper plant teaching [16]. In practice, however, three categories of herbs may be distinguished from a safety standpoint. There are a few plants in the first category that have near pharmaceutical quantities of toxic components and should not be used internally by untrained people unless in homoeopathic potencies. *Atropa belladonna, Arnica* sp., *Aconitum* spp, and *Digitalis* spp are among examples. Herbs in the second group have very strong effects, frequently producing nausea or vomiting. They are totally safe when used under the right circumstances. *Lobelia* and *Eonymus* spp. are two examples. Different countries have their own set of paradoxes. Ephedra, for example, is prohibited in the United Kingdom, maybe with good reason, yet is readily available in the United States. There is an idiosyncratic grouping of plants that display certain forms of toxicity in the third category, which has some scientific validity [17].

Commonly Used Medicinal Plants

Aconite, Alfa Alfa, Aloe Vera, Borage, Calamus, Chaparrel, Coltsfoot, Comfrey, Ephedra, Germander , Ginkgo biloba, Ginseng, Licorice, Isapghul, Sassafras, Senna, Silybum marianum, St. John's wort ,Neem, Tulsi etc.

s. no.	Common Name	Source and Family	Parts Used
1	Aleppo Oak	*Quercus infectoria Fagaceae*	*Galls*
2	Aloe/kumara	*Aloe vera. Aloe barbadensis Liliaceae*	*Aq. Extract & juice of leaves*
3	Bael	*Aegle marmelos (Rutaceae)*	*Methanolic extract of root.*
4	Ginkgo(GB)	*Ginkgo biloba Ginkgoaceae*	*Ethanolic extract of stem*
5	Liquorice	*Glycyrrhiza glabra Leguminosae*	*Vacuumdried Ethanolic extract of bark & root*
6	Myrobalan harda	*Terminala chebula Combretoreceae*	*Alcoholic extract of leaves & fruit*
7	Neem	*Azardica indica Meliaceae*	*Methanol extract of leaves*
8	St. John wort	*Hypericum mosorence Hypericaceae*	*Methanolic extract of leaves.*
9	Tulsi	*Ocimumtenuiflorum, Ocimumsanctum Labiateae*	*Ethanolic extract of whole part*
10	Turmeric	*Curcuma longa Zingiberaceae*	*Rhizomes*

Table 1: Medicinal plants with wound healing activity

Secondary Metabolites of Medicinal Plants

The vast array of secondary metabolites (SMs) that plants produce as living chemical factories serve as the foundation for many commercial pharmaceutical drugs and herbal remedies made from medicinal plants. In addition to having significant value in the food and pharmaceutical sectors, many of the chemical components of medicinal plants also contain biological activity that may increase human strength [18]. They also have significant value in the perfume, agrochemical, and cosmetic industries. Alkaloids, terpenoids, and

phenylpropanoids are only a few of the SMs whose potential as potential therapeutics are being investigated. There are a few fundamental points to mention regarding new technologies that are being used and that are required to further medicinal plant research [19]. To start, the word "metabolome" now refers to ALL of a cell's tiny molecules. A few more broad remarks on the new technologies that are being employed and that are required to further the study of medicinal plants are in order before we delve into how the plant retains its secondary metabolites. Starting with the definition, the word "metabolome" now refers to ALL of a cell's tiny molecules, and it is the fourth element of a "systems" approach to biology, after genomics (DNA), transcriptomics (RNA), and proteomics [20]. Given the complexity of these extracts and the fact that most assays can only handle a few dozen fractions at a time, it is obvious that our current abilities to link a particular chemical structure to a particular clinical effect are severely constrained if more than one compound is required to elicit an effect [21]. The best opportunity for such a study may be provided by recent developments in high-throughput model organisms with neurological systems resembling those of the brain (e.g., Drosophila). SMs have altered a plant's growth and ability to survive under stressful circumstances throughout time. In the plant domain, there are around 100,000 SMs that are exclusive to certain taxonomic collections. Based on their metabolic functions (isoprenoids), phenolic compounds (flavonoids and phenylpropanoids), nitrogen having chemicals and terpenes are the three primary families of SMs in plants [22]. Although SMs biosynthesis and growth research has advanced, it is still rare to find articles on the developmental and environmental variables that affect SM production and growth in medicinal plants. Based on their chemical structures, secondary plant metabolites are categorized into a variety of classes. Nature of secondary plant metabolites described in this chapter as a foundation for a study of the main groups of therapeutically important compounds. The following are examples: phenolic, alkaloids, saponins, terpenes, lipids and carbohydrates [23].

In-vitro Propagation of Medicinal Plants

Since beginning of time, medicinal plants have attracted man's interest [24]. Medicinal plant use has a long history in almost every society. with plant extracts accounting for approximately 85% of traditional medicine [25]. Combining *in-vitro* production and cryopreservation techniques might help preserve the richness of medicinal plants used in the area. Plants play an important part in discovery of novel healing agents, and medicines produced from greater plants have a long history in medicine . Many molecules utilized in modern medicine have a complicated structure, making chemically manufacturing these bioactive substances at a low cost difficult. Medicinal riches are quickly depleted as a result of deforestation [26].

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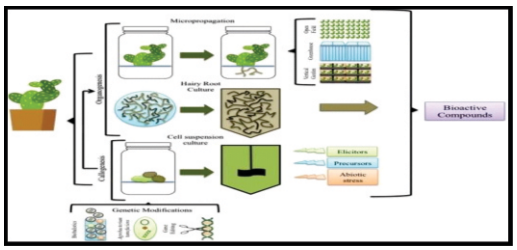

Figure 1: *In-vitro* plant tissue culture

Many important Chinese traditional medicinal herbs have shown to be beneficial when grown *in-vitro*. Each bioactive chemical has its unique set of properties. Due to its unique mode of action on the microtubular cell system, Taxol (plaxitaxol), a complex diterpene alkaloid discovered in the bark of the Taxus tree, is one of the most promising anticancer therapies [27].

Species	Mode of regeneration
Aconitum carmichaeli	Anther → Callus →SE →Plant STP, AB→ Multiple Shoot →Plant
Adenophora triphylla	SI→ Adventitious root → Multiple Shoot →Plant
Alpinia galangal	Rhizome bud → Multiple Shoot →Plant
Angelica acutiloba	Pedicel → Callus → Somatic embryos → Plant Pedicel → Callus → Cell clumps → Somatic embryos →Plant Flower Bud→ Callus→ Embryoid →Plant
Angelica sinensis (Oliv.) Diels	Immature embryos → Callus → Cell suspension → Cell clumps → Somatic embryos →Plant Immature embryos → Callus → Cell suspension → Somatic embryos →Plant
Aralia cordata	Inflorescence bud → Cell clumps → Somatic embryos →Plant
Artemisia annual	Leaf→ Callus,Shoot→Plant
Astragalus membranaceus	Shoot tip→ Multiple Shoot →Plant
Gentiana triflora	Leaf,S, Root→ Adventitious shoot →Plant
Gentiana triflora	Leaf→ Protoplast→ Shoot →Plant

Table 2: In-vitro studies in some of the important traditional Chinese medicinal plants and their related species

In-vitro Propagation of sea lavrnder (*Limonium wrightii)*

They are herbaceous perennial plants native to Japan's islands, as well as the southern Taiwanese islands of Lanyu and Lutao [28]. It produces naturally among the rocks, and a few farmers Lutao Island use seeds to cultivate it on a small scale [29]. Utilizing main and lateral branch tips, leaf

bases, and explants from inflorescence nodes, we created a standard procedure for the efficient in vitro cultivation of this medicinal plant. After two months of culture, the explants on Murashige and Skoog's media (MS basal medium)supplemented with 8.87 M N6 - benzyladenine(BA) and 1.07 M a-naphthaleneacetic acid generated adventitious shoots(NAA).

***In- vitro* Propagation of lady bell (*Adenophora triphylla*)**

Popular names for *Adenophora triphylla* include "sha shen" in Taiwan, China, and the Japanese Ryukyus and Bonin Islands. Triphylla's fleshy roots, which also contain saponins and insulin and have antifebrile pharmacological effect, have also been used as an expectorant to treat chronic bronchitis and whooping cough. It has an antimicrobial effect as well as stimulating cardiac contraction. It's also used as a general tonic to re-energize the body. Plant extracts induced apoptosis in human Jurkat T cells, which had tumoricidal effects[30].

***In-vitro* Propagation of Pinellia ternata (*Araceae*)**

It is a Japanese and Chinese medicinal plant that grows wild. *P. ternata* tubers contain homogentisic acid. The tubers are an essential component of "Sho- seiryuto," medication used to cure cold symptoms. Because seedling and bulbil collecting is difficult, the plants are not grown. Because seedling and bulbil collecting is difficult, the plants are not grown. Tubers obtained from naturally occurring plants in the highlands are insufficient. As a result, tissue culture experiments were conducted with the goal of widespread multiplication of this therapeutic plant [31].

CONCLUSIONS

Medicinal plants have long been harvested, eaten, and controlled by local norms and knowledge. They are inextricably linked to local livelihoods. Traditional treatments should be managed since they are empirical and knowledge-based, frequently culturally inherited, and vital to pharmacology and local livelihoods. Traditional treatments, on the other hand, are being undermined as a result of shifting lifestyles, views, societal changes, and acculturation. Plants are abundant sources of pharmaceutically significant chemicals; however, these molecules must be synthesised in the laboratory. Because many secondary plant metabolites cannot be synthesised chemically, micropropagation is an essential technique. Many plant species have yet to be identified, and their therapeutic qualities are unknown; even traditional medicinal treatments are being forgotten. To conserve nature's natural pharmaceuticals, more research and protection of all plant species, especially medicinal plants, is required. Plant tissue culture advances will allow for fast replication and long-term usage of therapeutic plants for future generations. Even if the plant parent material is overexploited, slow-growing, or low-yielding plant, *in-vitro* tissue culture remains a viable technique for the creation of structurally complex and high-value natural products. Nonetheless, due to difficult costs, an in vitro culture cost-benefit analysis is advised before implementation. Similarly, employing plant culture systems to produce medicines can have a number of advantages, including cost savings, speed, minimal human pathogen load, and scalability. All of these benefits are particular to herb products and reliant on manufacturing efficiency as compared to alternatives. Moreover *in-vitro* propagation is a promising strategy to obtain valuable medicinal plants irrespective geographical or seasonal constraints.

Conflicts of Interest

The authors declare no conflict of interest

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REFERENCES

[1] Ahvazi M, Khalighi-Sigaroodi F, Charkhchiyan MM, Mojab F, Mozaffarian VA, Zakeri H. Introduction of medicinal plants species with the most traditional usage in Alamut region. Iranian Journal of Pharmaceutical Research: IJPR. 2012 Aug; 11(1): 185-194.

[2] Thatoi H and Patra JK. Biotechnology and pharmacological evaluation of medicinal plants: An overview. Journal of Herbs, Spices & Medicinal Plants. 2011 Jul;17(3):214-48. doi: 10.1080/10496475.2011.602471

[3] Singh R. Medicinal plants: A review. Journal of Plant Sciences. 2015 May;3(1-1):50-5.

[4] Pal SK and Shukla Y. Herbal medicine: current status and the future. Asian Pacific Journal of Cancer Prevention. 2003 Aug; 4(4): 281-8.

[5] Larhsini M, Oumoulid L, Lazrek HB, Wataleb S, Bousaid M, Bekkouche K, *et al.* Antibacterial activity of some Moroccan medicinal plants. Phytotherapy Research. 2001 May; 15(3): 250–2. doi: 10.1002/ptr.815

[6] Ekanem AP and Udoh FV. The diversity of medicinal plants in Nigeria: An Overview. 2010 Jan. doi: 10.1021/bk-2009-1021.ch007

[7] Kumar V. Potential medicinal plants for CNS disorders: an overview. Phytotherapy Research: An International Journal Devoted to Pharmacological and Toxicological Evaluation of Natural Product Derivatives. 2006 Dec; 20(12): 1023-35. doi: 10.1002/ptr.1970

[8] Art Spomer L and Smith MA. Direct measurement of

water availability in gelled plant tissue culture media. In Vitro Cellular & Developmental Biology-Plant. 1996 Jul; 32(3): 210-5. doi

[9] De Silva T. medicinal plants in developing countries. Medicinal Plants for Forest Conservation and Health Care. 1997; 92: 34-158.

[10] Wanjari AS and Wanjari DS. An overview on herbal medicine. Research Journal of Pharmacognosy and Phytochemistry. 2019 Mar; 11(1): 14-7. doi: 10.5958/0975-4385.2019.00003.7

[11] Chan K. Some aspects of toxic contaminants in herbal medicines. Chemosphere. 2003 Sep; 52(9): 1361-71. doi: 10.1016/S0045-6535(03)00471-5

[12] Aslam MS and Ahmad MS. Worldwide importance of medicinal plants: Current and historical perspectives. Recent Advances in Biology and Medicine. 2016 Sep; 2(2016): 88-96. doi: 10.18639/RABM.2016.02.338811

[13] Farnsworth NR and Soejarto DD. Global importance of medicinal plants. The Conservation of Medicinal Plants. 1991 Jul; 26:25-51. doi: 10.1017/CBO9780511753312.005

[14] Schmelzer GH and Gurib-Fakim A. Medicinal Plants, vol. 11. Wageningen, The Netherlands: Plant Resources of Tropical Africa (PROTA). 2008.

[15] Bisi-Johnson MA, Obi CL, Hattori T, Oshima Y, Li S, Kambizi L, *et al*. Evaluation of the antibacterial and anticancer activities of some South African medicinal plants. BMC Complementary and Alternative Medicine. 2011 Dec; 11(1): 1-5. doi: 10.1186/1472-6882-11-14

[16] Kutschera U and Ray PM. Forever young: stem cell and plant regeneration one century after Haberlandt 1921. Protoplasma. 2022 Jan; 259: 1-6. doi: 10.1007/s00709-021-01683-5

[17] Petrovska BB. Historical review of medicinal plants' usage. Pharmacognosy Reviews. 2012 Jan; 6(11): 1-5. doi: 10.4103/0973-7847.95849

[18] Dörnenburg H and Knorr D. Strategies for the improvement of secondary metabolite production in plant cell cultures. Enzyme and Microbial Technology. 1995 Aug; 17(8) :674-84. doi: 10.1016/0141-0229(94)00108-4

[19] Wink M. Medicinal plants: a source of anti-parasitic secondary metabolites. Molecules. 2012 Oct; 17(11): 12771-91. doi: 10.3390/molecules171112771

[20] Hussein RA and El-Anssary AA. Plants secondary metabolites: the key drivers of the pharmacological actions of medicinal plants. Herb. Med. 2019 Jan; 1(3): 12-30. doi: 10.5772/intechopen.76139

[21] Takshak S and Agrawal SB. Defense potential of secondary metabolites in medicinal plants under UV-B stress. Journal of Photochemistry and Photobiology B: Biology. 2019 Apr; 193: 51-88. doi: 10.1016/j.jphotobiol.2019.02.002

[22] Verma N and Shukla S. Impact of various factors responsible for fluctuation in plant secondary metabolites. Journal of Applied Research on Medicinal and Aromatic Plants. 2015 Dec; 2(4): 105-13. doi: 10.1016/j.jarmap.2015.09.002

[23] Kharchoufa L, Bouhrim M, Bencheikh N, Addi M, Hano C, Mechchate H, *et al*. Potential toxicity of medicinal plants inventoried in northeastern Morocco: an ethnobotanical approach. Plants. 2021 May; 10(6): 1108. doi: 10.3390/plants10061108

[24] Constabel F. Medicinal plant biotechnology1. Planta Medica. 1990 Oct; 56(05): 421-5. doi: 10.1055/s-2006-961002

[25] Sidhu Y. In vitro micropropagation of medicinal plants by tissue culture. 2011 Jan ;4(1): 432-449.

[26] Zhou LG and Wu JY. Development and application of medicinal plant tissue cultures for production of drugs and herbal medicinals in China. Natural Product Reports. 2006 Sep; 23(5): 789-810. doi: 10.1039/b610767b

[27] Kothar SL and Varshney A. Morphogenesis in long-term maintained immature embryo-derived callus of wheat (Triticum aestivum L)-histological evidence for both somatic embryogenesis and organogenesis. Journal of Plant Biochemistry and Biotechnology. 1998 Feb; 7(2): 93-8. doi: 10.1007/BF03263042

[28] Scherwinski-Pereira JE, Costa FH, Camillo J, Silva DB, Alves RB, Vieira RF. Tissue culture storage of Brazilian medicinal plants germplasm. InIV International Symposium on Breeding Research on Medicinal and Aromatic Plants-ISBMAP2009 860 2009 Jun 17 (pp. 211-214). doi: 10.17660/ActaHortic.2010.860.31

[29] Sharma S and Thokchom R. A review on endangered medicinal plants of India and their conservation. Journal of Crop Weed. 2014 Sep; 10(2):205-18.

[30] Sahu J and Sahu RK. A review on low cost methods for in vitro micropropagation of plant through tissue culture technique. Pharmaceutical and Biosciences Journal. 2013 Nov; 1(1): 38-41. doi: 10.20510/ ukjpb/1/i1/91115

[31] Patel H and Krishnamurthy R. Elicitors in plant tissue culture. Journal of Pharmacognosy and Phytochemistry. 2013 Jul; 2(2):60-5.

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Original Article

Antibacterial Susceptibility Pattern of Gram Negative Bacteria Isolated from Patients with Urinary Tract Infection

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ABSTRACT

In clinical setting, urinary tract infections (UTIs) rank second among infectious presentations. Each year, around 150 million people receive a UTI diagnosis worldwide. **Objectives:** To determine the antibacterial sensitivity of meropenem, imipenem and ciprofloxacin against bacteria isolated from patients presented with Urinary Tract Infection. **Methods:** This Descriptive type Cross-sectional study was carried out at Clinical Microbiology Laboratory, Pathology Department of King Edward Medical University. A total of 100 patients were included that had Urinary Tract Infections. Urine culture was done using standardized Cysteine lactose electrolyte deficient (CLED) agar. Gram reaction, morphology, and biochemical properties were used to identify bacterial pathogens. Antibiotic sensitivity of the selected Gram negative bacteria was observed by using the Kirby Bauer method for antibiotic susceptibility. **Results:** The bacteria isolated were *E. coli* (62%), *Klebsiella* spp. (11%), *Candida* spp. (8%), *Pseudomonas aeruginosa* (7%), *Proteus* spp. (4%), *Staphylococcus* spp. (3%), *Staphylococcus saprophyticus* (3%) and *Enterobacter* spp. (2%). Sensitivity pattern for Ciprofloxacin was 20 (23.3%) sensitive and 66 (76.7%) resistant. Similarly 87.2% were sensitive to Meropenem and 12.8% were resistant. While for Imipenem 95.3% were sensitive and 4.7% were resistant. **Conclusions:** It was concluded that UTI most commonly caused by *E. coli* which is a gram negative bacteria followed by *Klebsiella*, *Pseudomonas*, *Proteus* and *Enterobacter*. Other microorganisms such as *Staphylococcus* spp., *Staphylococcus saprophyticus* and *Candida* spp. were also isolated in least amount. Antimicrobial susceptibility showed that Gram negative isolates of uropathogens were highly resistant against Ciprofloxacin and showed sensitivity against Imipenem and Meropenem.

INTRODUCTION

Infection of the urinary tract (UTI) is a general term that refers to both asymptomatic microbial colonization of the urine and symptomatic infection, in which microbial invasion and inflammation of the urinary tract organs, such as the kidneys, ureters, bladder, prostate, and urethra, occur [1]. The second most frequent infectious presenting in community practice is a urinary tract infection (UTI). The worldwide economy loses close to 6 billion US dollars each year due to the estimated 150 million UTI diagnoses [2]. Urinary Tract Infections were the cause of 100,000 hospitalizations and nearly 7 million office visits, according to the 1997 National Ambulatory Medical Care Survey and National Hospital Ambulatory Medical Care Survey. However, because urinary tract infections are not recognized as diseases in the United States, it is challenging to determine their frequency and prevalence [3]. According to estimates, urinary tract infections affect 18 people per 1000 each year, making it one of the most prevalent infections in the general population [4]. Urinary tract infections, which make up approximately 25% of all infections in non-institutionalized older people, are the second-most prevalent type of infection [5]. Nearly 10% of people may encounter a urinary tract infection at some point in their lives, making them one of the most prevalent infectious disorders [6]. Similar to most epithelia, the bladder wall is covered in a range of cationic antimicrobial peptides such defensins and cathelicidin that damage bacterial cell walls [7]. Uropathogenic *Escherichia coli*

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invades superficial umbrella cells during cystitis and multiplies quickly to create intracellular bacterial communities, which compromise innate defenses [8]. Gram-negative pathogens like *Escherichia coli* are more prevalent than other gram-negative pathogens including *Klebsiella pneumonia*, *Enterobacter* spp., *Proteus mirabilis*, *Pseudomonas aeruginosa*, gram-positive *Staphylococcus saprophyticus*, and *Candida albicans* in urinary tract infections [9]. *Escherichia coli* is the most common pathogen isolate which is responsible for urinary tract infection [10]. Acute cystitis may be accompanied by dysuria (painful urination), urgency, hesitation, polyuria, and incomplete voiding. Hematuria, suprapubic or low back discomfort, and urinary incontinence could also be present. Fever, pain in the costovertebral angle, nausea, and vomiting are signs of pyelonephritis. Reduced functional capacity, which is shown in dementia patients, cardiovascular accidents, neurological abnormalities, and faecal incontinence are risk factors [11]. Multidrug resistance of antimicrobial classes is common among the uropathogenic bacteria like *E. coli*, *Klebsiella pneumonia* and *Pseudomonas aeruginosa* [12]. This study aim to determine the antibacterial sensitivity of meropenem, imipenem and ciprofloxacin against bacteria isolated from patients with Urinary Tract Infection.

METHODS

This Descriptive type Cross-sectional study was carried out for 4 months duration at Clinical Microbiology Laboratory, Pathology Department of King Edward Medical University, Mayo Hospital, Lahore. A total of 100 patients were included that had Urinary Tract Infections. A brief history and biodata was taken from all patients. Clean Catch or early morning Mid stream urine samples were collected from all patients. Mid stream urine was collected in a sterilized wide mouthed container after necessary precautions. Only Mid stream urine samples received at Microbiology Lab Pathology dept. King Edward Medical University were included. Samples other than mid stream urine e.g. Catheter tips and supra pubic puncture sample and sample having mix growth of bacteria on culture plates were excluded. CLED agar, Muller Hinton agar, Triple sugar iron agar, Urease test medium, Oxidase test medium, Indole test medium and Citrate test medium were obtained from Oxoid Ltd., England. Commercially prepared Crystal violet solution, Iodine solution, Acetone-alcohol decolorizer solution and Safranine solution were used. Drug discs of Ciprofloxacin (5ug), Meropenem (10ug) and Imipenem (10ug) were obtained from Oxoid Ltd., UK. A commercially available reagent strip (DIRUI, Jilin, China) was used to detect pH, proteins, and sugar in urine after a physical examination for color. Then, using a microscope, it was possible to identify casts, red blood cells (RBCs), epithelium cells, pus cells, and RBCs. A calibrated loop was used to cultivate urine. For the development of microorganisms in the case of pyurea, urine was cultured for an entire day on standardized Cysteine lactose electrolyte deficient (CLED) agar. Gram reaction, appearance, and biochemical characteristics were used to identify bacterial pathogens. A drop of distilled water was applied to a spotless glass slide for Gram staining. Using a sterile loop in a drop of water on a slide, bacteria were cultured for 24 hours. On the slide, smear was equally distributed before air drying. Heat removes smears. Smear was cleaned with distilled water, dyed with crystal violet solution for 1 minute, and then submerged in iodine solution for 1 minute. After being cleaned with water, the smear was soaked in 95% alcohol to get rid of any remaining discoloration. The smear was then water washed, air dried, and counter dyed with safranine for 30 or 1 minute. seen using an oil immersion lens and a microscope. Biochemical tests like Urease, Oxidase, Indole and Citrate utilization test for Gram negative bacteria and Coagulase test for Gram positive isolates were done for further confirmation. Antibiotic sensitivity of the selected Gram negative bacterias was observed by using the Kirby Bauer method for antibiotic susceptibility. Muller Hinton agar plates were prepared and a single colony of selected strain was taken and swabbed on to agar surface by means of a sterile cotton swab. The antibiotic discs were then aseptically applied on the surface of agar plate at well spaced intervals (30 mm apart). The plates were incubated for 24 hours. According to the disc manufacturer's recommendations, the sizes of the zones that impede bacterial growth were used to determine whether a disc was "Sensitive" or "Resistant." Imipenem (10 ug), Meropenem (10 ug), and Ciprofloxacin (5 ug) drug discs were commercially manufactured and used in accordance with the National Committee for Clinical Laboratory Standards' standard disc diffusion procedure (NCCLS). According to the NCCLS criteria, the diameter of the zone of growth inhibition was measured and classified as either sensitive or resistant [13]. Statistical analysis was performed using SPSS, version 13.

RESULTS

A total of 100 urine samples were collected from which, 54% are females and 46% are males. The 52% patients were from 21-40 years age group, 28% were from 41-60 years, 15% were from 1-20 years and 5% were from 61-80 years age group as shown in table 1.

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Demographic characteristics	n (%)
Gender	
Male	46 (46%)
Female	54 (54%)
Age group	
1-20	15 (15%)
21-40	52 (52%)
41-60	28 (28%)
61-80	5 (5%)

Table 1: Gender and age wise frequency of UTI

Over the study period, total samples cultured on CLED medium, of these the isolated causative pathogens were *E. coli* (62%), *Klebsiella* spp. (11%), *Candida* spp. (8%), *Pseudomonas aeruginosa* (7%), *Proteus* spp. (4%), *Staphylococcus* spp. (3%), *Staphylococcus saprophyticus* (3%) and *Enterobacter* spp. (2%) (Table 2).

Growth on culture	n (%)
E. coli	62 (62 %)
Klebsiella spp.	11 (11 %)
Pseudomonas aeruginosa	8 (8 %)
Candida spp.	7 (7 %)
Proteus spp.	4 (4 %)
Staph. spp.	3 (3 %)
Staph. Saprophyticus	3 (3 %)
Enterobacter spp.	2 (2 %)
Total	100 (100&)

Table 2: Frequency of different Microbes on Culture.

E. coli was the most common uropathogen detected equally in females (31%) and in males (31%). *Klebsiella* species, *Candida* species, *Pseudomonas aeruginosa*, *Proteus* species, *Staphylococcus* species, *Staphylococcus saprophyticus* , *Enterobacter* species were also detected in decreasing frequencies. *Candida* species and *Staphylococcus saprophyticus* were detected only in females, while on the other hand *Staphylococcus* aureus was detected only in males as shown in figure 1.

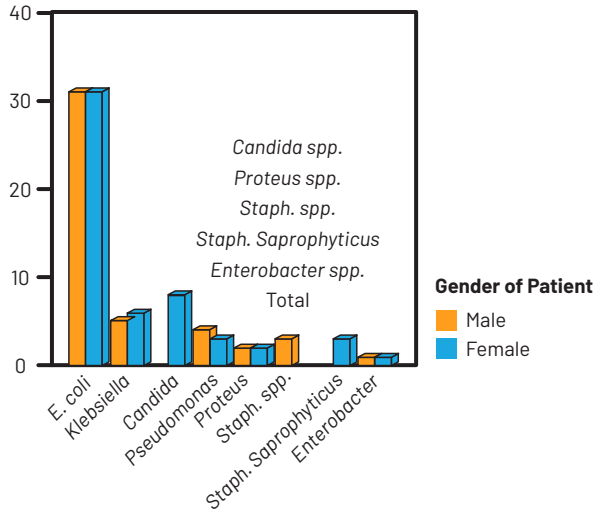

Figure 1: Frequency of microbes in male and female patients

In second part of the study the sensitivity pattern of gram negative uropathogens against Ciprofloxacin, Meropenem and Imipenem were detected. Out of 100 cultural growths, Gram negative isolates were 86%. Which showed, sensitivity pattern for Ciprofloxacin was 20 (23.3%) sensitive and 66 (76.7%) resistant. Out of 86% gram negative isolates 12 *E. coli*, 4 *Klebsiella* spp., 3 *Pseudomonas aeruginosa* and 1 *Proteus* spp. were sensitive to Ciprofloxacin while 50 *E. coli*, 7 *Klebsiella* spp., 4 *Pseudomonas aeruginosa*, 3 *Proteus* spp. and 2 *Enterobacter* spp. showed resistance (table 3). The p value for Ciprofloxacin was 0.44 which is > 0.05, it is not significant. It showed, increasing resistance pattern of uropathogens against Ciprofloxacin. Similarly, out of 86% gram negative isolates sensitivity pattern for Meropenem and Imipenem were showed in table no. 8 and 9, according to which 87.2% were sensitive to Meropenem and 12.8% were resistant. While for Imipenem 95.3% were sensitive and 4.7% were resistant. Only 1 *E. coli* isolate showed resistant to meropenem while 7 isolates of *Klebsiella* spp. showed resistance. In (table 11) only 3 *E. coli* isolates and 1 *Enterobacter* spp. showed resistance against Imipenem (table 3). The p value for Imipenem and Meropenem was < 0.05, which is significant.

Growth on culture	Sensitivity pattern of ciprofloxacin		Sensitivity pattern of meropenem		Sensitivity pattern of imipenem	
	Sensitive	Resistant	Sensitive	Resistant	Sensitive	Resistant
E. coli	12	50	61	1	59	3
Klebsiella spp.	4	7	4	7	11	0
Pseudomonas aeruginosa	3	4	6	1	7	0
Proteus spp.	1	3	3	1	4	0
Enterobacter spp.	0	2	1	1	1	1
Total	20	66	75	11	82	4

Table 3: Sensitivity pattern of ciprofloxacin, meropenem, and imipenem in gram negative isolates

DISCUSSION

In terms of bacteriology, a UTI occurs when pathogenic germs are found in the urine, urethra, bladder, kidney, or prostate. When a correctly taken midstream clean catch sample grows more than 105 organisms per ml, infection is usually the cause [14]. Because females have shorter urethras than males, germs can more easily travel up the urinary canal in females [15]. According to our study's findings, 54% more women than men were infected, and this is a common pattern that UTIs all across the world follow. The Enterobacteriaceae were the pathogens most frequently found to be responsible for 84.3% of UTIs[16]. In this Research UTI causing microbial agents were isolated.The results also revealed that gram negative isolates were the major cause of UTI. Among them most

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prevalent strain *E. coli* detected to be 62.0% and the second most prevalent strain was *Klebsiella* spp. 11%, while *Pseudomonas aeruginosa*, *Proteus* and *Enterobacter* spp. were least prevalent in causing UTI. *Candida* spp., *Staphylococcus* spp. and *Staphylococcus saprophyticus* also isolated in 8%, 3% and 3% respectivly. Amin *et al*. and Ziad Daoud showed similar percentage of uropathogens isolates [17, 18]. In this study, 86% gram negative isolates were isolated out of which 20(23.3%)were sensitive and 66 (76.7%) were resistant (Graph no.8). It showed high resistance pattern for uropathogens. The right antibiotic should be chosen based on factors such regional patterns of antibiotic resistance, pharmacokinetics, once-daily versus multiple-daily dosing, impact on the body's normal vaginal and intestinal flora, and antibiotic safety [19]. *E. coli* bacteria that are resistant to antibiotics are on the rise globally [20]. Despite improvements in the detection and treatment of infectious infections, the persistent growth of antibiotic-resistant strains in microorganisms continues to provide a challenge to antimicrobial chemotherapy. Millions of people around the world now live in harsher conditions as a result of these [21]. Third generation Cephalosporins and Quinolones are substantially less effective against isolates of *E. coli* in our country due to multidrug resistance [22]. Consuming quinolones has been linked to people in hospitals developing more resistance [23]. This has also been shown in other parts of the world, particularly Spain, where 22% of the *E. coli* isolates tested positive for ciprofloxacin resistance [21]. In both wealthy and developing nations, including Pakistan, antibiotic resistance has become a significant issue. In a developing nation like Pakistan, the price of efficient wide spectrum antibiotics is extremely exorbitant and out of the grasp of the average middle class individual. This has made things more difficult and contributed to the emergence of diseases with increased resistance. People who have common infections that were once easily treatable with straightforward medications may experience severe danger and suffering as a result. The free availability of medicines over the counter, the prescription of antibiotics without considering susceptibility, and other variables may be the main causes of the rising degree of resistance to commonly used antibiotics in these isolates in this region. The resistance genes carried by these pathogens need to be further studied at the molecular level, and the sequences can be compared to those of genes reported from other parts of the world.

CONCLUSIONS

The microbiological causative agents of UTI's are Gram negative & positive bacteria and fungai. It was also observed that UTI most commonly caused by *E. coli* which is a gram negative bacteria followed by *Klebsiella*, *Pseudomonas*, *Proteus* and *Enterobacter*. Gram positive bacteria like *Staphylococcus* spp., *Staphylococcus saprophyticus* and *Candida* spp. were also isolated in least amount. Urinary Tract Infections are most frequent in females as compared to males. Antimicrobial susceptibility showed that Gram negative isolates of uropathogens were highly resistant against Ciprofloxacin and showed sensitivity against Imipenem and Meropenem. It showed that resistance against Ciprofloxacin increased among gram negative uropathogens.

Conflicts of Interest

The authors declare no conflict of interest

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REFERENCES

[1] Kunin CM. Urinary tract infections and pyelonephritis. Cecil text book of medicine. 2000: 613-7.

[2] Gonzalez CM and Schaeffer AJ. Treatment of urinary tract infection: what's old, what's new, and what works. World journal of urology. 1999 Dec; 17(6): 372-82. doi: 10.1007/s003450050163

[3] Schappert SM. Ambulatory care visits to physician offices, hospital outpatient departments, and emergency departments. United States,1997. Vital Health Statistic. 1997; 143: 1-39.

[4] Laupland KB, Ross T, Pitout JD, Church DL, Gregson DB. Community-onset urinary tract infections: a population-based assessment. Infection. 2007 Jun; 35(3): 150-3. doi: 10.1007/s15010-007-6180-2

[5] Foxman B. Epidemiology of urinary tract infections: incidence, morbidity, and economic costs. Disease-a-month. 2003 Feb; 49(2): 53-70. doi: 10.1067/mda.2003.7

[6] Delanghe JR, Kouri TT, Huber AR, Hannemann-Pohl K, Guder WG, Lun A, *et al*. The role of automated urine particle flow cytometry in clinical practice. Clinica Chimica Acta. 2000 Nov; 301(1-2): 1-8. doi: 10.1016/S0009-8981(00)00342-9

[7] Ali AS, Townes CL, Hall J, Pickard RS. Maintaining a sterile urinary tract: the role of antimicrobial peptides. The Journal of urology. 2009 Jul; 182(1): 21-8. doi: 10.1016/j.juro.2009.02.124

[8] Justice SS, Hunstad DA, Seed PC, Hultgren SJ. Filamentation by *Escherichia coli* subverts innate defenses during urinary tract infection. Proceedings of the National Academy of Sciences. 2006 Dec 26; 103(52): 19884-9. doi: 10.1073/pnas.0606329104

DOI: https://doi.org/10.54393/fbt.v1i01.1

[9] Blair KA. Evidence-based management of urinary tract infections across the lifespan: Current update. The Journal for Nurse Practitioners. 2007 Oct; 3(9): 629-32. doi: 10.1016/j.nurpra.2007.07.020

[10] Farajnia S, Alikhani MY, Ghotaslou R, Naghili B, Nakhlband A. Causative agents and antimicrobial susceptibilities of urinary tract infections in the northwest of Iran. International journal of infectious diseases. 2009 Mar; 13(2): 140-4. doi: 10.1016/j.ijid.2008.04.014

[11] Karram M and Siddighi S. Lower urinary tract infection. Ostergards's urogynecology and pelvic floor dysfunction. 2008: 148-69.

[12] Yüksel S, Öztürk B, Kavaz A, Özçakar ZB, Acar B, Güriz H, *et al*. Antibiotic resistance of urinary tract pathogens and evaluation of empirical treatment in Turkish children with urinary tract infections. International journal of antimicrobial agents. 2006 Nov; 28(5): 413-6. doi: 10.1016/j.ijantimicag.2006.08.009

[13] Wayne PA. National committee for clinical laboratory standards. Performance standards for antimicrobial disc susceptibility testing. 2002; 12: 01-53.

[14] Khattak AM, Khan HU, Mashud IU, Shah SH, Ashiq B. Antimicrobial sensitivity pattern of urine isolates from asymptomatic bacteriuria during pregnancy. Biomedica. 2006; 22(1): 67-70.

[15] Awaness AM, Al-Saadi MG, Aadoas SA. Antibiotics resistance in recurrent urinary tract infection. Kufa medical journal. 2000; 3: 159.

[16] Gales AC, Jones RN, Gordon KA, Sader HS, Wilke WW, Beach ML, *et al*. Activity and spectrum of 22 antimicrobial agents tested against urinary tract infection pathogens in hospitalized patients in Latin America: report from the second year of the SENTRY antimicrobial surveillance program (1998). Journal of Antimicrobial Chemotherapy. 2000 Mar; 45(3): 295-303. doi: 10.1093/jac/45.3.295

[17] Daoud Z, Afif C. *Escherichia coli* isolated from urinary tract infections of Lebanese patients between 2000 and 2009: epidemiology and profiles of resistance. Chemotherapy Research and Practice. 2011; 2011. doi: 10.1155/2011/218431

[18] Amin, M., M. Manijeh and P. Zohreh. Study of bacteria isolated from urinary tract infections and determination of their susceptibility to antibiotics. Jundishapur journal of Microbiology. 2009; 2: 118-23.

[19] Officer H and Zareef S. The Culture and Sensitivity Pattern of Uropathogens Detected at Benazir Bhutto Hospital. Annals of Pakistan Institute of Medical Sciences. 2009; 5(2): 121-5.

[20] Greer LG, Roberts SW, Sheffield JS, Rogers VL, Hill JB, Mcintire DD, *et al*. Ampicillin resistance and outcome differences in acute antepartum pyelonephritis. Infectious diseases in obstetrics and gynecology. 2008 Oct; 2008. doi: 10.1155/2008/891426

[21] Jones ME, Karlowsky JA, Draghi DC, Thornsberry C, Sahm DF, Nathwani D. Epidemiology and antibiotic susceptibility of bacteria causing skin and soft tissue infections in the USA and Europe: a guide to appropriate antimicrobial therapy. International journal of antimicrobial agents. 2003 Oct; 22(4): 406-19. doi: 10.1016/S0924-8579(03)00154-7

[22] Shah SH. Susceptibility patterns of *Escherichia coli*: prevalence of multidrug-resistant isolates and extended spectrum beta-lactamase phenotype. Journal of Pakistan Medical Association. 2002; 52(407).

[23] Sabir N, Khan E, Sheikh L, Hasan R. Impact of antibiotic usage on resistance in microorganisms; urinary tract infections with E-coli as a case in point. Journal of Pakistan Medical Association. 2004; 54(9): 472.

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Original Article

Effect of Different Media Types on *In Vitro* Wheat Germination

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ABSTRACT

Wheat is a very crucial cereal for us as we highly depend on it and in Pakistan it is a crop which is mostly consumed. Wheat prevents colon cancer as it is a good source of dietary fiber. In vitro plant tissue culture is an alternative way to produce high yielding crops. Therefore, through the in vitro propagation of the wheat we can produce wheat with better quality and high nutritional value. **Objective:** To determine the rate of germination of the seeds of a wheat cultivar on different types of nutrient media. **Methods:** In the current study, two different types of media containing different hormones were used to investigate their effect on seed germination. For this purpose, M1 media with 2,4-D and M2 media with a combination of 2,4-D and BAP was used. Plant growth regulators were added in order to induce the germination process. The wheat cultivar Galaxy-2013 was used where 60 seeds were used for each medium. **Results:** The germination frequency on M1 was 41.66% while the germination frequency on M2 media was 61.66%. **Conclusions:** Hence, the results indicate that the combination of the PGRs showed better germination on MS media. This experiment could help in the selection of media which have a better germination capability for future research.

INTRODUCTION

Wheat (*Triticum aestivum L*) is a staple food which is widely and most commonly used as a cereal grain, it belongs to the family of the poaceae. Wheat is one of the few crops which is grown globally. The area devoted to wheat cultivation is 220.4 million hectares [1]. Due to its gluten protein, nutritional value, viscos elastic properties wheat is considered to be the most essential crop and due to its increasing demand, it is aimed to create a gene revolution in such a way that it could be made tolerant to various biotic and abiotic stresses [2]. To preserve the genetic diversity in the seed bank in vitro tissue culture is most important. Through in vitro tissue culture techniques better understanding of the metabolism and the effects of plant hormones could be understood which could eventually help in the production of the better-quality yield [3]. As compared to the conventional propagation techniques this technology has more advantages. Growth and development of the in vitro propagated plants is dependent on the genetic expression of the plant [4]. Genotype and expressions of the in vitro propagated plants are also affected by the environmental conditions. In vitro propagation can help in the production of the disease-free plantlets, decrease the period of acclimatization, fast clonal propagation, plantlets obtained through in vitro propagation have high survival rate when transferred to ex vitro conditions, reduced cost of the micropropagation plantlets etc. We can adjust the temperature, air movement, light as these physical environmental factors is predetermined in the culture medium and these factors can be varied or made constant during the growth cycle [5]. We can also adjust the chemical environmental conditions as these are also predetermined, in chemical conditions we

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can adjust the pH as well as the composition of the medium in such a way that young propagules are nourished properly [6]. The plant tissue culture room and the physical parameters in it could be optimized by changing the humidity and temperature in the room and to alter the growth conditions in a better way physically moving the culture [7]. B5, WPM and MS are the most common culture media used. Among these MS basal media is the most commonly used culture media [8]. In the MS basal media, the nitrogen content and the amount of the total salt is the highest [9]. For the growth of the explant nitrogen is the most crucial element because in the cell it affects the nucleic acid and amino acid production [10]. In this experiment MS media was used for the germination of the seeds. This study aimed to select a wheat variety i.e., Galaxy-2013 for in vitro propagation and analyzing the effect of the two PGRs i.e., 2, 4- Dichlorophenoxy acetic acid and 6- Benzyl aminopurine in MS medium on seed germination. Galaxy-2013 (*Triticum aestivum L.*) is a rust and lodging resistant, heat tolerant and high yielding variety of the wheat. The rate of germination of the seeds of a wheat cultivar was also observed by using different types of nutrient media.

METHODS

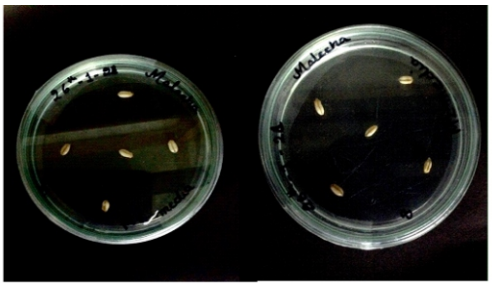
The seeds used for this experimental study were obtained from the Ayub Agricultural Research Centre and the variety of the seeds used were Galaxy-2013seeds. For the seed sterilization, the seeds were rinsed with auto calved dH20 for one minute with the half falcon filled. In the second step, ethanol 70% was used for the sterilization for about two minutes. In the third step 50% Clorox bleach was used for 15 to 20 minutes, whereas in the last step 8 to 10 times washing was done with the autoclaved dH20. Germination media (M1) was used for the seed germination purpose of the galaxy seeds which included the growth hormone 2, 4-Dichlorophenoxy acetic acid (2,4D)(3mg/L) Other nutrients in the media included were MS basal medium (1.1g), maltose (73g), Gelzan/ Gelrite (1g) for 250ml of the media. Whereas, germination media (M2) included the growth hormones 2, 4- Dichlorophenoxy acetic acid (2,4D)(3mg/L) and 6- Benzyl aminopurine (BAP) (5mg/L) [11]. Other nutrients in the M2 media included were Maltose (8.75g), MS basal media (1.1g), gelrite/gelzan (1g). These media were then autoclaved and then further subjected for the germination procedure. After the autoclave procedure, the media was allowed to be cooled down for about 10 minutes and was poured in autoclaved petri plates. For the seed placement, the seeds were first sterilized in the laminar flow with the half falcon filled with the seeds. After the last step of the washing dH20 was discarded carefully and the rest of the seeds were used for the placement. The seeds were picked with the sterilized forceps one at a time and were placed on the petri plate which contained the media in such a way that the embryonic part of the seeds was touching the media. The petri plate was then covered, wrapped with the Para film and properly labelled. For the placement of the Petri plates containing the seeds in the tissue culture lab, the rack was first properly sterilized and for the proper germination of the seeds the light above the rack was kept on whereas the other lights surrounding it were turned off. Frequency of germination was determined by following formula:

$$\text{frequency of germination\%} = \frac{\text{Number of seeds germinated}}{\text{Number of seeds cultured}} \text{x100}$$

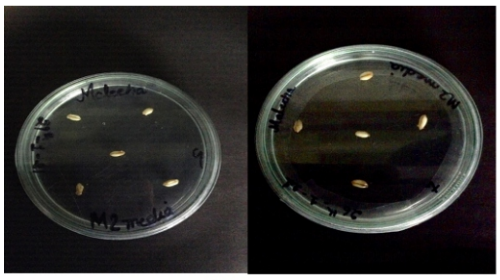
It is ration between the seeds placed and the number of seeds germinated. Multiplying the figures by 100 gives us the percentage and efficiency of our experiment.

RESULTS

Two different types of media were used to investigate their effect on seed germination of Galaxy-2013 cultivar. A total of 60 seeds were sterilized and placed on each medium. Out of 60, 25 seeds were germinated on M1 medium while 37 seeds were germinated on M2 medium (Figure 1).



(A)



(B)

Figure 1: (A) Seeds of Galaxy-2013 cultivar placed on M1 media. (B) Seeds of Galaxy-2013 cultivar placed on M2 media

Based on these observations, germination percentage was calculated. It was noticed that germination percentage was 41.66 % on M1 while it was 61.66 % on M2 media (Table 1; Figure 2)

	Total no. of seeds	No. of seeds germinated (%)	No. of days of germination
M1	60	25 (41.66%)	8
M2	60	37 (61.66%)	5

Table 1: Data on seed germination

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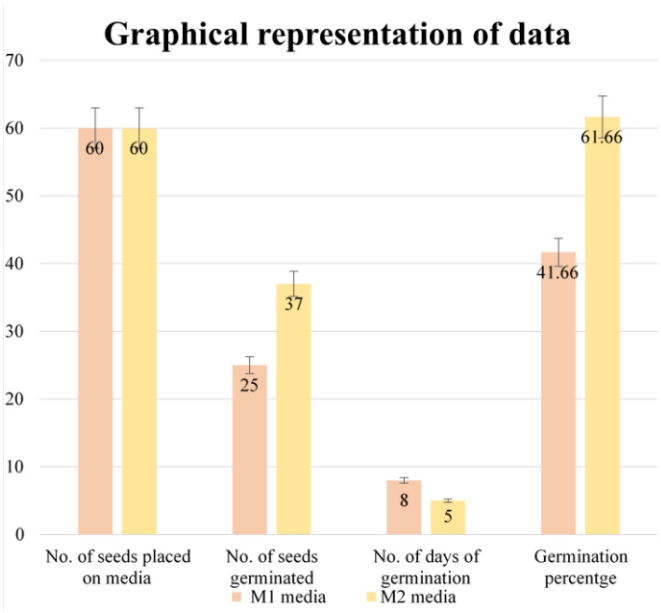

Figure 2: Graphical representation of germination data

Different stages of the seed germination were observed in M1 and M2 media as shown in Figure 3.

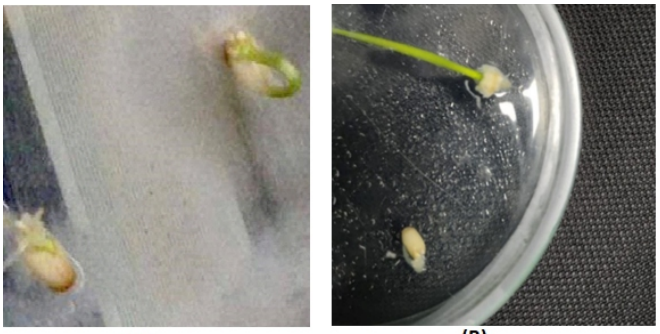

Figure 3: (A) Germination of the Galaxy-2013 seeds on M1 medium. (B) Germination of the Galaxy-2013 seeds on M1 medium

DISCUSSION

This experimental research work reveals that seed germination of wheat could be obtained by using the protocol appropriately. We used MS basal media for the germination of the Galaxy-2013 cultivar with different hormones (3mg/L of the 2,4-D and 5mg/L of the BAP). Another research work done on the germination of the (*Triticum aestivum L*) seeds used varying amounts of 2,4-D concentration which indicated that using higher than the normal concentration of the 2,4-D causes a significant decline in the seed germination [12]. A similar study indicated that the use of low dose of 2,4-D slowed down the seed germination process of wheat [13]. Therefore, in our study, a concentration of 3mg/ml of the 2,4-D was an optimal concentration for the in vitro Galaxy-2013 seed germination. In our study we studied the effect of the single PGR and combination of different PGRs on the germination of the wheat seeds. In a similar study done on wheat (*Triticum aestivum L.)* different hormonal combinations were used to observe the regeneration efficiency. In this study the BAP was used alone as well as different combinations were also observed. The RM14 media which is known as regeneration media was used which contained 2.5 mg/L of the BAP, kinetin 0.5 mg/l and IAA 0.1 mg/l. 80 to 84.5% and 83.4 to 87.9% was the regeneration efficiency obtained by it whereas 80.73% regeneration was obtained by using (0.1 mg/l) of the 2,4-D on the RM24 media. A combination of the 5 mg/l zeatin with the 2,4-D also had a good effect on shoot regeneration as well [14]. On the other hand, one of the studies observed showed that on MS medium when 0, 5 and 50 µM 2,4-D was used in combination with 0 and 0.5 µM TDZ, the shoot multiplication was slow. Whereas the root growth was also inhibited due to this combined effect. Similar results were obtained as in our study by using 2,4-D. Separately or in combination with the TDZ the shoot multiplication response was slow towards this plant growth regulator also the high concentration of the auxin: cytokinin was not so suitable for the propagation [15]. In the in vitro propagation experiments the most crucial step is the selection of a suitable media. We used MS media for the seed germination of the Galaxy cultivar. In a study conducted by Paul *et al.*, four different media have been used to study their effect on the seed germination. In this study MS medium, B5, KC and Mitra had been used and the speed of the seed germination varied according to the media which have been used [16]. After 2 weeks the greening of the embryo started to occur as compared to the other media used in which the signs of the germination started to occur later which indicates that MS media is best suited for the in vitro germination of the seeds and hence we were also able to carry out the in vitro propagation process successfully on the MS media. In our study we used 5 mg/L BAP in combination with 2,4-D in the M2 media on which the seeds of the Galaxy cultivar germinated faster followed by the rapid growth of the shoot. In many studies different concentrations of the BAP is used to assess the concentration at which the higher germination percentage occurs. Different concentrations of the BAP effect the germination of the seeds differently. A slightly different protocol followed by Gomes *et al.*, to assess the effect of the different concentrations of the BAP on the in vitro propagation of the soybean cultivar was used [17]. In our study we kept the seeds of the Galaxy cultivar placed on the media in the petri plates under the light condition whereas in this study the seeds placed on the media in the test tubes was kept under the dark. 0, 1, 3 and 5 µg / L BAP were the concentrations used followed by the different days of the pre-soaking of the seeds, this study concluded that 100% of the germination was obtained by the seeds of the

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soybean cultivar by using the 3 µg / L BAP concentration after the seven days with the pre-soaking of the seeds for 1 day. This study also concluded that increasing the concentration of the BAP followed by the increase in the days of the pre-soaking of the seeds lead to the decrease in the germination percentage. Whereas in our study we were able to obtain favourable amount (66.66 %) of the germination percentage by using 5 mg/L BAP within 5 days. In many studies the combined effect of the 2,4-D and BAP are used to investigate its effect on in vitro propagation. In a study the different concentrations of the combined effect of the 2,4-D and BAP was studied for the in vitro propagation of the saffron [18]. The concentrations used for both the hormones were (0, 0.25, 0.5, 1, 2, 4 and 8 mg L(-1)). This procedure was carried out in the darkness. This study showed that 2.0 mg L(-1) 2,4-D and 1.0 mg L(-1) BAP was the most suitable concentration for the explant [19]. In our study, we used cytokinin (BAP) along with 2,4-D which had a better germination ability. This indicates that the use of cytokinin (BAP) as a plant growth hormone accelerates the germination process. Similar results were obtained in a study, in which BAP was used for the in vitro propagation of the *Dendrobium aphyllum* (Orchidaceae) seeds [20]. The Galaxy-2013 seeds placed on M2 media containing 2,4-D (3mg/L) and BAP (5mg/L) had a better and faster germination ability as compared to the media containing only 2,4-D (3mg/L). This indicates that using a combination of the PGRs has a positive effect on the in vitro germination of the wheat seeds. Therefore, our study concluded that the concentration ranging from 3-5 mg/L is optimum for wheat germination under in vitro condition.

CONCLUSIONS

The results indicated that M2 media which contained BAP as well as 2,4-D had a better effect on the seed germination compared to M1 media. This shows that the proper amount of the cytokinin in combination with auxin should be used to achieve the better germination. Since 61.66% germination frequency was achieved by using M2 media. Hence, we can conclude that a combination of 2,4-D and BAP gives better germination. Different combinations of these auxins and cytokinin can be used in future studies.

Conflicts of Interest

The authors declare no conflict of interest.

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REFERENCES

[1] Kheyrodin H. Importance of Wheat farming in Iran: An Overview. Annals of Agricultural and Environmental Sciences. 2016 Feb; 1(2): 40-8. doi: 10.20936/AAE/160205.

[2] Gill BS, Li W, Sood S, Kuraparthy V, Friebe BR, Simons KJ, *et al*. Genetics and genomics of wheat domestication-driven evolution. Israel Journal of Plant Sciences. 2007 May; 55(3-4): 223-9. doi: 10.1560/IJPS.55.3-4.223.

[3] Krikorian AD. Hormones in tissue culture and micropropagation. In: Plant hormones 1995; 774-796. Springer, Dordrecht. doi: 10.1007/978-94-011-0473-9_35.

[4] Yaseen M, Ahmad T, Sablok G, Standardi A, Hafiz IA. Role of carbon sources for in vitro plant growth and development. Molecular Biology Reports. 2013 Apr; 40(4): 2837-49. doi: 10.1007/s11033-012-2299-z.

[5] Kozai T and Smith MA. Environmental control in plant tissue culture—general introduction and overview. In: Automation and environmental control in plant tissue culture 1995; 301-318. Springer, Dordrecht. doi: 10.1007/978-94-015-8461-6_14.

[6] Sardar H, Ali MA, Ayyub CM, Ahmed R. Effects of different culture media, temperature and pH levels on the growth of wild and exotic Pleurotus species. Pakistan Journal of Phytopathology. 2015 Jan; 27(2): 139-45.

[7] Taji A, Dodd WA, Williams RR. Plant tissue culture practice. University of New England; 1992.

[8] Phillips GC, Garda M. Plant tissue culture media and practices: an overview. In Vitro Cellular & Developmental Biology-Plant. 2019 Jun; 55(3): 242-57. doi: 10.1007/s11627-019-09983-5.

[9] Gamborg OL, Murashige T, Thorpe TA, Vasil IK. Plant tissue culture media. In vitro. 1976 Jul; 12(7): 473-8. doi: 10.1007/BF02796489.

[10] Ramage CM and Williams RR. Inorganic nitrogen requirements during shoot organogenesis in tobacco leaf discs. Journal of Experimental Botany. 2002 Jun; 53(373): 1437-43. doi: 10.1093/jexbot/53.373.1437.

[11] Zameer Z, Mohsin S, Hasnain A, Maqbool A, Malik KA. Influence of Explant Sources on in vitro Callogenesis and Regeneration in Maize (*Zea mays L.*). Pakistan Journal of Agricultural Research. 2020 Dec; 33(4): 917-25. doi: 10.17582/journal.pjar/2020/33.4.917.925.

[12] Kaur A and Kaur N. In vitro evaluation of low dosages of 2, 4-D on germination and seedling growth of wheat and associated weeds. Indian Journal of Weed Science. 2019 Jun; 51(2): 178-82. doi: 10.5958/0974-8164.2019.00038.8.

[13] Perboni LT, Agostinetto D, Vargas L, Cechin J, Zandoná RR, Farias HD. Yield, germination and herbicide residue in seeds of preharvest desiccated

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wheat. Journal of Seed Science. 2018 Jul; 40: 304-12. doi: 10.1590/2317-1545v40n3191284.

[14] Kumar R, Mamrutha HM, Kaur A, Venkatesh K, Grewal A, Kumar R, *et al.* Development of an efficient and reproducible regeneration system in wheat (Triticum aestivum L.). Physiology and Molecular Biology of Plants. 2017 Oct; 23(4): 945-54. doi: 10.1007/s12298-017-0463-6.

[15] Wattanawikkit P, Bunn E, Chayanarit K, Tantiwiwat S. Effect of cytokinins (BAP and TDZ) and auxin (2, 4-D) on growth and development of Paphiopedilum callosum. Agriculture and Natural Resources. 2011 Feb; 45(1): 12-9.

[16] Paul S, Kumaria S, Tandon P. An effective nutrient medium for asymbiotic seed germination and large-scale in vitro regeneration of Dendrobium hookerianum, a threatened orchid of northeast India. AoB Plants. 2012 Jan; 2012: plr032. doi: 10.1093/aobpla/plr032.

[17] Gomes AP, Oliveira Nogueira AP, De Sá GD, Rodrigues B, Bonetti AM. BAP effect in vitro germination of soybean cultivars UFUS Xavante an UFUS Carajás. BMC Proceedings. 2014 Oct; 8(4): 1. doi: 10.1186/1753-6561-8-S4-P91.

[18] Shewry PR. Improving the protein content and composition of cereal grain. Journal of Cereal Science. 2007 Nov; 46(3): 239-50. doi: 10.1016/j.jcs.2007.06.006.

[19] Tahir SM, Samaila AA, Abdulrahman MD. Studies on the effects of 6-Benzylaminopurine and 1-Naphthaleneacetic acid on the in vitro regeneration of okra (Abelmoschus esculentus l.). Science World Journal. 2019 Jan; 14(1): 119-28.

[20] Hossain MM, Kant R, Van PT, Winarto B, Zeng S, Teixeira da Silva JA. The application of biotechnology to orchids. Critical Reviews in Plant Sciences. 2013 Mar; 32(2): 69-139. doi: 10.1080/07352689. 2012. 715984.

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FUTURISTIC BIOTECHNOLOGY

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Volume 1, Issue 1 (Jan-Jun 2021)

Original Article

Detection of Tuberculosis on Culture, Comparison of Findings with Fluorescence Microscopy and GeneXpert

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ABSTRACT

The GeneXpert MTB/RIF technique is used for the identification of tuberculosis and rifampicin (RIF) resistance. Xpert MTB / RIF provides patients with distinct advantages such as early diagnosis. **Objectives:** To compared the performance of fluorescence microscopy and GeneXpert with culture in TB samples from Narowal, Pakistan. **Methods:** A total of 299 TB positive specimens were obtained. Among these 54% (n = 160) were categorized to be obtained from male and 46% (n = 139) from female population. The sensitivity and specificity of fluorescence microscopy, GeneXpert and culture of TB samples were done. **Results:** The parameters including sensitivity and specificity calculated for GeneXpert were 73% and 100%, respectively, while the sensitivity and specificity calculated for culture was 100% and the sensitivity and specificity for FM microscope were 43% and 100%, respectively. **Conclusions:** We conclude that the GeneXpert is more sensitive than FM considering culture as a gold standard. Although the GeneXpert assay was also shown to be able to detect a limited number of bacillus from samples, the culture's sensitivity and specificity were both 100%.

INTRODUCTION

Lung is the main organ affected by *Mycobacterium tuberculosis*, but other organs of body may also be severely affected by it. In most cases, the ailment can reduce symptoms, in which case it is called drowsiness or inactive tuberculosis [1]. The identification of indolent tuberculosis depends on the special skin test called tuberculin skin test (TST) or by way of blood test [2]. Avoidance of tuberculosis includes vaccination against BCG, increased screening hazard, timely identification and correct management of the cases [3]. Mycobacteria are aerobic, non-motile, acidic alcohol or micro-curvature. These organisms have high molecular weight carbon atoms and mycolic acid in the cell wall, and after pyrolysis, they release C22-C26 linear saturated acidic chain are pathogenic bacteria of human tuberculosis [4]. Tuberculosis was reported nationwide in 1953, and incidence of tuberculosis declined steadily in 1984 [5]. *Mycobacterium tuberculosis* is transmitted mainly by inhalation of small infectious droplets (1-10 µm in diameter) of dry residue [6]. Based on the analysis of sequence of genome, *M. tuberculosis* does not recognize the characteristic virulence factors of bacteria [7]. A common host response to MTBC infection is cell-mediated activation of body's immune system. Infrequent infection

with intravesical instillation of *M. bovis* BCG is used to treat superficial bladder cancer [8]. AFB staining typically has a specificity of 99% or higher and a sensitivity of about 25% to about 75% [9]. Inoculation with solid and liquid media is suggested for optimum growth of *mycobacteria* from the samples [10]. AFB staining does occur in sputum in up to 75% of tuberculosis patients, less than 20% of *tuberculosis* children have significant AFB spread during sputum or stomach inhalation [11]. Traditionally, the identification of mycobacteria was based on the growth rate of solid media, biochemical test results and the morphology and coloration of colonies [12]. Recently Cepheid proposed GeneXpert MTB / RIF test [13]. GeneXpert assays, like real-time PCR assays, can simultaneously recognize MTB and specifically identify rifampicin resistance from sputum or other liquid samples [14].

METHODS

Cross sectional study was done in the tuberculosis department at DHQ Hospital in Narowal. The sample size of 299 was determined by formula as follows:

$$n = \frac{z^2_{1-a/b} * P * (1-p)}{d^2}$$

The patient's detailed clinical parameters were recorded and the patient was guided to collect the sputum sample in a defined container. Smears were prepared from samples after concentration and re-suspension of the pallet. Smear was covered with stain. After staining, the slides were examined by the microscopists. Lowenstein Jensen media was employed to detect the bacilli from samples. To prevent the growth of Gram-positive and Gram-negative bacteria as well as to restrict growth to Mycobacterium species only, low concentrations of penicillin and nalidixic acid are also added in LJ medium. Presence of malachite green in the medium inhibits most other bacteria. It is disinfected and solidified by a process of inspissation. Presence of glycerol enhances the growth of *M. tuberculosis*. For cultivation of *M. bovis,* glycerol is omitted and sodium pyruvate is added. Positive and negative results of samples as found by microscopy and GeneXpert were cultured on Lowenstein Jensen media. After inoculation, the plates were incubated for at least 6 weeks at 37°C. Any visible growth was observed and recorded as MTB and MOTT. For GeneXpert system. The sample reagent and the sputum collection container lids were opened. 02 volumes of sample reagent was added to 01 volume of sputum and lid was replaced. The mixture was thoroughly mixed over a vortex for at least 10 seconds. Then it was incubated for 10 minutes at room temperature and then mixed again. It was incubated for another 05 minutes. The sample was processed till it was perfectly liquid, if it was still viscous, a waiting time of 05-10 minutes was given. The side of the cartridge was labelled with the sample id before its lid was opened. Sample (2ml) was slowly transferred to the sample chamber of the cartridge taking care that care that bubbles don't form. The lid was firmly closed and the test was run on GeneXpert instrument. Using the 2×2 table in the SPSS-20 software and considering the sputum culture as gold standard. The sensitivity, specificity, PPV and NPV for each assay were calculated to diagnose TB in patients. The kappa(k) test was used to assess the consistency between the tests. Using the formula, the sensitivity was found as follows: Sensitivity % = true positive(TP)/(true positive(TP)+false negative(FN))X 100. Specificity was calculated using the formula given below: Specificity % = true negative (TN) / (true negative (TN) + false positive(FP))X 100.

RESULTS

The current study was conducted (in Narowal, Pakistan) to compare the diagnosis of tuberculosis using GeneXpert and fluorescence microscopy with culture. Total processed samples were 299 of which 54% (n = 160) were obtained from male and 46% (n = 139) from female population. Culture method declared 43% (n=128) samples as positive (Table 1).

n(%)	Methods		
	FM	GeneXpert	Culture
	55 (18%)	93 (31%)	128 (43%)

Table 1: Detection of tuberculosis on culture

The sensitivity and specificity recorded for culture were 100% and 100%, respectively. Furthermore, positive predictive value (PPV) and negative predictive value (NPV) estimated for culture methods were found to be 100% and 100%, respectively (figure 1). Also, no samples were found that had positive results on culture and negative on GeneXpert. Culture was taken as standard and had sensitivity of 100% as compared to FM technique that had 43% sensitivity. Sensitivity for culture was found to be half fold more than FM technique. It was noted that culture was more sensitive than FM in detecting tubercle bacilli. Culture was taken as standard which had a sensitivity of 100% as compared to GeneXpert technique that had 73% sensitivity. It was found that culture was more sensitive than GeneXpert (figure 1).

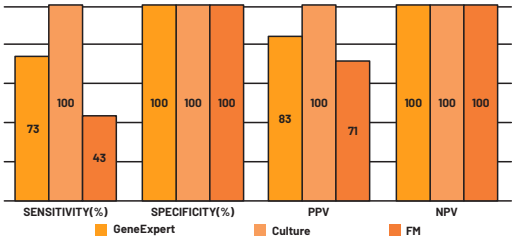

Figure 1: Comparison of sensitivity and specificity of FM,

GeneXpert and Culture

DISCUSSION

In this study total of 299 sputum samples were examined, with an FM detection rate of 18% (n = 55), GeneXpert of 31% (n = 93) and a standard culture technique of 43% (n = 128). The specificity and sensitivity of the GeneXpert assay were known to be 100% and 73%, respectively, in addition the sensitivity and specificity of the FM microscope were 43% and 100%, respectively. The findings showed culture as better than the two techniques used. This is in contrast with another study comparing the GeneXpert findings and stated GneXpert to be better [15]. As a reference standard, the culture revealed that the smear positive samples had a sensitivity of 98.4% (60/61) and the smear negative samples had a sensitivity of 93.7% (30/32)[16]. The result of this study are similar to our study which aimed to evaluate GeneXpert for culture and fluorescence microscopy, and GeneXpert analysis showed sensitivity and specificity of 73% and 100%, correspondingly. The specificity, sensitivity, positive predicted value and negative predicted value of XpertTB/RIF detection were 93%, 93.3%, 82.3% and 93.3%, respectively. Respectively. Xpert determination was significantly higher than the sensitivity of rapid smear of citric acid ($p < 0.001$). GeneXpert detected 50% additional positive cases compared to LJ culture and smear microscopy [17, 18]. Results of this study are quite comparable to our study which analyzed 299 samples. LJ culture cases are twice times higher than AFB smear cases, with sensitivity and specificity of 45.7% and 100%. In addition, from the clinically diagnosed 81 urinary tract tuberculosis cases, 51 were processed by the Xpert technique, showing the sensitivity of 63% that is considerably higher than AFB smear microscopy and LJ culture method. GeneXpert was only detected in 5 patients with RIF resistance, and all patients had a phenotypic sensitivity test with a sensitivity of 100% [19, 20]. This study is quiet similar to present study in which GeneXpert is more sensitive and specific as compared to FM.

CONCLUSIONS

We conclude that the GeneXpert is more sensitive than FM considering culture as a gold standard. The culture sensitivity and specificity was 100% but the GeneXpert assay was also found to detect small number of bacillus from samples. In addition, the estimated PPV and NPV values for the culture method were found to be 100% and 100%, respectively.

Conflicts of Interest

The authors declare no conflict of interest

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REFERENCES

[1] Bennett JE, Dolin R, Blaser MJ. Mandell, douglas, and bennett's principles and practice of infectious diseases E-book. Elsevier health sciences; 2019 Aug.

[2] Neely AH. Internal ecologies and the limits of local biologies: A political ecology of tuberculosis in the time of AIDS. Annals of the Association of American Geographers. 2015 Jul; 105(4): 791-805. doi: 10.1080/00045608.2015.1015097

[3] Hawn TR, Day TA, Scriba TJ, Hatherill M, Hanekom WA, Evans TG, *et al*. Tuberculosis vaccines and prevention of infection. Microbiology and Molecular Biology Reviews. 2014 Dec; 78(4): 650-71. doi: 10.1128/MMBR.00021-14

[4] Castets M, Boisvert H, Grumbach F, Brunel M, Rist N. Tuberculosis bacilli of the African type: preliminary note. Revue de tuberculose et de pneumologie. 1968 Mar; 32(2): 179-84.

[5] Rieder HL, Cauthen GM, Comstock GW, Snider DE. Epidemiology of tuberculosis in the United States. Epidemiologic reviews. 1989 Jan; 11: 79-98. doi: 10.1093/oxfordjournals.epirev.a036046

[6] Munch Z, Van Lill SW, Booysen CN, Zietsman HL, Enarson DA, Beyers N. Tuberculosis transmission patterns in a high-incidence area: a spatial analysis. International journal of tuberculosis and lung disease. 2003 Mar; 7(3): 271-7.

[7] Woods GL, Brown-Elliott BA, Conville PS, Desmond EP, Hall GS, Lin G, *et al*. Susceptibility testing of mycobacteria, nocardiae, and other aerobic actinomycetes. 2011. doi: 10.1128/9781555816728.ch73

[8] Lamm DL, Van Der Meijden AP, Morales A, Brosman SA, Catalona WJ, Herr HW, *et al*. Incidence and treatment of complications of bacillus Calmette-Guerin intravesical therapy in superficial bladder cancer. The Journal of urology. 1992 Mar; 147(3): 596-600. doi: 10.1016/S0022-5347(17)37316-0

[9] Strumpf IJ, Tsang AY, Sayre JW. Re-evaluation of sputum staining for the diagnosis of pulmonary tuberculosis. American Review of Respiratory Disease. 1979 Apr; 119(4): 599-602.

[10] Tenover FC, Crawford JT, Huebner RE, Geiter LJ, Horsburgh Jr CR, Good RC. The resurgence of tuberculosis: is your laboratory ready?. Journal of clinical microbiology. 1993 Apr; 31(4): 767-70. doi: 10.1128/jcm.31.4.767-770.1993

[11] Singhal R, Myneedu VP. Microscopy as a diagnostic

tool in pulmonary tuberculosis. International journal of mycobacteriology. 2015 Mar; 4(1): 1-6. doi: 10.1016/j.ijmyco.2014.12.006

[12] Kent PT. Public health mycobacteriology: a guide for the level III laboratory. US Department of Health and Human Services, Public Health Service, Centers for Disease Control; 1985.

[13] Fred T. Two-hour detection of MTB and resistance to rifampicin. Invitro diagnostic medical device. 2009.

[14] Helb D, Jones M, Story E, Boehme C, Wallace E, Ho K, *et al*. Rapid detection of Mycobacterium tuberculosis and rifampin resistance by use of on-demand, near-patient technology. Journal of clinical microbiology. 2010 Jan; 48(1): 229-37. doi: 10.1128/JCM.01463-09

[15] Abarca Tomás B, Pell C, Bueno Cavanillas A, Guillén Solvas J, Pool R, *et al*. Tuberculosis in migrant populations. A systematic review of the qualitative literature. PLOS one. 2013 Dec; 8(12): e82440. doi: 10.1371/journal.pone.0082440

[16] Ahmad S, Afzal S, Ullah A, Sheed A. Evaluation of GeneXpert MTB/RIF assay for detection of pulmonary tuberculosis on sputum samples. Journal of the College of Physicians and Surgeons Pakistan. 2019 Jan; 29(1): 66-9. doi: 10.29271/jcpsp.2019.01.66

[17] Bajrami R, Mulliqi G, Kurti A, Lila G, Raka L. Comparison of GeneXpert MTB/RIF and conventional methods for the diagnosis of tuberculosis in Kosovo. The Journal of Infection in Developing Countries. 2016 Apr; 10(04): 418-22. doi: 10.3855/jidc.7569

[18] Bajrami R, Mulliqi G, Kurti A, Lila G, Raka L. Assessment of diagnostic accuracy of GeneXpert Mycobacterium tuberculosis/rifampicin in diagnosis of pulmonary tuberculosis in Kosovo. Biomedical and Biotechnology Research Journal (BBRJ). 2018 Jul; 2(3): 191. doi: 10.4103/bbrj.bbrj_78_18

[19] Pang Y, Shang Y, Lu J, Liang Q, Dong L, Li Y, Zhao L, *et al*. GeneXpert MTB/RIF assay in the diagnosis of urinary tuberculosis from urine specimens. Scientific reports. 2017 Jul; 7(1): 6181. doi: 10.1038/s41598-017-06517-0

[20] Chen Y, Wu P, Fu L, Liu YH, Zhang Y, Zhao Y. Multicentre evaluation of Xpert MTB/RIF assay in detecting urinary tract tuberculosis with urine samples. Scientific reports. 2019 Jul; 9(1): 1-6. doi: 10.1038/s41598-019-47358-3

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Original Article

Evaluation of Gene Expression of TNF-α in Healthy Subjects

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ABSTRACT

Tumor necrosis factor-alpha (TNF-α) is a cytokine involved in the immune response, inflammation, and apoptosis. Dysregulation of TNF-α expression has been associated with various diseases, including autoimmune disorders, cancer, and chronic inflammatory conditions. Understanding the regulation of TNF-α expression in healthy individuals can help identify potential therapeutic targets for these diseases. **Objective:** To evaluate of gene expression of TNF-α in healthy subjects. **Methods:** The cross-sectional study conducted on 40 individuals on healthy individuals. RNA was extracted and TNF-α gene expression was evaluated using PCR and statistical analysis was done using SPSS software. **Results:** The evaluation of TNF-α gene expression in healthy individuals has also led to the identification of potential biomarkers of disease and new therapeutic targets. **Conclusion:** In conclusion, the evaluation of TNF-α gene expression in healthy individuals is an important tool for identifying potential biomarkers of disease and understanding the physiological role of this cytokine.

INTRODUCTION

Tumor Necrosis Factor-alpha (TNF-α) is a cytokine that plays a critical role in the immune response, inflammation, and apoptosis. In healthy individuals, TNF-α is produced in response to various stimuli, including infections, injury, and stress. It has been shown that dysregulation of TNF-α expression can lead to the development of various diseases, including autoimmune disorders, cancer, and chronic inflammatory conditions [1-3]. Therefore, the evaluation of TNF-α expression in healthy individuals is of great importance for understanding the physiological role of this cytokine and for identifying potential biomarkers of disease. Gene expression analysis is a powerful tool for evaluating the expression of TNF-α in healthy individuals [4, 5]. This analysis allows researchers to determine the amount of TNF-α mRNA produced by different cell types in response to different stimuli. This information can be used to identify the factors that regulate TNF-α expression and to determine the molecular mechanisms that control its production. Furthermore, gene expression analysis can be used to identify potential therapeutic targets for diseases associated with dysregulated TNF-α expression [6, 7]. Several methods are available for the evaluation of TNF-α gene expression in healthy subjects. These include quantitative polymerase chain reaction (qPCR), microarray analysis, and next-generation sequencing (NGS) [8-10]. Each of these methods has its advantages and limitations and must be chosen based on the research question, available resources, and experimental design. Studies evaluating TNF-α gene expression in healthy individuals have provided valuable insights into the regulation of this cytokine and its physiological role [11, 12]. For example, it has been shown that TNF-α expression is regulated by a complex network of signaling pathways that involve various transcription factors, cytokines, and other molecules.

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Moreover, the evaluation of TNF-α expression in healthy individuals has led to the identification of potential biomarkers of disease and new therapeutic targets. In conclusion, the evaluation of TNF-α gene expression in healthy individuals is an essential tool for understanding the physiological role of this cytokine and for identifying potential biomarkers and therapeutic targets for diseases associated with dysregulated TNF-α expression [13-14]. The use of gene expression analysis methods such as qPCR, microarray analysis, and NGS has provided valuable insights into the regulation of TNF-α expression and its molecular mechanisms, which can be used to improve human health.

METHODS

It was a cross sectional case-control study. The research work was carried out in the department of Immunology and Resource lab University of Health Sciences, Lahore. The calculated sample size for each group is 40. A total of 40 subjects were tested for this study. Five ml venous blood was collected in EDTA coated vacationers from healthy subjects was brought to the Resource lab within four hours of the sample collection to avoid genomic RNA degradation. The primers were suspended using low TAE buffer in a calculated amount to achieve concentration 1µg/µl as stock. A working solution of 10pm/µl diluted from stock were used for all further PCR experiments. Primers were optimized for reaction conditions of annealing temperature, Mg concentration, amount of buffer and dNTPs. These optimum conditions were in further experimentation. The following primers was used:

Gene	Primer	GC content (%)	Product Size
TNF α –F	5' CGAGTGACAAGCCTGTAGC 3'	45	453
TNF α –R	5' GGTGTGGGTGAGGAGCACAT 3'	50	

Table 1: Primer used for PCR

RNA was extracted from blood samples within 6 hours of sample collection. Samples was stored in trizol if extraction is delayed. Extracted RNA samples were stored at -80◦C. Quantity and quality of RNA were checked by nano drop. After pcr reaction gel electrophoresis was done. All statistical analysis was done using SPSS software (version 20.0).

RESULTS

In healthy subject group, which consisted of 40 individuals, 69.38% (n= 28) were male, and 32.61% (n= 13) were female. The mean age of the participants was 47.49 ± 2.72 years. The mean alanine aminotransferase (ALT) level was 22.71 ± 5.22 U/L, and the mean aspartate aminotransferase (AST) level was 32.24 ± 7.54 U/L.

Variables		Healthy Subject Group N= 40
Gender	Male	69 (69.38%)
	Female	32 (32.61%)
Age		47.49 ±2.72
ALT		22.71 ± 5.22
AST		32.24 ± 7.54

Table 2: Demographic variables of healthy subjects

Sample	Conc. ng/µl of Healthy Subject	Amount used for cDNA (1.5µg) of Healthy Subject
1	1327	2.39617
2	1578	1.85644
3	530	2.92398
4	1225	2.29709
5	576	0.66578
6	876	1.94805
7	484	2.39234
8	650	1.27334
9	743	1.90355
10	1021	1.50301
11	1097	1.94805
12	2016	2.39234
13	1786	1.27334
14	987	1.90355
15	644	1.95313
16	443	1.49701
17	544	1.36861
18	793	0.74368
19	2264	0.8394
20	564	0.66578
21	455	0.4178
22	1678	1.19338
23	928	1.54834
24	1733	2.38626
25	2254	1.9664
26	3597	2.95388
27	1255	1.36544
28	976	2.68743
29	627	1.6943
30	807	3.25266
31	514	2.34891
32	786	1.8059
33	894	1.3870
34	497	1.4676
35	554	0.6336
36	654	0.8564
37	2254	1.83976
38	3597	2.83845
39	1257	3.9467
40	976	2.6853

Table 3: Amount of cDNA used for experiment

The expression of TNF-*a* gene was observed by RT-PCR by using gene specific primers and the dye which is SYBR Green mix of the ferments. For internal control GAPDH gene was applied. Each PCR assay of real time was

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performed in triplicate. Increased expression was observed in healthy subjects. Almost 1-fold induced expression of TNF-*a* was observed in healthy subjects

DISCUSSION

Rodenburg *et al.*, study have evaluated TNF-α gene expression in healthy individuals to understand its physiological role and identify potential biomarkers of disease. These studies have shown that TNF-α expression is regulated by a complex network of signaling pathways and can be influenced by various factors, such as age, sex, and environmental stimuli [14]. For example, one study found that exposure to air pollution was associated with increased TNF-α expression in healthy individuals, suggesting a potential link between environmental factors and the regulation of TNF-α expression [15]. Another Buha *et al.*, study evaluated the association between TNF-α gene expression and the risk of developing cardiovascular disease (CVD). The study found that higher TNF-α expression was associated with an increased risk of CVD, suggesting that TNF-α may serve as a potential biomarker of disease. Moreover, the study identified several genetic variants that regulate TNF-α expression and may be used to predict disease risk [16, 17]. A recent study used microarray analysis to evaluate the gene expression profile of TNF-α in healthy individuals and in patients with type 2 diabetes mellitus (T2DM). The study found that TNF-α expression was significantly upregulated in patients with T2DM compared to healthy controls. Moreover, the study identified several genes that were co-expressed with TNF-α and may be involved in the pathogenesis of T2DM [18]. These previous studies like de Oliveira *et al.*, highlight the importance of evaluating TNF-α gene expression in healthy individuals to understand its physiological role and identify potential biomarkers of disease. Moreover, these studies demonstrate the potential of gene expression analysis to identify novel regulatory mechanisms and potential therapeutic targets. The use of methods such as qPCR, microarray analysis, and NGS can provide valuable insights into the molecular mechanisms that control TNF-α expression and help improve our understanding of the pathogenesis of various diseases [19, 20]. The use of gene expression analysis methods, such as qPCR, microarray analysis, and NGS, can provide valuable insights into the regulation of TNF-α expression and its molecular mechanisms. Future studies can build on previous research to identify new biomarkers of disease, potential therapeutic targets, and novel regulatory mechanisms that control TNF-α expression.

CONCLUSIONS

In conclusion, the evaluation of TNF-α gene expression in healthy individuals is an important tool for identifying potential biomarkers of disease and understanding the physiological role of this cytokine.

Conflicts of Interest

The authors declare no conflict of interest.

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REFERENCES

[1] Nourian M, Chaleshi V, Pishkar L, Azimzadeh P, Baradaran Ghavami S, *et al*. Evaluation of tumor necrosis factor (TNF)-α mRNA expression level and the rs1799964 polymorphism of the TNF-α gene in peripheral mononuclear cells of patients with inflammatory bowel diseases. Biomedical Reports. 2017 Jun; 6(6): 698-702. doi: 10.3892/br.2017.908

[2] Akhtari M, Zargar SJ, Vojdanian M, Jamshidi A, Mahmoudi M. Monocyte-derived and M1 macrophages from ankylosing spondylitis patients released higher TNF-α and expressed more IL1B in response to BzATP than macrophages from healthy subjects. Scientific Reports. 2021 Sep; 11(1): 17842. doi: 10.1038/s41598-021-96262-2

[3] Groote DE. Tumour necrosis factor (TNF) gene polymorphism influences TNF-α production in lipopolysaccharide (LPS)-stimulated whole blood cell culture in healthy humans. Clinical & Experimental Immunology. 1998 Sep; 113(3): 401-6. doi: 10.1046/j.1365-2249.1998.00662.x

[4] Bobińska K, Gałecka E, Szemraj J, Gałecki P, Talarowska M. Is there a link between TNF gene expression and cognitive deficits in depression?. Acta Biochimica Polonica. 2016 Dec; 64(1): 65-73. doi: 10.18388/abp.2016_1276

[5] Carneiro JR, Fuzii HT, Kayser C, Alberto FL, Soares FA, Sato EI, *et al*. IL-2, IL-5, TNF-α and IFN-γ mRNA expression in epidermal keratinocytes of systemic lupus erythematosus skin lesions. Clinics. 2011; 66: 77-82. doi: 10.1590/S1807-59322011000100014

[6] Zhao Y, Yang J, Zhang L, Li Z, Yang Y, Tang Y, *et al*. Association between TNF-α– 308G/A polymorphism and diabetic nephropathy risk: a meta-analysis. International Urology and Nephrology. 2013 Dec; 45: 1653-9. doi: 10.1007/s11255-013-0490-3

[7] Zheng Z and Zheng F. Immune cells and inflammation in diabetic nephropathy. Journal of Diabetes Research. 2016 Oct; 2016: 1-10. doi: 10.1155/2016/1841690

[8] Hashizume M and Mihara M. Atherogenic effects of TNF-α and IL-6 via up-regulation of scavenger receptors. Cytokine. 2012 Jun; 58(3): 424-30. 10.1016/

DOI: https://doi.org/10.54393/fbt.v1i01.15

j.cyto.2012.02.010

[9] Kim HY. Nutritional intervention for a patient with diabetic nephropathy. Clinical Nutrition Research. 2014 Jan; 3(1): 64-8. doi: 10.7762/cnr.2014.3.1.64

[10] Sturgeon PhD LP, Bragg-Underwood DNP TM, Blankenship DNP M. Practice matters: Prevention and care of individuals with type 2 diabetes. International Journal of Faith Community Nursing. 2016; 2(1): 32-39.

[11] Mogensen CE, Christensen CK, Vittinghus E. The stages in diabetic renal disease: with emphasis on the stage of incipient diabetic nephropathy. Diabetes. 1983 Jun; 32(Supplement_2): 64-78. doi: 10.2337/diab.32.2.S64

[12] STÜBER F. Effects of genomic polymorphisms on the course of sepsis: is there a concept for gene therapy?. Journal of the American Society of Nephrology. 2001 Feb; 12(suppl 1): S60-4. doi: 10.1681/ASN.V12suppl_1s60

[13] López-Contreras AK, Martínez-Ruiz MG, Olvera-Montaño C, Robles-Rivera RR, Arévalo-Simental DE, Castellanos-González JA, *et al*. Importance of the use of oxidative stress biomarkers and inflammatory profile in aqueous and vitreous humor in diabetic retinopathy. Antioxidants. 2020 Sep; 9(9): 891. doi: 10.3390/antiox9090891

[14] Rodenburg RJ, Van den Hoogen FH, Van de Putte LB, Van Venrooij WJ. Peripheral blood monocytes of rheumatoid arthritis patients do not express elevated TNF α, IL-1β, and IL-8 mRNA levels. A comparison of monocyte isolation procedures. Journal of Immunological Methods. 1998 Dec; 221(1-2): 169-75. doi: 10.1016/S0022-1759(98)00183-5

[15] Bilen A, Calik I, Yayla M, Dincer B, Tavaci T, Cinar I, *et al*. Does daily fasting shielding kidney on hyperglycemia-related inflammatory cytokine via TNF-α, NLRP3, TGF-β1 and VCAM-1 mRNA expression. International Journal of Biological Macromolecules. 2021 Nov; 190: 911-8. doi: 10.1016/j.ijbiomac.2021.08.216

[16] Reidy K, Kang HM, Hostetter T, Susztak K. Molecular mechanisms of diabetic kidney disease. The Journal of Clinical Investigation. 2014 Jun; 124(6): 2333-40. doi: 10.1172/JCI72271

[17] Buha I, Škodrić-Trifunović V, Adžić-Vukičević T, Ilić A, Blanka-Protić A, Stjepanovic M, *et al*. Relevance of TNF-α, IL-6 and IRAK1 gene expression for assessing disease severity and therapy effects in tuberculosis patients. The Journal of Infection in Developing Countries. 2019 May; 13(05): 419-25. doi: 10.3855/jidc.10949

[18] Kalantarinia K, Awad AS, Siragy HM. Urinary and renal interstitial concentrations of TNF-α increase prior to the rise in albuminuria in diabetic rats. Kidney International. 2003 Oct; 64(4): 1208-13. doi: 10.1046/j.1523-1755.2003.00237.x

[19] de Oliveira JG, Rossi AF, Nizato DM, Cadamuro AC, Jorge YC, Valsechi MC, *et al*. Influence of functional polymorphisms in TNF-α, IL-8, and IL-10 cytokine genes on mRNA expression levels and risk of gastric cancer. Tumor Biology. 2015 Dec; 36: 9159-70. doi: 10.1007/s13277-015-3593-x

[20] Caughey GE, Cleland LG, Gamble JR, James MJ. Up-regulation of endothelial cyclooxygenase-2 and prostanoid synthesis by platelets: role of thromboxane A2. Journal of Biological Chemistry. 2001 Oct; 276(41): 37839-45. doi: 10.1074/jbc.M010606200

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Original Article

Sensitivity and Resistivity of Various Antibiotics Against of P*seudomonas aeruginosa* in Clinical Isolates

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ABSTRACT

Antibiotics are an essential therapy for a variety of bacterial infections, but misuse and overuse of them is encouraging bacterial resistance. **Objective:** To check different drugs' antibacterial effects on *Pseudomonas aeruginosa* was the goal. **Methods:** For this experiment, samples were taken from patients in the pathology division of the Fatima Memorial Hospital in Lahore, Pakistan. From all of the samples gathered, 170 clinical isolates of *P. aeruginosa* were discovered. To identify bacteria, traditional culture and biochemical techniques were performed. Antibacterial activity was determined by comparing the antibiotic susceptibility patterns of all clinical isolates to commercial antibiotic discs (cefazolin, cefepime, cefixime, cefoxitin, ceftriaxone, ceftazidime, cefuroxime, cephalothin, amikacin, amoxycillin, ampicillin, Augmentin, ciprofloxacin, clindamycin, gentamycin, imipenem. **Results:** Imipenem (100% sensitivity), Ceftazidime (99%), Linezolid (99%), Clindamycin (99%), Gentamycin (92%), Ciprofloxacin (88%), Levofloxacin (78%), and Cefotaxime (71%), among other antibiotics, shown remarkable sensitivity against Pseudomonas aeruginosa. **Conclusions:** We came to the conclusion that all clinical isolates of *P. aeruginosa* exhibited broad resistance to meropenem, ampicillin, cefuroxime, and cefepime. To reduce antibiotic resistance, technical infrastructure must be improved. Appropriate antibiotic selection and advised hand washing are two such measures.

INTRODUCTION

P. aeruginosa is a kind of bacterium that may infect the skin, urinary system, respiratory tract, and bloodstream, among other areas of the body. Because of this bacterium's inherent and acquired resistance to antibiotics, infections caused by it are often difficult to cure. Therefore, finding novel and potent medicines is essential for stopping and containing the spread of *P. aeruginosa* infections. This review discusses the antibacterial effects of several drugs on *P. aeruginosa* clinical isolates. Numerous clinical issues are caused globally by multi-drug resistant microorganisms. It is recognized that the extensive use of antibiotics contributes to bacteria that cause nosocomial and community-acquired illnesses developing increased resistance [1]. Particularly in developing nations, infectious illnesses brought on by resistant bacteria are to blame for increasing morbidity and death rates as well as rising healthcare expenses. Nearly 10% of all surgical site, respiratory tract, and urinary tract infections acquired in hospitals are caused by the opportunistic gramme negative bacteria *Pseudomonas aeruginosa,* which often develops in conjunction with significant underlying illnesses [2, 3]. It is a primary source of morbidity owing to burn wound infection and is often linked to otitis media, nasal infections, and other conditions [4, 5]. To treat systemic infections, *P. aeruginosa* is naturally resistant to the majority of antibiotics now on the market, including

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aminoglycosides, anti-pseudomonal penicillin's, newer cephalosporins, imipenem, and fluoroquinolones [6-8]. Antibiotic resistance has now become a health problem worldwide. Every year, approximately 7 billion causalities throughout the world are as a result of infections that have developed resistance to the antibiotics used to treat them. Resistance is a common occurrence in nature. Only a few germs survive being exposed to pharmaceuticals that are supposed to kill them, and these microbes pass on their drug resistance to others. In view of the findings that overuse and abuse of antibiotics, as well as inadequate disease control, is hastening antibiotic resistance, this issue has gained prominence [9, 10]. Studies have been done to determine how well different antibiotics work against *P. aeruginosa*. According to research by Gill, carbapenems like imipenem and meropenem were very efficient against clinical isolates of *P. aeruginosa* [11, 12]. The fluoroquinolones ciprofloxacin and levofloxacin were similarly effective against *P. aeruginosa*, according to different research in North America.

METHODS

A cross-sectional investigation was conducted. The study was conducted in Lahore, Pakistan, in the pathology division of the Fatima Memorial Hospital. A total of 1,400 samples, including blood, pus, swabs, sputum, urine, CSF, and semen, were gathered from different wards at Fatima Memorial Hospital over the course of a year. Each sample was taken in a sterile container. Within an hour after being collected, the sample container was labelled with the collection time, source, and date and transported to the lab for analysis. On certain medium plates, samples from the sample container were grown (Eosin thiazine Agar, Mannitol Salt agar, TCBS Agar, MSA agar, MacConkey Agar, enteric bacteria enteric bacteria Agar). After that, the plates were stored for 24 hours at 37 °C in an incubator. The colonies were then injected onto agar plates to create pure cultures that could be stored. The colony morphology of clinical isolates on Mac-Conkey agar was used to identify them. Isolated colonies were used to study the characteristics of colonies. Standard identification and susceptibility techniques were used to the identification of these species. In gramme stained smears, gramme negative bacteria were seen as pink-colored organisms. Antibiotic susceptibility testing was used to examine the sensitivity or susceptibility of bacteria to various antibiotics as well as their patterns of resistance. The susceptibility of the bacteria to antibiotics was evaluated in this research using the Kirby-Bauer disc diffusion method. In a tube containing sterile saline solution, a colony from the plate was thoroughly mixed and aseptically emulsified. The agar plates were created by Muller Hinton. A sterile cotton swab was used to streak the dried MHA plate surface at least four to six times after being dipped into the organism's broth culture. Using sterile forceps, the antibiotic discs were positioned. After all of the discs were positioned correctly, the MHA plates were inverted and incubated at 37 0C for 24 hours. Bacterial growth was seen around each disc after incubation. A distinct region of "no growth" was seen around that particular disc if the clinical isolate was susceptible to an antibiotic. To establish whether an isolate is susceptible, intermediately susceptible, or resistant to an antibiotic, the size of the zone of inhibition for each drug is measured in millimeters using a metric ruler and compared to a standard interpretation chart. To tabulate and analyze the data, SPSS version 22.0 was utilized. Both antibiotic sensitivity and resistance were statistically evaluated. The proportion of sensitivity and resistance was used to calculate an antibiotic's antibacterial activity.

RESULTS

Antibiotics which showed high sensitivity against *Pseudomonas* species were Imipenem (100%), Ceftazidime (99%), Linezolid (99%), Clindamycin (99%), Gentamycin (92%), Ciprofloxacin (88%), Levofloxacin (78%), and Cefotaxime (71%). *Pseudomonas* species had shown high resistance to Meropenem (100%), Cefoxitin (99%), Ampicillin (99%) and Nalidixic acid (99%). Other antibiotics showing high resistance were Augmentin (99%), Cefazolin (97%). Cefepime (94%), Cefuroxime (93%), Cephalothin (92%), Cefixime (87%), Norfloxacin (70%) (table 1).

Antibacterial agent	*Pseudomonas aeruginosa* (144)	
	Sensitive n (%)	Resistance n (%)
Amikacin	141 (98.0%)	3 (2.0%)
Ampicillin	1 (1.0%)	143 (99.0%)
Augmentin	1 (1.0%)	143 (99.0%)
Cefazolin	4 (3.0%)	140 (97.0%)
Cefepime	6 (4.0%)	135 (94.0%)
Cefixime	19 (13.0%)	125 (87.0%)
Cefotaxime	101 (70.0%)	43 (30.0%)
Cefoxitin	1 (1.0%)	143 (99.0%)
Ceftazidime	143 (99.0%)	1 (1.0%)
Cefuroxime	10 (7.0%)	134 (93.0%)
Cephalothin	12 (8.0%)	132 (92.0%)
Ciprofloxacin	127 (88.0%)	17 (12.0%)
Clindamycin	143 (99.0%)	1 (1.0%)
Gentamycin	143 (99.0%)	1 (1.0%)
Imipenem	144 (100.0%)	1 (1.0%)
Levofloxacin	112 (78.0%)	32 (22.0%)
Linezolid	143 (99.0%)	1 (1.0%)
Meropenem	0 (0.0%)	144 (100.0%)
Nalidixic Acid	1 (1.0%)	143 (99.0%)
Nitrofurantoin	65 (45.0%)	79 (55.0%)
Norfloxacin	43.2 (30.0%)	100.8 (70.0%)
Ofloxacin	94 (65.0%)	50 (35.0%)

Table 1: Antibacterial activities against *Pseudomonas aeruginosa*

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DISCUSSION

It has been shown that certain resistant clinical isolates of *P. aeruginosa* are susceptible to the inhibitory effects against Imipenem, Ceftazidime, Linezolid, Clindamycin, Gentamycin (92%), Ciprofloxacin, Levofloxacin, and Cefotaxime. The fact that none of the antimicrobial drugs were successful in treating all of the multi-drug tested strains showed the present difficulty in treating nosocomial infections that are multi-drug resistant [14, 15]. *P. aeruginosa* isolates from earlier research by Servin shown partial or total resistance to antibiotics [16]. Unluckily, *P. aeruginosa* strains completely resisted ampicillin and cephalexin. Its sensitivity to other strong antibiotic substances, such as those more often used to treat hospital infections, such as ceftriaxon, chloramphenicol, cefotaxime, ceftazidime, tobramycin, piperacilin, imipenem, gentamicin, and amikacin, was also examined by Fluit *et al.*, [17-19]. Natural sources of lactic acid bacteria include dairy products, seafood, vegetables, and cereals. They guard against urinary tract infections and are a part of healthy vaginal flora. In actuality, several strains of the genus Lactobacillus are capable of colonizing certain areas of the body, such as the gastrointestinal tract, uro-genital tract, and oral cavity, where they are crucial to the competitive exclusion of pathogens. It turns out that the antimicrobial action of Lactobacillus strains against bacterial pathogens is multifaceted and involves the generation of compounds including bacteriocin-like molecules, hydrogen peroxide, lactic acid, and unknown heat-stable, non-lactic acid chemicals. Competition for nutrients, adhesion inhibition of infections to surfaces, and immune system modelling have all been postulated as additional pathways for their function. Imipenem (100% sensitivity), Gentamycin (99%), Ceftazidime (99%), Linezolid (99%), and Clindamycin (99%) were among the antibiotics that shown great sensitivity against Pseudomonas aeruginosa. Barakoti *et al.*, saw comparable outcomes [20].

CONCLUSIONS

The findings of the current investigation of *P. aeruginosa* had a higher prevalence of antibiotic resistance. In this investigation, the most effective antibacterial agents against *P. aeruginosa* bacterial infections were Linezolid, Imipenem, Amikacin, and Gentamycin. By using preventative measures, antibiotic resistance should be managed and avoided.

Conflicts of Interest

The author declares no conflict of interest.

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REFERENCES

[1] Hasan B, Perveen K, Olsen B, Zahra R. Emergence of carbapenem-resistant Acinetobacter baumannii in hospitals in Pakistan. Journal of Medical Microbiology. 2014 Jan; 63(1): 50-5. doi: 10.1099/jmm.0.063925-0

[2] Gould IM and Bal AM. New antibiotic agents in the pipeline and how they can help overcome microbial resistance. Virulence. 2013 Feb; 4(2): 185-91. doi: 10.4161/viru.22507

[3] Iredell J, Brown J, Tagg K. Antibiotic resistance in Enterobacteriaceae: mechanisms and clinical implications. Bmj. 2016 Feb; 352. doi: 10.1136/bmj.h6420

[4] Haq FU, Imran M, Saleem S, Aftab U, Ghazal A. Investigation of Morchella esculenta and Morchella conica for their antibacterial potential against methicillin-susceptible Staphylococcus aureus, methicillin-resistant Staphylococcus aureus and Streptococcus pyogenes. Archives of Microbiology. 2022 July; 204(7): 1-13. doi: 10.1007/s00203-022-03003-8

[5] Arnott A, Wang Q, Bachmann N, Sadsad R, Biswas C, Sotomayor C, *et al*. Multidrug-Resistant Salmonella enterica 4,[5], 12: i: -Sequence Type 34, New South Wales, Australia, 2016-2017. Emerging Infectious Diseases. 2018 Apr; 24(4): 751-53. doi: 10.3201/eid2404.171619

[6] Kaleem F, Usman J, Hassan A, Omair M, Khalid A, Uddin R. Sensitivity pattern of methicillin resistant Staphylococcus aureus isolatedfrom patients admitted in a tertiary care hospital of Pakistan. Iranian Journal of Microbiology. 2010; 2(3): 141-3.

[7] Walsh TR. Emerging carbapenemases: a global perspective. International journal of antimicrobial agents. 2010 Nov; 36: S8-14. doi: 10.1016/S0924-8579(10)70004-2

[8] Klemm EJ, Shakoor S, Page AJ, Qamar FN, Judge K, Saeed DK, *et al*. Emergence of an extensively drug-resistant Salmonella enterica serovar Typhi clone harboring a promiscuous plasmid encoding resistance to fluoroquinolones and third-generation cephalosporins. MBio. 2018 Mar; 9(1): e00105-18. doi: 10.1128/mBio.00105-18

[9] Siegel RE. Emerging gram-negative antibiotic resistance: daunting challenges, declining sensitivities, and dire consequences. Respiratory Care. 2008 Apr; 53(4): 471-9.

[10] Giske CG, Monnet DL, Cars O, Carmeli Y. Clinical and economic impact of common multidrug-resistant gram-negative bacilli. Antimicrobial Agents and Chemotherapy. 2008 Mar; 52(3): 813-21. doi:

DOI: https://doi.org/10.54393/fbt.v1i01.7

10.1128/AAC.01169-07

[11] Gill HS, Rutherfurd KJ, Cross ML, Gopal PK. Enhancement of immunity in the elderly by dietary supplementation with the probiotic Bifidobacterium lactis HN019. The American Journal of Clinical Nutrition. 2001 Dec; 74(6): 833-9. doi: 10.1093/ajcn/74.6.833

[12] Slama TG. Gram-negative antibiotic resistance: there is a price to pay. Critical Care. 2008 May; 12: 1-7. doi: 10.1186/cc6817

[13] Chopra I, Schofield C, Everett M, O'Neill A, Miller K, Wilcox M, *et al*. Treatment of health-care-associated infections caused by Gram-negative bacteria: a consensus statement. The Lancet Infectious Diseases. 2008 Feb; 8(2): 133-9. doi: 10.1016/S1473-3099(08)70018-5

[14] Antonio MA, Hawes SE, Hillier SL. The identification of vaginal Lactobacillus species and the demographic and microbiologic characteristics of women colonized by these species. Journal of Infectious Diseases. 1999 Dec; 180(6): 1950-6. doi: 10.1086/315109

[15] Redondo-Lopez V, Cook RL, Sobel JD. Emerging role of lactobacilli in the control and maintenance of the vaginal bacterial microflora. Reviews of Infectious Diseases. 1990 Sep; 12(5): 856-72. doi: 10.1093/clinids/12.5.856

[16] Servin AL. Antagonistic activities of lactobacilli and bifidobacteria against microbial pathogens. FEMS Microbiology Reviews. 2004 Oct; 28(4): 405-40. doi: https://doi.org/10.1016/j.femsre.2004.01.003

[17] McFarland LV. Normal flora: diversity and functions. Microbial Ecology in Health and Disease. 2000 Jan; 12(4): 193-207. doi: 10.1080/08910600050216183

[18] Reid G and Burton J. Use of Lactobacillus to prevent infection by pathogenic bacteria. Microbes and infection. 2002 Mar; 4(3): 319-24. doi: 10.1016/S1286-4579(02)01544-7

[19] Fluit A, Verhoef J, Schmitz F, Sentry Participants TE. Frequency of isolation and antimicrobial resistance of gram-negative and gram-positive bacteria from patients in intensive care units of 25 European university hospitals participating in the European arm of the SENTRY Antimicrobial Surveillance Program 1997-1998. European Journal of Clinical Microbiology and Infectious Diseases. 2001 Sep; 20: 617-25. doi: 10.1007/s100960100564

[20] Barakoti A, Guragain A, Adhikari RP, Amatya R. Profile and antimicrobial susceptibility pattern of aerobic bacterial isolates from pus/wound swab samples in a tertiary care hospital, Kathmandu. Nepal Medical College Journal. 2017 Jan; 19(4): 179-83.