Evaluation of Bacteriological Quality
Comparison of CHROMagar Orientation

Highlights

Signs of Herd immunity in COVID-19
Lung Development and Tumorigenesis

Discovering Thoughts, Inventing Future
# Editorial Board

**Global Journal of Medical Research**

<table>
<thead>
<tr>
<th>Name</th>
<th>Title/Position</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Dr. Apostolos Ch. Zarros</strong></td>
<td>DM, Degree (Ptychio) holder in Medicine, National and Kapodistrian University of Athens, MRes, Master of Research in Molecular Functions in Disease, University of Glasgow FRNS, Fellow, Royal Numismatic Society Member, European Society for Neurochemistry Member, Royal Institute of Philosophy Scotland, United Kingdom</td>
</tr>
<tr>
<td><strong>Dr. William Chi-shing Cho</strong></td>
<td>Ph.D., Department of Clinical Oncology, Queen Elizabeth Hospital, Hong Kong</td>
</tr>
<tr>
<td><strong>Dr. Alfio Ferlito</strong></td>
<td>Professor Department of Surgical Sciences, University of Udine School of Medicine, Italy</td>
</tr>
<tr>
<td><strong>Dr. Michael Wink</strong></td>
<td>Ph.D., Technical University Braunschweig, Germany, Head of Department Institute of Pharmacy and Molecular Biotechnology, Heidelberg University, Germany</td>
</tr>
<tr>
<td><strong>Dr. Jixin Zhong</strong></td>
<td>Department of Medicine, Affiliated Hospital of Guangdong Medical College, Zhanjiang, China, Davis Heart and Lung Research Institute, The Ohio State University, Columbus, OH 43210, US</td>
</tr>
<tr>
<td><strong>Dr. Pejcić Ana</strong></td>
<td>Assistant Medical Faculty Department of Periodontology and Oral Medicine University of Nis, Serbia</td>
</tr>
<tr>
<td><strong>Rama Rao Ganga</strong></td>
<td>MBBS, MS (University of Health Sciences, Vijayawada, India), MRCS (Royal College of Surgeons of Edinburgh, UK), United States</td>
</tr>
<tr>
<td><strong>Dr. Ivandro Soares Monteiro</strong></td>
<td>M.Sc., Ph.D. in Psychology Clinic, Professor University of Minho, Portugal</td>
</tr>
<tr>
<td><strong>Dr. Izzet Yavuz</strong></td>
<td>MSc, Ph.D., D Ped Dent., Associate Professor, Pediatric Dentistry Faculty of Dentistry, University of Dicle Diyarbakır, Turkey</td>
</tr>
<tr>
<td><strong>Sanguansak Rerksuppaphol</strong></td>
<td>Department of Pediatrics Faculty of Medicine, Srinakharinwirot University, Nakorn Nayok, Thailand</td>
</tr>
<tr>
<td><strong>Dr. Sanjay Dīxit, M.D.</strong></td>
<td>Director, EP Laboratories, Philadelphia VA Medical Center, Cardiovascular Medicine - Cardiac Arrhythmia, Univ of Penn School of Medicine, Web: pennmedicine.org/wagform/MainPage.aspx?</td>
</tr>
<tr>
<td><strong>Anthony Simone Laganà</strong></td>
<td>M.D. Unit of Gynecology and Obstetrics, Department of Human Pathology in Adulthood and Childhood “G. Barresi” University of Messina, Italy</td>
</tr>
<tr>
<td><strong>Dr. Han-Xiang Deng</strong></td>
<td><strong>Dr. Pina C. Sanelli</strong></td>
</tr>
<tr>
<td>------------------------</td>
<td>------------------------</td>
</tr>
<tr>
<td>MD, Ph.D</td>
<td>Associate Professor of Radiology</td>
</tr>
<tr>
<td>Associate Professor and Research Department</td>
<td>Associate Professor of Public Health</td>
</tr>
<tr>
<td>Division of Neuromuscular Medicine</td>
<td>Weill Cornell Medical College</td>
</tr>
<tr>
<td>Davee Department of Neurology and Clinical Neurosciences</td>
<td>Associate Attending Radiologist</td>
</tr>
<tr>
<td>Northwestern University Feinberg School of Medicine</td>
<td>NewYork-Presbyterian Hospital</td>
</tr>
<tr>
<td>Web: neurology.northwestern.edu/faculty/deng.html</td>
<td>MRI, MRA, CT, and CTA</td>
</tr>
<tr>
<td></td>
<td>Neuroradiology and Diagnostic Radiology</td>
</tr>
<tr>
<td></td>
<td>M.D., State University of New York at Buffalo, School of Medicine and Biomedical Sciences</td>
</tr>
<tr>
<td></td>
<td>Web: weillcornell.org/pinasanelli/</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>Dr. Roberto Sanchez</strong></th>
<th><strong>Dr. Michael R. Rudnick</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>Associate Professor</td>
<td>M.D., FACP</td>
</tr>
<tr>
<td>Department of Structural and Chemical Biology</td>
<td>Associate Professor of Medicine</td>
</tr>
<tr>
<td>Mount Sinai School of Medicine</td>
<td>Chief, Renal Electrolyte and Hypertension Division (PMC)</td>
</tr>
<tr>
<td>Ph.D., The Rockefeller University</td>
<td>Penn Medicine, University of Pennsylvania</td>
</tr>
<tr>
<td>Web: mountsinai.org/</td>
<td>Presbyterian Medical Center, Philadelphia</td>
</tr>
<tr>
<td></td>
<td>Nephrology and Internal Medicine</td>
</tr>
<tr>
<td></td>
<td>Certified by the American Board of Internal Medicine</td>
</tr>
<tr>
<td></td>
<td>Web: uphs.upenn.edu/</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>Dr. Feng Feng</strong></th>
<th><strong>Dr. Seung-Yup Ku</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>Boston University</td>
<td>M.D., Ph.D., Seoul National University Medical College, Seoul, Korea</td>
</tr>
<tr>
<td>Microbiology</td>
<td>Department of Obstetrics and Gynecology</td>
</tr>
<tr>
<td>72 East Concord Street R702</td>
<td>Seoul National University Hospital, Seoul, Korea</td>
</tr>
<tr>
<td>Duke University</td>
<td>United States of America</td>
</tr>
<tr>
<td>United States of America</td>
<td></td>
</tr>
<tr>
<td>Web: uphs.upenn.edu/</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>Dr. Hrushikesh Aphale</strong></th>
<th><strong>Santhosh Kumar</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>MDS- Orthodontics and Dentofacial Orthopedics. Fellow- World Federation of Orthodontist, USA.</td>
<td>Reader, Department of Periodontology, Manipal University, Manipal</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>Gaurav Singhal</strong></th>
<th><strong>Dr. Aarti Garg</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>Master of Tropical Veterinary Sciences, currently pursuing Ph.D in Medicine</td>
<td>Bachelor of Dental Surgery (B.D.S.) M.D.S. in Pedodontics and Preventive Dentistry Pursuing Phd in Dentistry</td>
</tr>
<tr>
<td>Name</td>
<td>Title/Qualifications</td>
</tr>
<tr>
<td>-----------------------------</td>
<td>--------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Sabreena Safuan</td>
<td>Ph.D (Pathology) MSc (Molecular Pathology and Toxicology) BSc (Biomedicine)</td>
</tr>
<tr>
<td>Arundhati Biswas</td>
<td>MBBS, MS (General Surgery), FCPS, MCh, DNB (Neurosurgery)</td>
</tr>
<tr>
<td>Getahun Asebe</td>
<td>Veterinary medicine, Infectious diseases, Veterinary Public health, Animal Science</td>
</tr>
<tr>
<td>Rui Pedro Pereira de Almeida</td>
<td>Ph.D Student in Health Sciences program, MSc in Quality Management in Healthcare Facilities</td>
</tr>
<tr>
<td>Dr. Suraj Agarwal</td>
<td>Bachelor of dental Surgery Master of dental Surgery in Oromaxillofacial Radiology. Diploma in Forensic Science &amp; Odoontology</td>
</tr>
<tr>
<td>Dr. Sunanda Sharma</td>
<td>B.V.Sc.&amp; AH, M.V.Sc (Animal Reproduction, Obstetrics &amp; gynaecology), Ph.D.(Animal Reproduction, Obstetrics &amp; gynaecology)</td>
</tr>
<tr>
<td>Osama Alali</td>
<td>PhD in Orthodontics, Department of Orthodontics, School of Dentistry, University of Damascus, Damascus, Syria. 2013 Masters Degree in Orthodontics.</td>
</tr>
<tr>
<td>Shahanawaz SD</td>
<td>Master of Physiotherapy in Neurology PhD- Pursuing in Neuro Physiotherapy Master of Physiotherapy in Hospital Management</td>
</tr>
<tr>
<td>Prabudh Goel</td>
<td>MCh (Pediatric Surgery, Gold Medalist), FISPU, FICS-IS</td>
</tr>
<tr>
<td>Dr. Shabana Naz Shah</td>
<td>PhD. in Pharmaceutical Chemistry</td>
</tr>
<tr>
<td>Raouf Hajji</td>
<td>MD, Specialty Assistant Professor in Internal Medicine</td>
</tr>
<tr>
<td>Vaishnavi V.K Vedam</td>
<td>Master of dental surgery oral pathology</td>
</tr>
<tr>
<td>Surekha Damineni</td>
<td>Ph.D with Post Doctoral in Cancer Genetics</td>
</tr>
<tr>
<td>Tariq Aziz</td>
<td>PhD Biotechnology in Progress</td>
</tr>
</tbody>
</table>
CONTENTS OF THE ISSUE

i. Copyright Notice
ii. Editorial Board Members
iii. Chief Author and Dean
iv. Contents of the Issue

2. Comparison of CHROMagar Orientation versus CLED (cystine–lactose–electrolyte-deficient) Agar, VITEK-XL and MALDI-TOF in a Tertiary Laboratory Setting Processing Urine Culture Samples at Dr. Lal Path Labs, Delhi. 11-20
3. Evaluation of Bacteriological Quality of Periwinkle Snail (Tymanotonus Fuscatus) Collected from Ilaje, Nigeria and Isolates’ Susceptibility Pattern to Ocimum Gratissimum. 21-25

v. Fellows
vi. Auxiliary Memberships
vii. Preferred Author Guidelines
viii. Index
Characteristics Shared between Lung Development and Tumorigenesis: Mini Review Article

By Josenel Maria Barcelos Marçal, Júlia Iaroseski, Luiz Fillipe Pinto da Silva, Thales Augusto Della Torre Marzarotto, Giulia Righetti Tuppini Vargas, Vitória Morita Fukuoka, Luiza Carolina da Rosa Scherner, Rafael Fabiano Machado Rosa & Paulo Ricardo Gazzola Zen

Universidade Federal de Ciências da Saúde de Porto Alegre (UFCSPA)

Abstract- Cells with characteristics of embryonic stem cells, and cancer stem cells are at the basis of both embryo development and the cancer process. At the same time, they share signaling pathways, such as the hedgehog, Notch, Wnt, TGF beta, among others. This knowledge is important for understanding the pulmonary regeneration process and for the development of new target therapies.

Keywords: embryogenesis, alveolarization, lung, tumorigenesis, molecular biology, signaling pathways.

GJMR-C Classification: NLMC Code: WF 600

© 2021. Josenel Maria Barcelos Marçal, Júlia Iaroseski, Luiz Fillipe Pinto da Silva, Thales Augusto Della Torre Marzarotto, Giulia Righetti Tuppini Vargas, Vitória Morita Fukuoka, Luiza Carolina da Rosa Scherner, Rafael Fabiano Machado Rosa & Paulo Ricardo Gazzola Zen. This is a research/review paper, distributed under the terms of the Creative Commons Attribution-Noncommercial 3.0 Unported License http://creativecommons.org/licenses/by-nc/3.0/), permitting all non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.
**Abstract** - Cells with characteristics of embryonic stem cells, and cancer stem cells are at the basis of both embryo development and the cancer process. At the same time, they share signaling pathways, such as the hedgehog, Notch, Wnt, TGF beta, among others. This knowledge is important for understanding the pulmonary regeneration process and for the development of new target therapies.

**Keywords:** embryogenesis, alveolarization, lung, tumorigenesis, molecular biology, signaling pathways.

**Introduction**

The understanding of lung development during embryogenesis and the knowledge of several cell populations is essential for regenerative medicine and for the recognition of the cell of origin of lung neoplasms.

Several evidences suggest that the human lung contains a population of characteristic stem cells. This statement is explained by the fact that most patients with small cell lung cancer (CPCP) already have metastases, resistance or refractoriness to chemotherapy treatment at the moment. Likewise, patients with adenocarcinomas that express tyrosine kinase (EGFR) mutations, and who are initially sensitive to therapy, also acquire resistance. (Kobayashi et al. 2005; Pao et al. 2005; Kosaka et al. 2006).

Another piece of evidence was the identification of cells from the lateral population, isolated by their ability to efflux the Hoechst dye and which exhibit increased expression of drug transporters, tumor propagation capacity and resistance to multiple chemotherapies (Ho et al. 2007). It was identified that CD133 positive lung tumor cells formed self-renewing spheres in culture with tumor propagation, when transplanted subcutaneously in immunodeficient mice (Erasmus et al. 2008).

With this review we intend to define the cell types and molecular biology data of the lung in embryogenesis and in the adult lung, drawing points of comparison and trying to correlate with the development of neoplasms.

**1. Pulmonary Development Stages**

The lung has a large internal surface and an airway conduction system with several branches. Conductive airways are formed first, followed by the formation and enlargement of the gas exchange area. Alveolarization is the last stage of the fetal period and continues in the postnatal period.

In the embryonic period, between 4-7 gestational weeks organogenesis occurs. The left and right lungs have their own ring, an external pouch of the anterior intestine (Cardoso and Lu 2006). Each pulmonary bud initiates a repetitive process of growth and branched morphogenesis to form future airways (Schittny and Burri 2008). Epithelial cells are supported by a basement membrane, surrounded by an extracellular matrix that is produced by mesenchymal cells. The components of the extracellular matrix, including the basement membrane, are different in the terminal bud, in the branching points and in the most proximal portions of the bronchial tree, where epithelial differentiation has already started (Schittny and Burri2004). The branching is coordinated by epithelial and mesenchymal cells, growth factors and transcription factors that the cells are producing.

The fetal period includes the pseudoglandular, canalicular and saccular stages. The postnatal lung period comprises the stages of classic and continuous alveolarization, as well as microvascular maturation. As most processes during lung development begin in the proximal area and extend to the periphery, all phases of lung development overlap (Schittny and Burri 2008; Wood and Schittny 2016).

The expression of growth factors, such as fibroblast growth factor 10 (FGF-10), bone morphogenetic protein 4 (BMP-4), Sonic Hedgehog (Shh), retinoic acid, Notch and TGF-β provide the instructions for
ramification in the period of morphogenesis. (Cardoso and Lu 2006; Hines and Sun 2014; Schittny and Burri 2008)

The negative feedback mechanism involves signaling by Shh (Sonic hedgehog), its Ptc1 receptor (Patched 1) and transcription factors such as Gli1-3, belonging to the Shh signaling pathway. The Shh pathway acts by inhibiting local expression of Fgf10, preventing branching from occurring indefinitely. The process also depends on complex regulation by signaling pathways of the TgfB (Transforming Growth Factor Beta) family members, Wnt (Wingless-type) and Bmp4 (Bone Morphogenetic Protein 4, from the Tgf Beta family) (Park 1998; Rock and Hogan 2011; Katton and Morrisey 2014).

The Fgf (fibroblast Growth Factor) signaling pathway is activated by localized expression of Fgf10 in the mesoderm and its receptor Fgfr2 in the endoderm. This signaling induces branching, and Fgf10 stimulates the proliferation of epithelial cells. (Rock and Hogan 2011; Katton and Morrisey 2014).

Around the 10th gestational week, respiratory movements begin that cause additional stretching of the fetal lung tissue (Koos and Rajace2014). These stimuli positively regulate the release of serotonin, promoting epithelial differentiation (Pan et al 2006).

A continuous layer of positive cells for a smooth muscle actin begins to form around the future proximal airways, becoming discontinuous distally in the bronchial tree and ending in front of the terminal buds. These contractile cells begin to perform spontaneous contractions, pushing peristaltic waves of interbronchial fluid to the periphery. These movements, too, stimulate branched morphogenesis and prevent uncontrolled airway expansion as lung fluid is secreted into the lung (Schittny et al. 2000; Sparrow et al. 1994).

The canalicular stage occurs between 16-26 gestational weeks and comprises the differentiation of the epithelium that allows the morphological distinction between the airways (acino / ventilatory unit) (Winkelmann and Noack 2010).

At the junction of the bronchioalveolar duct (BADJ), there is an abrupt change in the epithelium from hair cells and from Clear cells to type I and type II alveolar epithelial cells (Winkelmann and Noack 2010). This junction is formed at the canalicular stage, when epithelial differentiation occurs and is of particular importance, because it represents a niche of stem cells (McQuuter et al. 2010). It has recently been shown that the junction bronchioalveolar remains constant throughout the lung development in the generation of the airways, where it was originally formed (Barre et al. 2014; 2016).

The saccular stage occurs between 24-38 gestational weeks and represents an intermediate stage, when the branching morphogenesis ceases and the alveolarization has not yet started (Cardoso and Lu 2006; Morrisey et al. 2013). They are coated by type 1 and type 2 cells (Cardoso; 2006; Rock and Hogan, 2011).

At the end of the saccular stage, the mesenchyme located between the future airways contains a loose three-dimensional vascular network in proliferation, due to intense angiogenesis, conferring a high capillary density. The future airways that will become alveolar ducts grow in width and length, change shape and appear as "canaliculi", which form the canalization of the mesenchyma through the airways and capillaries. The growth of the airways and apoptosis cause condensation of the mesenchyme, where the volume and the total number of mesenchymal cells decrease (Rogelj et al. 1989).

In parallel with alveolarization, the double layer capillary network of immature septa merges with a single layer network, resulting in an optimized configuration for gas exchange. Alveolarization still continues, because, in places where new septa are shedding pre-existing mature septa, the second necessary capillary layer will be formed instantly by angiogenesis, confirming a lifelong alveolarization capacity, which is important for any type of lung regeneration.

The lung mesoderm represents a source of essential paracrine instructional signals that regulate the proliferation and differentiation of the endoderm progenitor and also contributes to the various lung structures, including airway smooth muscle, vascular smooth muscle, endothelial cells, mesothelial cells and many less known mesodermal strains, such as pericytes, alveolar fibroblasts and lipofibroblasts. The lung mesoderm is believed to originate from the initial mesoderm that surrounds the ventral anterior intestine.

II. Pulmonary Cell Types

a) Embryonic Lung Cells

The embryonic pulmonary epithelium differentiates into hair, serous secretory cells, goblet cells, clear cells, basal cells and neuroendocrine (NE) cells. The proportions of these cells vary along the proximal-distal axis. In bronchioles, clear cells are more abundant than the ciliated ones, with some groupings of NE cells, called neuroepithelial bodies or NEBs. Goblet cells are marked by the expression of the transcription factor SPDEF and mucin-5ac (Muc5ac). (Morrisey and Hoghan, 2010).

Neuroendocrine (NE) cells are the first epithelial cells to appear in the lung and are more abundant in fetal and neonatal lungs than in the adult lung, basic ID2 helix loop. These multipotent cells have the ability to give rise to all major types of respiratory epithelial cells, including PNECs (pulmonary neuroendocrine cells) (Rawlins et al 2009).
The evidence suggests that the specification of the fate of PNECs is controlled by interference between bHLH activating and repressing genes, a conserved mechanism between Drosophila and mammals (Ito et al. 2006).

The ASCL1 complex activates NE differentiation, while the HES 1 gene suppresses this pathway, inhibiting the formation of the ASCL1 / TCF3 complex.

Notch signaling was also important in specifying the PNEC lineage. The delta-like Notch ligand 1 (DLL1) is expressed in NE cells in the proximal airways. Its activity may be under the control of ASCL1. Notch 2 mediates the fate of clear hair cells.

Finally, the migration control program for normal pulmonary neuroendocrine cells and malignant cells is extremely relevant for the understanding and treatment of metastasis of small cell lung cancer. Recently, a new form of epithelial cell migration shown by normal pulmonary neuroendocrine cells during pulmonary epithelial development has been demonstrated, called 'sliding', which is used to organize neuroendocrine cells into stereotyped groups (Kou 2015). The normal sliding program involves the transient activation of an epithelial-mesenchymal transition (EMT), in which the pulmonary neuroendocrine cells migrate over and around other epithelial cells to meet, without ever invading the lung mesenchyma. The pulmonary NE cells are distributed throughout the bronchial epithelium, interspersed between secretory cells (Claras) and hair cells, the two main types of airway epithelial cells (Rock and Hogan, 2011; Semenova 2015). The clusters of NE or (NEBs) are highly innervated (Brouns et al., 2008) and have sensory and neurosecretory functions; stem cell function, which helps to replace the bronchial epithelium after severe injury (Guha et al., 2012; Reynolds et al., 2000; Song et al., 2012); and function of small cell lung cancer cell initiator (Song et al., 2012; Kuo 2015).

Further investigation into the sliding program is likely to reveal molecular dependencies directed at small cell carcinoma to attenuate or perhaps even prevent metastasis to extrapulmonary organs, which is the main cause of patient death (Semenova 2015; Kuo, 2015).

III. Pulmonary Cells of Adults

Pulmonary epithelial cells are largely subdivided into airways (tracheal / bronchiolar) and alveolar types. The tracheobronchial airways are lined with pseudostratified epithelium in which each cell comes into contact with the basement membrane. Below the basement membrane are blood and lymph vessels, smooth muscle, cartilage, fibroblasts and nerves (Hogan et al., 2014). The most distal intrapulmonary conduction airways are lined by simple columnar epithelium. The gas exchange is performed inside the alveolar epithelium.

PNECs represent only 0.4% of adult epithelial cells and have endocrine and neuronal cell properties. They express neural markers, such as NCAM1 and ASCL1 neural cell adhesion molecules (Chanda et al. 2014). They are associated with intraepithelial nerve fibers and can transmit signals to the central nervous system. Generally, it contains electron-dense vesicles, which accumulate peptides, related to the bombesin and calcitonin gene (CGRP), which acts as a vasodilator; and to the amines, represented by serotonin, which act as a vasoconstrictor. The functions of PNECs include control of airway tone, pulmonary blood flow and immunomodulation.

Brush cells make up less than 1% of the airway epithelium and have recently been shown to have a chemosensory role that can allow the detection of bacterial infections (Tizzano et al., 2011). Basal cells are stem cells that self-renew and differentiate into secretory and hair cells during homeostasis and repair (Teixeira et al., 2013; Watson et al., 2015). Secretory cells are predominantly of the mucous subtype. It is not clear whether mucus-secreting cells retain the ability to proliferate and function as stem / progenitor cells (Teixeira et al., 2013).

The alveolar epithelium consists of type I and type II alveolar cells (AT1 and AT2 cells) that are surrounded by capillaries and fibroblasts (Weibel, 2015). AT1 cells are flat, highly extended and specialized for gas exchange. AT2 cells are cuboidal, more common and specialized in the production of surfactant, a complex mixture of proteins and phospholipids that reduces surface tension in the alveolar region (Crapo et al., 1982; Hogan et al., 2014; Weibel, 2015; Williams, 2003). AT2 cells are the main alveolar epithelial stem cells and can self-renew and differentiate into AT1 cells (Barkauskas et al., 2013; Desai et al., 2014; Rock et al., 2011).

Traditionally, alveolar fibroblasts have been characterized mainly as myofibroblasts and lipofibroblasts, but their exact roles have not yet been defined and there are controversies about the existence of lipofibroblasts in human lungs. (Bhattacharya and Westphalen, 2016).

The lung also contains a resident population of immune cells and alveolar macrophages, which play important roles in surfactant homeostasis and innate immunity (Bhattacharya and Westphalen, 2016).

IV. Molecular Regulation in Embryogenesis

The first indication of the respiratory precursor in the endoderm of the primitive intestinal tube is registered by the expression of TTF1 (Thyroid Transcriptional factor 1, homeobox or NKx2.1 type transcription factor) where the thyroid and lungs will be formed (Cardoso and Lu 2006).
CK8 / CK18 cytokeratins are the first keratins to appear in embryogenesis, already in pre-implantation embryos and also appear to be the oldest keratins during phylogenesis (Jackson, 1980; Blumenberg 1988). With respect to malignant tumors, K8 and K18 flush strongly the majority of adenocarcinomas, hepatocellular carcinomas, renal cell carcinomas and neuroendocrine carcinomas. These keratins can be useful in diagnostic immunohistochemistry in cases of carcinomas with low keratin content, such as small cell lung cancer, to prove their epithelial nature (Moll, 2008).

P63 plays a prominent role in controlling the functions of epithelial stem cells and in differentiating and stratifying tissue derived from ectoderm during embryonic development. (Guerrini, 2011)

The transcription factor Sox2 marks proximal epithelial progenitors and Sox9 marks distal epithelial progenitors. Additional markers of Sox9 positive distal progenitor cells include surfactant proteins, such as surfactant protein C (Stipc), the transcription factor Id2. Lineage screening studies have suggested that positive distal Id2 cells can generate distal and proximal cell lines. This capacity for multipotent differentiation is subsequently lost, and positive Id2 progenitor cells may form only distal alveolar epithelia (Rawlins 2018).

All early events in lung development are controlled by a variety of signaling pathways, including Fgf, Tgfb, Wnt, SOX, Hedgehog (Shh-Sonic hedgehog, its Patched 1 receptor and transcription factors like Gli1 - helix family) that is fundamental in the formation of this cell type (Guilhemont 1993).

Basal cells are identified by the expression of specific molecular markers Trp-63 (Transcription Factor Transformation-related Protein or P63), cytokeratin 14 (Krt14) and cytokeratin 5 (Krt5) (School 2004)

Evidence indicates that basal cells comprise a population of multipotent parents (Rowlins and Hogan 2006).

Antigens such as ICAM-I (Intercellular Adhesion Molecule) are abundantly expressed by type I pneumocytes and by expression of Type I Caveolins (Transmembrane Proteins). Type II pneumocytes express proteins associated with pulmonary surfactants, such as SP-A, SP-B, SP-C and SP-D (Costa 2001). Transcription factors such as cat-6, TTF1, Hnf3 / 3, C / ebpa, hormones glucocorticoids and Fgfs are involved in the differentiation of pneumocyte II (Cardoso 1997).

With aging, human lung functions decrease at a rate of 1% per year after the age of 25, even without lung diseases (Janssens et al., 1999; Sharma and Goodwin, 2006). The lung starts to exhibit several changes, including increased secretion of pro-inflammatory cytokines, attenuated immune response and changes in the structural proteins of the extracellular membrane (Meiners et al., 2015; Navarro and Driscoll, 2017). Structural changes occur, such as spaces increased air space, loss of surface area and decreased static elastic recoil, with the most significant decline in the number and functions of capillary endothelial cells (Thurlbeck and Angus, 1975).

Fases do desenvolvimento pulmonary e mediadores

V. Epigenetic Regulators of Fate and Differentiation of Pulmonary Epithelial Cells

Recent studies have also identified epigenetic mechanisms of histone changes in the control of lung development. Acetylation through Histone acetyltransferase (HATs) promotes genetic transcription, and deacetylated histone (HDACs) removes the acetyl group, leading to genetic silencing (Choudhary et al., 2009). There is evidence that HATs are necessary for embryonic lung development. The loss of Hdac in the pulmonary epithelium results in reduced expression of Sox2, preventing the development of multiple types of proximal cells (Wang et al., 2013). This change in Sox2
expression is, in part, mediated by increased expression of Bmp4, which also contributes to the severe branching defects seen in Hdac mutants. It has also been shown that hyperoxia during neonatal development results in decreased hdac activity, leading to alveolar hyperplasia and interrupted alveolarization (Zhu et al., 2012).

Although histone acetylation is known to play an important role in the lung, little is known about the roles of other epigenetic complexes during lung development. The methyltransferases Suv39H1 and Suv39H2, which induce transcriptional silencing through histone H3 lysine 9 methylation, directly repress the expression of the surfactant protein SP-A (Sftpa1) during hypoxia (Benlhabib and Mendelson, 2011). Suv39H1 and Suv39H2 are also highly expressed in early lung development, suggesting that they may inhibit SP-A transcription until later in development. During pulmonary fibrosis, DNA methylation by Dnmt1 represses the transcription of miR17–92, a microRNA cluster that regulates lung development (Dakhlallah et al., 2013). Likewise, Dnmt1 mediates the progression of lung cancer through the methylation of several promoter regions (Dakhlallah et al., 2013).

VI. Conclusion

Recent studies demonstrate that cell signaling and gene expression pathways, including PTEN, protein kinase C (iota), Wnt, hedgehog, c-kit, Akt and others that can play important roles in the transformation of endogenous progenitor cells into cancer cells lung. Pluripotent stem cells (PSCs) can be derived from the internal cell mass of the initial embryo (in the case of embryonic stem cells, ESCs) or can be reprogrammed from fully differentiated cells (in the case of iPSCs). They retain the potential to differentiate into any type of cell in the body. For this reason, we can say that our organoid system provides a genetically treatable tool and, therefore, specific human characteristics of lung development should be investigated investigated. (Nicolic 2017).

Disclosure Statement
The authors declare no conflict of interest

References Références Referencias


Characteristics Shared between Lung Development and Tumorigenesis: Mini Review Article


44. Li R., Herriges J. C., Chen L., Mecham R. P. and Sun X. FGF receptors control alveolar elastogenesis. Development.2017, 144, 4563-4572. 10.1242/dev.149443


Comparison of CHROMagar Orientation versus CLED (cystine-lactose-electrolyte-deficient) Agar, VITEK-XL and MALDI-TOF in a Tertiary Laboratory Setting Processing Urine Culture Samples at Dr. Lal Path Labs, Delhi

By Puneeta Singh, Shalabh Malik & Vandana Lal

Abstract- Aims: To comparatively assess the performance and evaluate the advantages of CHROMagar orientation vs. CLED agar for the detection and enumeration of the most common yeast, gram-positive and gram-negative urinary tract pathogens.

Methods: Five hundred and eighty-seven fully characterized isolates (372 Gram-negative bacteria, 106 Gram-positive bacteria, 13 Candida spp. and 96 mixed culture) were used to test for accuracy of organism identification. To assess isolation rates of common urine isolates and ability to detect mixed cultures, 2500 urine samples were tested by parallel inoculation on the two best-performing media, CHROMagar orientation and CLED.

Results: Of the 2550 urine specimens, 587 (23.1%) yielded positive cultures, of which 491 (83.6%) were pure cultures and 96 (16.4%) were mixed cultures. CLED, CHROMagar orientation agar gave detection rates of 78.8% and 99.4% respectively.

Keywords: urine culture, CHROMagar orientation, CLED agar, presumptive identification.

GJMR-C Classification: NLMC Code: QW 570, QW 4

Strictly as per the compliance and regulations of:

© 2021. Puneeta Singh, Shalabh Malik & Vandana Lal. This is a research/review paper, distributed under the terms of the Creative Commons Attribution-Noncommercial 3.0 Unported License http://creativecommons.org/licenses/by-nc/3.0/), permitting all non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.
Comparison of CHROMagar Orientation versus CLED (cystine–lactose–electrolyte-deficient) Agar, VITEK-XL and MALDI-TOF in a Tertiary Laboratory Setting Processing Urine Culture Samples at Dr. Lal Path Labs, Delhi

Puneeta Singh *, Shalabh Malik ‡ & Vandana Lal §

Abstract- Aims: To comparatively assess the performance and evaluate the advantages of CHROMagar orientation vs. CLED agar for the detection and enumeration of the most common yeast, gram-positive and gram-negative urinary tract pathogens.

Methods: Five hundred and eighty-seven fully characterized isolates (372 Gram-negative bacteria, 106 Gram-positive bacteria, 13 Candida spp. and 96 mixed culture) were used to test for accuracy of organism identification. To assess isolation rates of common urine isolates and ability to detect mixed cultures, 2500 urine samples were tested by parallel inoculation on two best-performing media, CHROMagar orientation and CLED.

Results: Of the 2550 urine specimens, 587(23.1%) yielded positive cultures, of which 491(83.6%) were pure cultures and 96(16.4%) were mixed cultures. CLED, CHROMagar orientation agar gave detection rates of 78.8% and 99.4% respectively. The main difference in non-detection between CLED agar and the CHROMagar orientation media concerned Gram-positive strains. Based on the total number of strains detected (N=587), the total identification rates of E. coli, Pseudomonas, Acinetobacter spp. and Enterococcus spp. on CHROMagar orientation were 100%, 100%, 85.7%, and 100%, and CLED agar were 98.8%, 90.7%, 42.8%, and 58.9% respectively. The most important finding of this study towards Enterococcus faecalis and Enterococcus faecium were easily differentiated on CHROMagar orientation with 99.9% accuracy. The CHROMagar orientation performing best and detected more mixed cultures than did the CLED medium, although the differences became largely in Enterococcus/isolation rates.

Conclusion: CHROMagar orientation was found useful as a primary urine culture medium in both higher rate of isolation and presumptive identification of uropathogens and use as a replacement of conventional CLED agar. It would improve the detection rate of contaminated urine samples to enhanced identification that helps to distinguish species, facilitating the monitoring of bacterial resistance in support of the national antibiotic strategy.

Keywords: urine culture, CHROMagar orientation, CLED agar, presumptive identification.

I. Introduction

Urinary tract infections (UTIs) are the second most common infections, only after respiratory tract infections. Conventionally, Blood agar (BA), Mac Conkey agar (MAC), and Cystine Lactose Electrolyte Deficient (CLED) medium used routinely for processing of urine samples[1]. Several chromogenic media are now available, which are used to allow more specific and direct differentiation of bacterial colonies on the primary plate itself [1-9]. The following study conducted to evaluate the advantages of CHROMagar orientation over isolation of most common urine isolates (E.coli, Enterococcus faecalis, E. faecium, Staphylococcus aureus, streptococcus spp. Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa, Proteus mirabilis and Enterobacter species& Candida species) represent a global threat to human health[2-6]. Urine samples contribute greatly to the daily workload of a microbiology laboratory. CHROMagar orientation has the advantage of being technically simple, rapid and cost-effective method for the diagnosis of urinary tract infections as compared to the conventional methods [6,9].

In our lab continually, we strive to streamline and improve their urine culture algorithms because we received high volumes of urine specimens and the modest numbers of different species of bacteria that are ultimately considered clinically significant. In the current study, we quantitatively measured the impact of CHROMagar orientation media used as tools in the early differentiation and identifying of bacterial isolates from urine specimens. We have evaluated the CHROMagar orientation, a newly introduced chromogenic medium, for its utility as primary isolation and identification medium for correctly identify more-frequently occurring bacteria and yeasts organism groups on primary culture with no further testing or a minimum number of confirmatory tests. Substrates present in chromogenic
media target specific classes of enzymes produced by certain bacteria and yeasts [6].

CHROMagar orientation media may facilitate improved sensitivity of identifying some Gram-positive cocci (e.g., Enterococci) in mixed cultures with Enterobacteriaceae. They may promote the unique interpretation of urine culture plates by less experienced bench technologists [4]. The purpose of the current study for implementing CHROMagar orientation could be realized by use as the primary medium for urine culture and reduce workload of test, turnaround time, and labor costs.

II. Material and Methods

An evaluation of two commercial media undertaken using isolates of known identity to assess the level of accuracy of presumptive identification. Subsequently, an assessment of the two best-performing media in our laboratory adopting a standardized protocol to determine isolation rates and detect mixed cultures. The study was conducted at Dr. Lal Path Labs largest clinical microbiology laboratory in North India, which collectively processes approximately 500,000 urine specimens per year.

a) Media preparation, inoculation, and incubation

CHROMagar orientation (CO) (CHROMagar company, Paris, France) and CLED agar (Hi-Media Laboratories Pvt. Ltd. Mumbai-400086, India) were obtained as a dehydrated powder form. All culture petri plates were prepared in house by following manufacturer’s instructions and recommendations. Every fresh batch of media was tested for its ability to support the growth of Escherechia coli ATCC (25922) to ensure the quality of the media. Urine samples were inoculated onto CLED agar and CO medium plates using a calibrated 0.001-ml loop and streaked manually. The inoculated plates (CLED agar or CO medium) were incubated at 37°C for 24 hrs and examined at the intervals of 6hrs, 12hrs, 18hrs, 24hrs, and 48 hrs. Samples showing significant bacterial growth were further recorded. This study was carried out in the Department of Microbiology, at Dr. Lal path Labs, Delhi from 1st November 2020 to 31st January 2021. In total, 2,550 routine urine samples (predominantly in boric acid) received in our laboratories during a three months in 2020-2021, from both hospital and general practice, were included in the study.

b) Plate reading

CHROMagar media utilize synthetic chromogenic enzyme substrates to specifically target pathogenic species (or groups of species) based on their enzyme activity. Such enzyme activity is never completely species-specific, necessitating complementary enzyme substrates and selective agents.

For the purpose of our study, plates were recorded according to colonial morphology. The numbers of each colony type were also recorded to support the evaluating of the contributing organism counts of mixtures. The organism obtained from the CHROMagar orientation agar media was of different colors. E.coli gives dark pink to reddish color colony, Klebsiella, Enterobacter, Citrobacter → metallic blue Proteus → brown halo, Pseudomonas → greenish translucent, Acinetobacter baumanii → cream, round translucent, bacterial isolates S. aureus → golden, opaque, small, S. saprophyticus → pink, opaque, small. However, MALDI-TOF techniques were used to confirm the identification of organism at species level of yeast and bacterial isolates.

c) Presumptive identification

Presumptive identification of bacterial growth was done on CHROMagar orientation agar according to colony morphology and colour as depicted by the manufacturer (Figure 1, 2) whereas when using CLED agar plates other tests and procedures were often required to differentiate between organisms. The final identification of the isolates was done using standard identification protocol such as VITEK -2XL (Biomerieux, France) and MALDI-TOF (Bruker Daltonics) as appropriate for the isolates.

d) Statistical methods

For the study, data were collected and entered into an Excel spreadsheet.

III. Results

The present study undertaken to validate the usefulness of CHROMagar orientation UTI agar as a primary urine culture medium for its rate of isolation and presumptive identification of uropathogens in comparison to CLED in a Dr. Lal Path Labs. Out of the 2550 urine samples processed, 587 samples were positive (23.1%) and 1963 samples (76.9%) were negative.

Among the 587 positive samples Escherichia coli was the predominant Gram-negative isolate and Enterococcus faecalis was the predominant Gram-positive isolate. This study included (587) positive isolates consecutively collected from both male and female population aged 0-100 midstream and/or catheter catch urine samples obtained from patients having bacteriuria in urinary tract infection. Based on data extracted from our Laboratory Information System from 2019-2020, the use of CHROMagar orientation medium resulted in a 28% reduction in workload for additional procedures such as Gram stains, subcultures, identification panels, agglutination tests, and biochemical tests and MALDI-TOF.

In the present study, CHROMagar Orientation was evaluated as a direct isolation medium for clinical
587 positive urine samples were tested by parallel inoculation on CHROMagar Orientation and on other reference media, CLED agar. The analysis of the data obtained from CLED, CHROMagar Orientation agar for the detection of different bacteria, result indicated that the growth pattern of the uropathogens were different. It could be due to the different constituents and properties of the media. From the study, it observed that the growth of organism over the media was according to the characteristics of the media. Mixed cultures were differentiate easily on CHROMagar orientation. On CLED agar lactose fermenting organism grows which gives yellow color colonies. However, The overall impression of the color changes produced on CHROMagar orientation media by E. coli (pink-red) which was the predominant species (32.5%). All these isolates grew on CHROMagar Orientation in reddish colonies and were very easy to distinguish. Since E. coli is responsible for many of the UTI in nosocomial patients Klebsiella spp., (blue) and the Acinetobacter spp. should be added to the list of gram-negative microorganisms that can be presumptively differentiated directly on CHROMagar Orientation. They grew in nontransparent, white, entire-edge colonies. These strains were very distinct from Pseudomonas isolates, which grew in diffuse, yellow-to-green colonies with serrated edges that they were distinct and easy to perceive. Similarly, tryptophan is also present in the medium to detect members of the Proteus group, which generates a diffuse brown coloration background because of tryptophan deaminase production.

In the study gram positive bacteria were also isolated as one chromogenic substrate cleaved by ß-glucosidase possessed by Enterococci resulting in formation of turquoise colonies and S.aureus gives golden yellow color colonies.

The results of the study to CHROMagar Orientation differentiate the most commonly encountered gram-negative pathogens gram-positive and fungal uropathogens because of color and morphology alone compared to CLED agar. CHROM agar supported the growth of all common routine urinary isolates can be recommended as a primary plating medium for recovery of uropathogens and the ease of distinguishing when multiple probable pathogens were present (Figure1).

![Different Gram-negative bacterial isolates on CHROMagar orientation isolated from urine culture](image-url)
Comparison between CHROMagar Orientation media, CLED agar VITEK 2-XL, and MALDI-TOF used for the identification of uropathogens: Patterns of 587 culture-positive samples yielded different bacterial isolates including 491 single and 96 (two bacteria in each plate account for polymicrobial growths from urine culture shown in Table 1 and Table 2 respectively.

For presumptive identification of bacterial species by colony characteristics on primary culture plate, of 491 bacterial and yeast isolates, 491(100%), 488(99.4%), 484 (98.5%) and 388(79%) could be differentially identified on MALDI-TOF, Vitek2-XL,CHROMagar Orientation and CLED agar respectively. The rate of presumptive identification of the isolates was found significantly higher on CHROMagar Orientation agar than CLED agar as primary urine culture medium (Table 1; Figure 2). E. coli was the leading bacteria isolated from 171 (34.8%) samples followed by Klebsiella pneumoniae 89 (18.1%), Enterococcus spp. 73 (14.8%), Pseudomonas aeruginosa 54 (10.9%), Acinetobacter spp. 21 (4.3%), Staph.aureus 16 (3.3%), Proteus mirabilis 13 (2.6%), Candida spp. 13 (2.6%), Enterobacter spp. 9 (1.8%), Staph. saprophyticus 11 (2.2%), and Streptococcus agalactiae 6 (1.2%) respectively.

Presumptive identification of mostly gram-negative and gram-positive common uropathogens such as E.coli, K.pneumoniae, Proteus, Pseudomonas, Morganella morganii, and Enterococci spp. was correct on the CHROMagar media. E. coli was correctly identify in 99 to 100% of the cases. 4-5 of total 54 isolates of Pseudomonas aeruginosa were not correctly presumptively identify on the CLED media. Six of Citrobacter spp., 9 of Enterobacter spp. isolates presumptively misidentified as E. coli on the CLED agars. The colony appearance of Serratia on the chromogenic media was either Red in 4 of the 9 isolates and 5 strains from the typical colony appearance of the Klebsiella- Enterobacter-Serratia group (i.e., blue, mucoid) as described by the manufacturers. The overall impression of the color changes produced on chromogenic media by E. coli, Enterococci, Klebsiella spp., Serratia spp., and the Proteus-Morganella-Providencia group that are distinct and easy to perceive. All the isolates of Enterococcus faecalis and E. faecium correctly identified at genus level and were easily distinguished from Streptococcus agalactiae isolates. Staphylococcus saprophyticus isolates were easy to identify only on the CHROMagar orientation medium whereas in CLED agar S. saprophyticus and E. faecalis have shown same colony characteristics (Figure 2). All of the gram-positive isolates were misidentified on CLED agar.

In this study, a total 21 isolates of Acinetobacter spp. we presumptively identified 18 isolates of Acinetobacter baumanii on CHROM agar whereas species level differentiation of Acinetobacter spp were showed difficulty in CHROMagar. Similarly remaining 3 isolates of Acinetobacter spp. were identify as A. junii (2), and A. iwoffii (1) by MALDITOF however, in CLED agar Acinetobacter spp were poorly identified. The identification results obtained from the Vitek-2XL system were not consistent with those from the MALDI-TOF for few Candida spp. Furthermore, identification results of 10 Candida spp. isolates from the MALDI-TOF system were the same as those from the Vitek 2 system (data not listed). In this study, we evaluate the identification performance of MALDI-TOF MS for identification of enteropathogens and yeast isolates with a lower identification error rate, MALDI-TOF MS has better performance than VITEK 2 in identifying yeast found routinely in the clinical laboratory.
Table 1: Comparison of two culture media, VITEK 2-XL and MALDI-TOF for the Rate of identification of Uropathogens

<table>
<thead>
<tr>
<th>Uropathogens</th>
<th>CHROMagar orientation N=484 (98.6%)</th>
<th>MALDI-TOF identification N=491 (100%)</th>
<th>VITEK-XL identification N=488 (99.4%)</th>
<th>CLEDagar N=388 (79%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Escherichia coli</em> (171)</td>
<td>171 (100%)</td>
<td>171 (100%)</td>
<td>171 (100%)</td>
<td>169 (98.8%)</td>
</tr>
<tr>
<td><em>Klebsiella pneumonia</em> (89)</td>
<td>89 (100%)</td>
<td>89 (100%)</td>
<td>89 (100%)</td>
<td>87 (97.8%)</td>
</tr>
<tr>
<td><em>Proteus mirabilis</em> (13)</td>
<td>13 (100%)</td>
<td>13 (100%)</td>
<td>13 (100%)</td>
<td>13 (100%)</td>
</tr>
<tr>
<td><em>Enterobacter</em> spp. (9)</td>
<td>9 (100%)</td>
<td>9 (100%)</td>
<td>9 (100%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td><em>Citrobacter koseri</em> (6)</td>
<td>6 (100%)</td>
<td>6 (100%)</td>
<td>6 (100%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em> (54)</td>
<td>54 (100%)</td>
<td>54 (100%)</td>
<td>54 (100%)</td>
<td>49 (90.7%)</td>
</tr>
<tr>
<td><em>Acinetobacter</em> spp. (21)</td>
<td>18 (85.7%)</td>
<td>21 (100%)</td>
<td>21 (100%)</td>
<td>9 (42.8%)</td>
</tr>
<tr>
<td><em>Serratia marcescens</em> (9)</td>
<td>9 (100%)</td>
<td>9 (100%)</td>
<td>9 (100%)</td>
<td>4 (44.4%)</td>
</tr>
<tr>
<td><em>Enterococcus faecalis</em> (52)</td>
<td>52 (100%)</td>
<td>52 (100%)</td>
<td>9 (100%)</td>
<td>43 (58.9%)</td>
</tr>
<tr>
<td><em>Enterococcus faecium</em> (21)</td>
<td>21 (100%)</td>
<td>21 (100%)</td>
<td>21 (100%)</td>
<td>9 (42.8%)</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em> (16)</td>
<td>16 (100%)</td>
<td>16 (100%)</td>
<td>16 (100%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td><em>Staphylococcus saprophyticus</em> (11)</td>
<td>11 (100%)</td>
<td>11 (100%)</td>
<td>11 (100%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td><em>Streptococcus agalactiae</em> (6)</td>
<td>6 (100%)</td>
<td>6 (100%)</td>
<td>11 (100%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td><em>Candida</em> spp. (13)</td>
<td>9 (69.2%)</td>
<td>13 (100%)</td>
<td>10 (76.9%)</td>
<td>5 (38.4%)</td>
</tr>
</tbody>
</table>
Comparison between CLED and CHROMagar Orientation media used for the isolation of uropathogens.

Table 2 shows the rate of presumptive identification of polymicrobial growth in different culture media. All 139 (100%) polymicrobial growths distinctly identified only on Chromagar Orientation agar medium, in contrast except in a single case consisting of *E. coli* and *Proteus mirabilis*, no mixed bacterial growths could be identified on CLED agar media. The detection of Gram positives and yeasts organisms diminishes in the presence of increasing numbers of Gram-negative organisms, because of the white or colorless appearance of the colonies on the CHROMagar orientation media for Gram-positive organisms and yeasts, CHROMagar performed better than other UTI medium such as CLED (Table 2).

In our study, CHROMagar showed a superior differentiation of mixed cultures because different species may generate colonies with different colors and may not easily differentiate on conventional agars. *Enterococci* spp. and *S. aureus* presumptively identified (Figure 3) on the CHROMagar and were not in CLED agar.
Table 2: Comparison of Rate of Isolation of polymicrobial Uropathogens on CHROMagar Orientation and CLED culture media

<table>
<thead>
<tr>
<th>Uropathogens</th>
<th>N=96 (16.4%)</th>
<th>CHROMagar orientation N=96 (100%)</th>
<th>CLED agar N =74 (77%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. coli and K. pneumoniae (23)</td>
<td></td>
<td>23(100%)</td>
<td>22(95.7%)</td>
</tr>
<tr>
<td>E coli and Enterococcus spp. (29)</td>
<td></td>
<td>29 (100%)</td>
<td>17 (58.6%)</td>
</tr>
<tr>
<td>K. pneumoniae and Pseudomonas aeruginosa (12)</td>
<td></td>
<td>12 (100%)</td>
<td>11 (91.6%)</td>
</tr>
<tr>
<td>E.coli and Pseudomonas aeruginosa (19)</td>
<td></td>
<td>19 (100%)</td>
<td>18 (94.7%)</td>
</tr>
<tr>
<td>Proteus mirabilis and E.faecalis (3)</td>
<td></td>
<td>3 (100%)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Proteus mirabilis and E.coli (6)</td>
<td></td>
<td>6 (100%)</td>
<td>6 (100%)</td>
</tr>
<tr>
<td>Staphylococcus aureus and E. coli (4)</td>
<td></td>
<td>4 (100%)</td>
<td>0 (0)</td>
</tr>
</tbody>
</table>

Therefore, CLED (cystine–lactose–electrolyte-deficient) agar, were used as the reference media. We also compared the interval of 6hrs incubation to 48hrs of incubation; to our knowledge, this has not done previously. The media evaluation were listed in (Table 3).

Figure 3: Comparison between CLED and CHROMagar Orientation media used for the isolation of polymicrobial uropathogens

In this study, we evaluated CHROMagar Orientation from The CHROMagar Company [Paris, France] for routine diagnosis of bacteriuria at our laboratory concerning isolation frequency and presumptive identification of urine isolates. CLED (cystine-, lactose-, and electrolyte-deficient) agar, were used as the reference media. We also compared the interval of 6hrs incubation to 48hrs of incubation; to our knowledge, this has not done previously. The media evaluation were listed in (Table 3).
Table 3: Comparison of culture media for the Rate of presumptive isolation as Primary culture plate of Uropathogens

<table>
<thead>
<tr>
<th>Bacterial isolates</th>
<th>CLED</th>
<th>CHROMagar orientation</th>
<th>CLED</th>
<th>CHROMagar orientation</th>
<th>CLED</th>
<th>CHROMagar orientation</th>
<th>CLED</th>
<th>CHROMagar orientation</th>
<th>CLED</th>
<th>CHROMagar orientation</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Incubation period</strong></td>
<td>0-6 hrs</td>
<td>7-12 hrs</td>
<td>13-18 hrs</td>
<td>19-24 hrs</td>
<td>0-6 hrs</td>
<td>7-12 hrs</td>
<td>13-18 hrs</td>
<td>19-24 hrs</td>
<td>0-6 hrs</td>
<td>7-12 hrs</td>
</tr>
<tr>
<td>E. coli (n=145)</td>
<td>No growth</td>
<td>No growth</td>
<td>10²cfu/ml</td>
<td>10³cfu/ml</td>
<td>10⁴cfu/ml</td>
<td>&gt;=10⁵cfu/ml</td>
<td>&gt;=10⁶cfu/ml</td>
<td>10⁷cfu/ml</td>
<td>&gt;=10⁷cfu/ml</td>
<td>10⁸cfu/ml</td>
</tr>
<tr>
<td>K. pneumoniae (n=85)</td>
<td>No growth</td>
<td>No growth</td>
<td>10³cfu/ml</td>
<td>10⁴cfu/ml</td>
<td>10⁵cfu/ml</td>
<td>&gt;=10⁶cfu/ml</td>
<td>&gt;=10⁶cfu/ml</td>
<td>10⁷cfu/ml</td>
<td>&gt;=10⁷cfu/ml</td>
<td>10⁸cfu/ml</td>
</tr>
<tr>
<td>P. mirabilis (n=13)</td>
<td>No growth</td>
<td>No growth</td>
<td>10³cfu/ml</td>
<td>10⁴cfu/ml</td>
<td>10⁵cfu/ml</td>
<td>&gt;=10⁶cfu/ml</td>
<td>&gt;=10⁶cfu/ml</td>
<td>10⁷cfu/ml</td>
<td>&gt;=10⁷cfu/ml</td>
<td>10⁸cfu/ml</td>
</tr>
<tr>
<td>P. aeruginosa (n=54)</td>
<td>No growth</td>
<td>No growth</td>
<td>10³cfu/ml</td>
<td>10⁴cfu/ml</td>
<td>10⁵cfu/ml</td>
<td>&gt;=10⁶cfu/ml</td>
<td>&gt;=10⁶cfu/ml</td>
<td>10⁷cfu/ml</td>
<td>&gt;=10⁷cfu/ml</td>
<td>10⁸cfu/ml</td>
</tr>
<tr>
<td>Enterococcus spp. (n=73)</td>
<td>No growth</td>
<td>No growth</td>
<td>10⁴cfu/ml</td>
<td>10⁵cfu/ml</td>
<td>10⁶cfu/ml</td>
<td>&gt;=10⁷cfu/ml</td>
<td>&gt;=10⁷cfu/ml</td>
<td>10⁸cfu/ml</td>
<td>&gt;=10⁸cfu/ml</td>
<td>10⁹cfu/ml</td>
</tr>
<tr>
<td>Acinetobacter baumannii (n=21)</td>
<td>No growth</td>
<td>No growth</td>
<td>10⁴cfu/ml</td>
<td>10⁵cfu/ml</td>
<td>10⁶cfu/ml</td>
<td>&gt;=10⁶cfu/ml</td>
<td>&gt;=10⁶cfu/ml</td>
<td>10⁷cfu/ml</td>
<td>&gt;=10⁷cfu/ml</td>
<td>10⁸cfu/ml</td>
</tr>
<tr>
<td>Enterobacter spp. (n=9)</td>
<td>No growth</td>
<td>No growth</td>
<td>10⁵cfu/ml</td>
<td>10⁶cfu/ml</td>
<td>10⁷cfu/ml</td>
<td>&gt;=10⁷cfu/ml</td>
<td>&gt;=10⁷cfu/ml</td>
<td>10⁸cfu/ml</td>
<td>&gt;=10⁸cfu/ml</td>
<td>10⁹cfu/ml</td>
</tr>
<tr>
<td>Streptococcus agalactiae (n=6)</td>
<td>No growth</td>
<td>No growth</td>
<td>10⁵cfu/ml</td>
<td>10⁶cfu/ml</td>
<td>10⁷cfu/ml</td>
<td>&gt;=10⁷cfu/ml</td>
<td>&gt;=10⁷cfu/ml</td>
<td>10⁸cfu/ml</td>
<td>&gt;=10⁸cfu/ml</td>
<td>10⁹cfu/ml</td>
</tr>
<tr>
<td>S. saprophyticus (n=11)</td>
<td>No growth</td>
<td>No growth</td>
<td>10⁵cfu/ml</td>
<td>10⁶cfu/ml</td>
<td>10⁷cfu/ml</td>
<td>&gt;=10⁷cfu/ml</td>
<td>&gt;=10⁷cfu/ml</td>
<td>10⁸cfu/ml</td>
<td>&gt;=10⁸cfu/ml</td>
<td>10⁹cfu/ml</td>
</tr>
<tr>
<td>S. aureus (n=16)</td>
<td>No growth</td>
<td>No growth</td>
<td>10⁵cfu/ml</td>
<td>10⁶cfu/ml</td>
<td>10⁷cfu/ml</td>
<td>&gt;=10⁷cfu/ml</td>
<td>&gt;=10⁷cfu/ml</td>
<td>10⁸cfu/ml</td>
<td>&gt;=10⁸cfu/ml</td>
<td>10⁹cfu/ml</td>
</tr>
<tr>
<td>Candida spp. (n=13)</td>
<td>No growth</td>
<td>No growth</td>
<td>10¹⁵cfu/ml</td>
<td>10¹⁶cfu/ml</td>
<td>10¹⁷cfu/ml</td>
<td>10¹⁸cfu/ml</td>
<td>10¹⁹cfu/ml</td>
<td>10²⁰cfu/ml</td>
<td>10²¹cfu/ml</td>
<td>10²²cfu/ml</td>
</tr>
</tbody>
</table>

According to the technical data, when the total number of isolates recovered from both of the media was compared to the number of isolates growing on the individual media types after an interval of 6-48 hours incubation period. The percentage for CHROMagar Orientation media shows approximately 20% high in colony count in 13-18 hours incubation that was evident in the present study. Although incubation longer than overnight (up to 24-48 hours) does not significantly increase the yield of common, urine isolates on CHROMagar orientation or traditional media CLED. In this study, we found that most common gram-negative isolates such as E.coli, K.pneumoniae, Proteus mirabilis, Pseudomonas aeruginosa and Acinetobacter spp. in 0-6 hrs incubation period no growth were seen in both media (Table 3, Figure 4). However, 7-18 hrs incubation period showed that CHROMagar Orientation performing better growth than CLED whereas after 18hrs incubation, there growth pattern were similar in both media. CHROMagar Orientation, performed better growth of Gram-positive isolates in a short incubation period and easily identified after 18 hrs incubation (Table 3). Similarly, CHROMagar Orientation given the best result for isolation of yeast species in 18-24hrs incubation period (Table-3).
IV. Discussion

Every clinical microbiology laboratory's daily workload of urine cultures accounts for a diagnosis of urinary tract infection because only 20 to 30% of urine samples result in significant growth[1,3]. Therefore, any new medium or method with the ability to streamline urine culture processing in a meaningful way, such as reducing technologist workload, improving result turnaround times (TATs), or reducing laboratory costs, would be welcomed and has the potential to have considerable laboratory impact. Our study confirmed the superiority of CHROMagar orientation over CLED agar in detecting mixed cultures, Gram-positive organisms, and yeasts; these results corroborate earlier studies [2-6].

Traditionally conventional media like Blood agar (BA) the majority of urine isolates as an enriched medium but its performance in the identification of bacteria is very deficient. Similarly, differentiation of lactose fermenter and non-fermenter is possible on MAC and CLED agar. Moreover, none of these media singly or in combination can support the growth and identification of possible urine isolates [1,7]. As a result, further species identification necessitates subculture or divergent tests with longer reporting time and cost. The present findings were in concordance with the findings of (Aspevall et al., 2002) observed that the CHROMagar Orientation media tested in this study was better than CLED agar. A similar observation was also reported by (Fallon et al., 2003) using BBL CHROMagar, UTI medium, or CPS ID2 chromogenic agar, as a replacement for Cystine Lactose Electrolyte Deficient agar (CLED) would improve the detection rate of contaminated urine samples. “A cost comparison of the agars suggests that as the use of chromogenic agar in laboratories increases, the purchase cost is decreasing” (Fallon et al., 2003)[6].

In the present study, the time interval between plating and final organism identification was decreased on CHROMagar orientation and it was seen that were evident within 18-hours versus CLED using the entire required standard microbiological tests; it was an average of 38 hours. Using CHROMagar orientation, clinically significant cultures required less hands-on time. Similarly in a study by Bajoria et al., concluded that conventional media requires 24-48 hours to give positive results [3]. Articles reported the effect of incubation time on results of urine culture on traditional media [2]. All agree that common urine isolates detected after overnight incubation and that a longer incubation time is required for the detection of yeasts.

Hence, it concluded that the cost comparison of the agars suggests that the use of CHROMagar orientation in laboratories increases, the purchase cost is decreasing due to the needs for repeat samples, and avoided antimicrobial therapy because of improved mixture detection [1,2]. In a few studies comparing CHROMagar Orientation media with traditional ones, its advantages including a 20% reduction in time for identification, reduction in workload [5, 6, 8]. When using traditional media requires a great deal of experience for presumptive identification of isolates, whereas CHROMagar media, is easier, requiring less training and interpreted by personnel with less
experience in microbiology. Thus, the use of CHROMagar Orientation media may improve the quality of urine culture by contributing to a uniform interpretation of urine culture plates by the different personnel engaged in this task at the laboratory. All these factors have a direct impact on ultimate cost reduction. Our data support the findings of these investigators [2-8]. Also, MALDI-TOF MS showed to be simple, rapid, and accurate tool for the identification of enteropathogens and rare yeast species. At the same time the Vitek 2 XL system is a popular commercial method commonly used in clinical microbiology laboratories for bacterial identification.

Most of the isolates analyzed in our study largely commonly found pathogens, and the construction of the MALDI-TOF MS database may offer higher identification accuracies for these pathogens. Additionally, MALDI-TOF MS dramatically shortened identification time from 6-8 hours to just a few minutes. However, MALDI-TOF MS made no errors at the genus and species level while VITEK -2XL made 0.6% errors at the species level of rare yeast species[10, 11].

V. Conclusion

CHROMagar Orientation provided the highest overall organism recovery rates, convenient for rapid identification, and the greatest ability to detect mixed cultures. The use of CHROMagar orientation medium as a replacement for Cystine Lactose Electrolyte Deficient (CLED) agar would improve the detection rate of contaminated urine samples and has the potential to streamline urine culture processing in a meaningful way, such as reducing technologist workload, improving result of turnaround times and reducing costs. It would improve identification that helps to distinguish species, facilitating the monitoring of bacterial resistance in support of the national antibiotic strategy.

Ethical Approval: It is not applicable.

Conflicts of interest: There are no conflicts of interest.

Acknowledgements: We are thankful to Dr Reena Nakra, Lab director operations National Reference Laboratory, Dr. Lal Path Labs, Delhi for providing us operational support and team Microbiology for technical assistance, Dr. Navaneeth Saxena from [r-biopharm neugen pvt ltd & Subs- Chromogenic life science India pvt.Ltd. Hyderabad, Advices and helps in conducting the study.

References Références Referencias


Evaluation of Bacteriological Quality of Periwinkle Snail (Tympa
notonus Fuscatus) Collected from Ilaje, Nigeria and Isolates’ Susceptibility Pattern to Ocimum Gratissimum
By Jeremiah Abiola Adesanya, Chukwuma G. Udensi, Daniel Juwon Arotupin & Emmanuel K. Amanze

Abstract- The incessant upsurge of antibiotic resistance and adverse side effects of many commercially accessible antibiotics has undoubtedly warranted the screening of diverse parts of plants to discover new ingredients that can serve as a substitute in prevention and cure of infections and diseases. Fresh leave samples of Ocimum gratissimum were collected, air-dried at room temperature and then blended to powder. The extraction was done using a vacuum evaporator with distilled water and absolute ethanol as the solvents. The extracts were reconstituted with dimethyl sulphoxide (DMSO) to obtain concentrations of 25 mg/ml, 50 mg/ml, 100 mg/ml and 200 mg/ml. The extracts were tested against bacterial isolates obtained from the meat of periwinkle snail (Tympa notonus fuscatus). Agar well diffusion technique was used to evaluate the antibacterial susceptibility of the extracts.

Keywords: infections, inhibition, antibacterial activity, antibiotic resistance, susceptibility.

GJMR-C Classification: NLMC Code: QW 50
Evaluation of Bacteriological Quality of Periwinkle Snail (*Tympanotonus Fuscatus*) Collected from Ilaje, Nigeria and Isolates’ Susceptibility Pattern to *Ocimum Gratissimum*

Jeremiah Abiola Adesanya a, Chukwuma G. Udensi b, Daniel Juwon Arotupin c & Emmanuel K. Amanze d

**Abstract** - The incessant upsurge of antibiotic resistance and adverse side effects of many commercially accessible antibiotics has undoubtedly warranted the screening of diverse parts of plants to discover new ingredients that can serve as a substitute in prevention and cure of infections and diseases. Fresh leave samples of *Ocimum gratissimum* were collected, air-dried at room temperature and then blended to powder. The extraction was done using a vacuum evaporator with distilled water and absolute ethanol as the solvents. The extracts were reconstituted with dimethyl sulphoxide (DMSO) to obtain concentrations of 25 mg/ml, 50 mg/ml, 100 mg/ml and 200 mg/ml. The extracts were tested against bacterial isolates obtained from the meat of periwinkle snail (*Tympanotonus fuscatus*). Agar well diffusion technique was used to evaluate the antibacterial susceptibility of the extracts. The isolates include *Escherichia coli*, *Salmonella typhi*, *Enterobacter aerogenes*, *Pseudomonas aeruginosa* and *Bacillus cereus*. The plant extracts showed selective inhibition against the isolates, which was most on *Salmonella typhi* at 100 mg/ml and least on *Enterobacter aerogenes* at 100 mg/ml for the ethanol extract of *Ocimum gratissimum*. *Bacillus cereus* was most susceptible at 200 mg/ml, and *Escherichia coli* was the least susceptible at 200 mg/ml to aqueous extract of *Ocimum gratissimum*. The ethanol extract exhibited more inhibitory effects compared to the aqueous extract. The findings showed that *Ocimum gratissimum* possess some antibacterial activity against the isolates and could be effective in treating infections and diseases that may be caused by these isolates.

**Keywords:** infections, inhibition, antibacterial activity, antibiotic resistance, susceptibility.

1. **Introduction**

Periwinkle Snail (*Tympanotonus fuscatus*) are members of phylum Mollusca and class Gastropoda, comprising over 40,000 species of snails, snails, and their relatives. The genus *Tympanotonus* known locally as periwinkle includes single species with two varieties: *Tympanotonus fuscatus* var. *fuscatus* and *Tympanotonus fuscatus* var. *radula*. The genus *Tympanotonus* is characterized by turreted granular and spiny shells, and tapering ends. *Tympanotonus fuscatus* var. *radula* is distinguished from the other variety by the absence of spiny tubercles on the surface (11). The two species are edible and can be found in different parts of Nigeria (9). This snail is referred to as "isam" in Igbo, "mfi" in Akwalbom, and "Udekana" in Niger Delta, Nigeria (3). Nigeria is much endowed with snail species (2). Snails have been a significant constituent in the nourishment of many Africans living in the tropics. Snail farming in Nigeria has a lot of potential. It serves many purposes coupled with its ability to alleviate acute protein deficiency. This seafood, just like any other, shelters series of microorganisms. Some of these microorganisms are opportunistic and pathogenic, leading to infections and challenging diseases with symptoms such as diarrhoea, typhoid fever, tuberculosis, cholera, and so on (2). The origin of these diseases is not farfetched. The arise from improper processing of the meat. Also, microorganisms can be contacted during the collection, transportation, and sales of the periwinkle or habitat, including the rocky shores creeks, swamps, and unclean environments (11). This study focuses on the isolation and characterization of bacterial species associated with periwinkle snail meat (*Tympanotonus fuscatus*) and determine the isolates’ susceptibility to *Ocimum gratissimum*.

11. **Materials and Methods**

a) **Study Area Description**

The periwinkle snails (*Tympanotonus fuscatus*) were collected at the Ilaje Local Government Area, Ondo State, Nigeria. Ilaje is located on the southern part of Ondo State, Nigeria. The principal occupation of the inhabitants of Ilaje local government is fishing (8). Ilaje is situated along the Atlantic coast of Nigeria between longitude 4° 28’ and 5° 1’ east of the Greenwich Meridian and 5° 51’ and 6° 21’ north of the equator (7).

b) **Sample Collection**

*Ocimum fuscatus var. fuscatus* for this research was obtained from the Ilaje area of Ondo State, Nigeria at...
about 8:00 am on Saturday 6th July 2019. *Tymp
donotus fuscatus* was easy to get as the rainy season enhanced their availability. The snails were washed in rushing tap water to remove mud, debris and other particles attached to the snail’s shell. The snails were then rinsed in distilled water and disinfected with cotton wool moistened in 70% ethanol. The snails were de-shellled aseptically, and the meat was separated from the intestinal portion. The meat of the snail was placed in distilled water in a sterile test tube for further study.

Isolation, identification and characterization of microbial isolates

Nutrient agar, MacConkey agar, Eosin methylene blue agar and *Salmonella Shigella* agar were prepared following the manufacturer’s specifications. Glass wares used for the experiment were autoclaved at 121°C for 15 minutes. Bacteria were cultured from the meat of the periwinkle using the pour plate method according to (1). The culture plates were incubated at 37°C for 24 hours and then distinct colonies sub-cultured to obtain pure isolates. The isolates were characterized using standard biochemical techniques. The isolates were identified using Bergey’s Manual of Determinative Bacteriology.

c) Preparation of plant extracts

The solvents used for the extraction were absolute ethanol and cold distilled water. Two hundred grams of the powdered plant leaves were weighed separately. A litre of absolute ethanol and distilled water was poured into the samples in separate bottles. The bottles were covered and placed on the laboratory bench for 72 hours, sieved using clean muslin cloths, Whatman filter paper and membrane Millipore of pore size 0.22 µm. The filtrate was concentrated using vacuum rotary evaporator.

d) Test for sterility of plant extracts

The plant extracts were tested for sterility according to (10) by introducing 1 ml of the reconstituted plant extract into 5 ml of sterile nutrient broth and incubated at 37°C for 24 hours. The absence of the broth’s turbidity compared to McFarland standard after incubation confirmed the sterility of the extract.

e) Determination of the antibacterial sensitivity of the plant extracts

The antibiotic sensitivity test was carried out using Kirby-Bauer’s disc diffusion method, while the antibacterial sensitivity test was carried out using the agar well diffusion method as described by (4). The minimum inhibitory concentration was also determined, according to (12).

f) Statistical Analysis of Data

Data obtained were compared using Statistical Packages for Social Sciences (SPSS) software version 25.

III. Results

The antibiotics sensitivity tests results revealed that the most effective antibiotics against the Gram negative isolates were ciprofloxacin while augmentin was least effective as described in table 2. Ciprofloxacin was also the most effective antibiotics against *Bacillus cereus* while Rocephin was least effective as defined in table 3. The extracts’ antibacterial activity shows *Salmonella typhi* was the most susceptible while *Enterobacteraerogenes* was the least sensitive to the ethanol extract. *Bacillus cereus* was the most vulnerable, while *Escherichia coli* was the least susceptible to the aqueous extract of *Ocimum gratissimum* (Table 4 and 5).

### Table 1: Biochemical and cultural characteristics of bacterial isolates

<table>
<thead>
<tr>
<th>H₂S</th>
<th>Ind</th>
<th>Mot</th>
<th>Lac</th>
<th>Man</th>
<th>Glu</th>
<th>Ox</th>
<th>Mann</th>
<th>Sor</th>
<th>Suc</th>
<th>Cit</th>
<th>Cat</th>
<th>Gram Staining</th>
<th>Suspected Isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td><em>Escherichia coli</em></td>
</tr>
<tr>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td><em>Salmonella typhi</em></td>
</tr>
<tr>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td><em>P. aeruginosa</em></td>
</tr>
<tr>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Bacillus cereus</td>
</tr>
</tbody>
</table>

**Keys:** In – Indole test, Mot – Motility test, Cat – Catalase test, Lac – Lactose, Man – Mannose, Suc – Sucrose, Cit – Citrate test, Glu – Glucose, Ox – Oxidase, Mann – Mannitol, Sor – Sorbitol

### Table 2: Antimicrobial activity of the Gram-negative antibiotic disc against the isolates

<table>
<thead>
<tr>
<th>Isolates</th>
<th>SXT</th>
<th>CH</th>
<th>SP</th>
<th>CPX</th>
<th>AM</th>
<th>AU</th>
<th>CN</th>
<th>PEF</th>
<th>OFX</th>
<th>S</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Escherichia coli</em></td>
<td>21.3±0.67&lt;sup&gt;a&lt;/sup&gt;</td>
<td>18.0±1.16&lt;sup&gt;b&lt;/sup&gt;</td>
<td>21.3±0.67&lt;sup&gt;a&lt;/sup&gt;</td>
<td>21.3±0.67&lt;sup&gt;a&lt;/sup&gt;</td>
<td>17.3±0.67&lt;sup&gt;c&lt;/sup&gt;</td>
<td>17.3±0.67&lt;sup&gt;a&lt;/sup&gt;</td>
<td>24.6±1.33&lt;sup&gt;a&lt;/sup&gt;</td>
<td>23.7±1.20&lt;sup&gt;a&lt;/sup&gt;</td>
<td>23.3±1.33&lt;sup&gt;b&lt;/sup&gt;</td>
<td>15.3±1.76&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td><em>Salmonella typhi</em></td>
<td>17.3±0.67&lt;sup&gt;a&lt;/sup&gt;</td>
<td>26.7±0.67&lt;sup&gt;a&lt;/sup&gt;</td>
<td>26.0±1.16&lt;sup&gt;a&lt;/sup&gt;</td>
<td>27.3±0.67&lt;sup&gt;a&lt;/sup&gt;</td>
<td>21.3±0.67&lt;sup&gt;a&lt;/sup&gt;</td>
<td>24.7±1.33&lt;sup&gt;a&lt;/sup&gt;</td>
<td>26.7±0.67&lt;sup&gt;a&lt;/sup&gt;</td>
<td>26.0±1.15&lt;sup&gt;a&lt;/sup&gt;</td>
<td>25.3±1.33&lt;sup&gt;a&lt;/sup&gt;</td>
<td>26.7±0.67&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td><em>P. aeruginosa</em></td>
<td>15.3±1.76&lt;sup&gt;a&lt;/sup&gt;</td>
<td>25.3±1.76&lt;sup&gt;a&lt;/sup&gt;</td>
<td>25.3±1.76&lt;sup&gt;a&lt;/sup&gt;</td>
<td>10.7±0.67&lt;sup&gt;b&lt;/sup&gt;</td>
<td>14.7±0.67&lt;sup&gt;a&lt;/sup&gt;</td>
<td>17.3±1.33&lt;sup&gt;b&lt;/sup&gt;</td>
<td>28.0±0.00&lt;sup&gt;a&lt;/sup&gt;</td>
<td>27.3±0.67&lt;sup&gt;a&lt;/sup&gt;</td>
<td>26.0±0.00&lt;sup&gt;a&lt;/sup&gt;</td>
<td>22.7±1.33&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td><em>E. aerogenes</em></td>
<td>16.7±0.67&lt;sup&gt;a&lt;/sup&gt;</td>
<td>22.0±2.00&lt;sup&gt;b&lt;/sup&gt;</td>
<td>20.7±0.60&lt;sup&gt;b&lt;/sup&gt;</td>
<td>26.7±1.33&lt;sup&gt;c&lt;/sup&gt;</td>
<td>18.7±1.33&lt;sup&gt;c&lt;/sup&gt;</td>
<td>18.0±1.15&lt;sup&gt;b&lt;/sup&gt;</td>
<td>18.0±0.00&lt;sup&gt;b&lt;/sup&gt;</td>
<td>26.0±0.00&lt;sup&gt;b&lt;/sup&gt;</td>
<td>26.0±0.00&lt;sup&gt;b&lt;/sup&gt;</td>
<td>22.7±1.33&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

(Mean ± SEM n = 3). Values with the same superscript letter(s) along the same column are significantly different (P<0.05).
Fig. 1 shows the sensitivity tests carried out using Gram-negative antibiotic discs on each of the isolates.

**Table 3:** Antimicrobial activity of the Gram-positive antibiotic disc against the isolate

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Mean of the zone of inhibition (mm) (Mean ± SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacillus cereus</td>
<td>E: 23.3±1.76, PEF: 25.3±0.67, CN: 22.67±1.76, APX: 16.0±1.15, Z: 14.7±1.76, AM: 15.3±1.76, R: 12.7±0.67, CPX: 26.7±0.67, S: 20.7±0.67, SXT: 24.3±0.33</td>
</tr>
</tbody>
</table>

(values with the same superscript letter(s) along the same column are significantly different (P<0.05).

Fig. 2: Antibiotic sensitivity patterns of Bacillus spp. (Gram-positive) bacterium isolated from the meat of the snail.

**Table 4:** Antimicrobial activity of the ethanol extracts of Ocimum gratissimum against the isolates

<table>
<thead>
<tr>
<th>Isolates</th>
<th>25 mg/ml</th>
<th>50 mg/ml</th>
<th>100 mg/ml</th>
<th>200 mg/ml</th>
<th>Control (distilled water)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Escherichia coli</td>
<td>0.0±0.00a</td>
<td>3.6±0.33c</td>
<td>7.0±0.57c</td>
<td>7.3±0.88c</td>
<td>0.0±0.00a</td>
</tr>
<tr>
<td>Salmonella typhi</td>
<td>8.3±0.88a</td>
<td>9.0±0.58b</td>
<td>12.3±0.68b</td>
<td>11.3±1.20b</td>
<td>0.0±0.00a</td>
</tr>
<tr>
<td>P. aeruginosa</td>
<td>0.0±0.00a</td>
<td>0.0±0.00a</td>
<td>6.7±0.58b</td>
<td>4.3±0.33b</td>
<td>0.0±0.00a</td>
</tr>
<tr>
<td>E. aerogenes</td>
<td>0.0±0.00a</td>
<td>0.0±0.00a</td>
<td>3.0±0.00a</td>
<td>3.3±0.33b</td>
<td>0.0±0.00a</td>
</tr>
<tr>
<td>Bacillus cereus</td>
<td>4.3±0.33a</td>
<td>6.3±0.89a</td>
<td>8.3±0.33b</td>
<td>10.7±0.89b</td>
<td>0.0±0.00a</td>
</tr>
</tbody>
</table>

(values with the same superscript letter(s) along the same column are significantly different (P<0.05).
Table 5: Antimicrobial activity of the aqueous extracts of Ocimum gratissimum against the isolates

<table>
<thead>
<tr>
<th>Isolates</th>
<th>25 mg/ml (Mean±SEM)</th>
<th>50 mg/ml (Mean±SEM)</th>
<th>100 mg/ml (Mean±SEM)</th>
<th>200 mg/ml (Mean±SEM)</th>
<th>Control (distilled water) (Mean±SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Escherichia coli</td>
<td>0.0±0.00a</td>
<td>0.0±0.00a</td>
<td>3.3±0.33b</td>
<td>4.7±0.33c</td>
<td>0.0±0.00a</td>
</tr>
<tr>
<td>Salmonella typhi</td>
<td>0.0±0.00a</td>
<td>0.0±0.00a</td>
<td>4.3±0.89b</td>
<td>5.7±0.33b</td>
<td>0.0±0.00a</td>
</tr>
<tr>
<td>P. aeruginosa</td>
<td>0.0±0.00a</td>
<td>0.0±0.00a</td>
<td>3.3±0.33b</td>
<td>4.7±0.33c</td>
<td>0.0±0.00a</td>
</tr>
<tr>
<td>E. aerogenes</td>
<td>0.0±0.00a</td>
<td>0.0±0.00a</td>
<td>4.7±0.33b</td>
<td>5.3±0.33b</td>
<td>0.0±0.00a</td>
</tr>
<tr>
<td>Bacillus cereus</td>
<td>0.0±0.00a</td>
<td>0.0±0.00a</td>
<td>4.0±0.58b</td>
<td>6.7±0.67c</td>
<td>0.0±0.00a</td>
</tr>
</tbody>
</table>

(Mean ± SEM n = 3). Values with the same superscript letter(s) along the same column are significantly different (P<0.05).

Fig. 3: Antimicrobial activity of the ethanol extracts of Ocimum gratissimum against the isolates.

Fig. 4: Antimicrobial activity of the aqueous extracts of Ocimum gratissimum against the isolates.

Minimum Inhibitory Concentration

Table 6: Antimicrobial activity of the ethanol and aqueous extracts of Ocimum gratissimum against the isolates

<table>
<thead>
<tr>
<th>Minimum Inhibitory Concentration (mg/ml)</th>
<th>Ethanol</th>
<th>Aqueous</th>
</tr>
</thead>
<tbody>
<tr>
<td>Escherichia coli</td>
<td>50</td>
<td>100</td>
</tr>
<tr>
<td>Salmonella typhi</td>
<td>50</td>
<td>100</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Enterobacter aerogenes</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Bacillus cereus</td>
<td>50</td>
<td>100</td>
</tr>
</tbody>
</table>

IV. Discussion

The results of this research agree with (6) who recorded the occurrence of Enterobacter, Escherichia coli, Pseudomonas aeruginosa, Salmonella, Shigella, Bacillus and Micrococcus in Tympanotonus fuscatus. The ethanol extracts showed more significant inhibitory activity against the isolates than the aqueous extract. This may be due to the higher volatility of ethanol, which tends to extract more active ingredients in the leaves sample than water. Salmonella typhi was the most susceptible to the ethanol extracts from the results obtained while Enterobacter aerogenes was the least vulnerable. Escherichia coli was the least sensitive to...
aqueous extract of while Bacillus cereus was the most susceptible.

The antibacterial activities of the extracts, both ethanol and aqueous, increased with increase in concentration. The resistance at lower concentration may be due to lipid contents in gram-positive bacteria membranes which may prevent the active phytochemicals’ permeability into the cell (1). However, (5) suggested that difference in planting locations, seasonal and environmental variations may differ in quantity and quality of phytochemical constituents. The results established good support for the use of Ocimum gratissimum leaves in herbal medicine and as base for the development of new drugs in phyto-medicine especially in the prevention and treatment of gastrointestinal diseases from consumption of contaminated meat of Tympanotonus fuscatus.

V. Conclusion

The result demonstrated high enteric bacteria in Tympanotonus fuscatus. Hence, adequate cooking is encouraged to kill the microorganisms in the snail to prevent infections. This study’s findings revealed that Ocimum gratissimum exhibited significant antimicrobial activity on the isolates (Escherichia coli, Salmonella typhi, Enterobacter aerogenes, Pseudomonas aeruginosa and Bacillus cereus). This indicates the presence of antimicrobial agents in the leaf samples and can serve a potential source of treatment for enteric diseases. Further investigations in the area of toxicological studies and purification of active substances in the plant extract are necessary for drug development as the plant may contain both beneficial and non-beneficial chemicals.

References Références Referencias

This page is intentionally left blank
Prospective Promising Signs of Herd Immunity in COVID-19 Transmission Suppression Via Vaccination
By Michael Tosin Bayode, Adedoyin Taofiq Oseni, Covenant Femi Adeboboye, Ruth Ebunoluwa Bodunrinde & Jeremiah Abiola Adesanya

Federal University of Technology

Abstract- Reports of the COVID-19 pandemic show an elevated level of mortality among patients, with some inclining hazards distinguished as age, underlying infection conditions; hypertension, diabetes, and so on, immune-compromised conditions and viral dose during exposure. Different investigations portray an elevated level of super-spread ing occasions, which proposes that heterogeneity in infectivity may altogether affect the elements of its transmission. This review is intended to make the perception herd immunity needs to play in COVID-19 transmission concealment inferable from its circumspect viability for the destruction of numerous maladies and indeed, give the premise to vaccines and their applications serving as a proviso for immune individuals that will prompt a huge decrease in disease event and spread. On account of the ebb and flow of the COVID-19 scourge, this may give the perfect viewpoint to totally eradicate the illness in our local communities in the event that a vaccine is before long evolved.

Keywords: COVID-19, transmission, herd immunity, vaccine, immune.

GJMR-C Classification: NLMC Code: QW 4
Prospective Promising Signs of Herd Immunity in COVID-19 Transmission Suppression Via Vaccination

Michael Tosin Bayode α, Adedoyin Taofiq Oseni α, Covenant Femi Adeboboye ρ, Ruth E bunoluwa Bodunrinde ο & Jeremiah Abiola Adesanya ¥

Abstract - Reports of the COVID-19 pandemic show an elevated level of mortality among patients, with some inclining hazards distinguished as age, underlying infection conditions; hypertension, diabetes, and so on, immune-compromised conditions and viral dose during exposure. Different investigations portray an elevated level of super-spreading occasions, which proposes that heterogeneity in infectivity may altogether affect the elements of its transmission. This review is intended to make the perception herd immunity needs to play in COVID-19 transmission concealment inferable from its circumspect viability for the destruction of numerous maladies and indeed, give the premise to vaccines and their applications serving as a proviso for immune individuals that will prompt a huge decrease in disease event and spread. On account of the ebb and flow of the COVID-19 scourge, this may give the perfect viewpoint to totally eradicate the illness in our local communities in the event that a vaccine is before long evolved.

Keywords: COVID-19, transmission, herd immunity, vaccine, immune.

I. Introduction

As of now, the COVID-19 pandemic is a bane to the entire world populace, because of the pace of infectivity, inclined danger of acquiring the airborne viral disease and combined with the way that there is as of now no endorsed corrective control/vaccine for this viral infection. Arrays of investigations have set up that the general population most predisposed and powerless against the ailment are the older individuals, males, people with hidden sickness conditions including; hypertension, diabetic condition, immuno-weakened people, and people dependent on immunosuppressant use [1]. Equally, considering the already infected populace, the possibility of being asymptomatic could vary, contingent upon the conditions and populace, going from 5% to 80% [2]. Three likely method of spread have been portrayed through droplet transmission, express contact spread, via airborne transmission. By and by, of late the digestive tract has additionally been demonstrated as a likely method of spread dependent on abdominal clinical indications and manifestation of diarrhea brought about by the viral infection, just as the viral RNA saw in faeces [3]. Various elements may control the infection's transmission given the infectivity levels of the host, for example, sanitary behaviours including quarantine, hand washing, and appropriate routine care which are needed to soothe the affinity of the viral infection and infectivity. It has been definite that community wellbeing mediation that stifles over 60% of transmissions are productive to deal with the concealment levels of the COVID-19 pandemic [3].

The term herd immunity was initially utilized in 1923 by Topley and Wilson [4]. It subsequently gave the need to vaccines and their purposes, vaccine program expenditure efficacy-breakdown, and the concealment of ailments, for example, smallpox and different irresistible diseases like polio and diphtheria [5, 6, 7]. Herd immunity is additionally affected by elements, for example, populace immunity and the methods of the spread of causal agents [6, 8].

Divergent investigations have broke down the helpfulness of vaccination projects to accomplish herd immunity in, and in this way shield the unvaccinated against different maladies [9]. The primary embodiments are the immunizations for cholera, hepatitis A, hepatitis B, human papillomavirus, haemophilus flu, meningococcal, flu, pneumococcal, polio, challenging hack, measles, chickenpox, rotavirus, and yellow fever [10, 11, 12, 13] a few of which are represented in Table 1. The ramifications of herd immunity on the viability of infection transmission were as of late observed in America with the Chikungunya and Zika infections, which caused a pandemic in an altogether weak populace. By and by, after the surge of these arboviruses, herd immunity confined their spread [14]. In like manner, the United Kingdom of late executed a plan for the COVID-19 pandemic that caused controversy, giving space for the infection to be transmitted in the populace before charging social seclusion to boost herd immunity [15].
Table 1: Historical perception of epidemic spread suppression via vaccination and herd effects

<table>
<thead>
<tr>
<th>Viral vaccines</th>
<th>Age range (yrs) of vaccine coverage</th>
<th>Sample population</th>
<th>Vaccination recipients</th>
<th>Percentage reduction of herd immuned</th>
<th>Location/year</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Seven-valent pneumococcal conjugate vaccine (PCV7)</td>
<td>&lt;5 (vaccinated) &gt;65 (not vaccinated)</td>
<td>851</td>
<td>2,105</td>
<td>98% reduction 65% reduction</td>
<td>UK/2006</td>
<td>[16]</td>
</tr>
<tr>
<td>Measles conjugate vaccine (MCV)</td>
<td>18 months (neonates) 10-13 (congenital)</td>
<td>3,115</td>
<td>2,105</td>
<td>85% reduction 100% reduction</td>
<td>Australia/2005</td>
<td>[17]</td>
</tr>
<tr>
<td>Live attenuated influenza vaccine (LAIV)</td>
<td>4-12</td>
<td>15,721</td>
<td>9021</td>
<td>60% reduction</td>
<td>Canada/2008</td>
<td>[18]</td>
</tr>
<tr>
<td>Rotavirus vaccine (Rotarix)</td>
<td>0-4</td>
<td>66,000</td>
<td>64,000</td>
<td>15% reduction</td>
<td>United States/2008</td>
<td>[19]</td>
</tr>
</tbody>
</table>

II. Elements of Herd Immunity in a Population with Covid-19

Deriving the orderly model of group immunity as the resistance that a specific populace has against a disease, Fox and his partners itemized four conditions under which such resistance can happen [20]. Initially, the transmittable microbe must be traced, found and restricted to a solitary host. For extreme intense respiratory disorder Covid 2 (SARS-Cov-2), the central method of spread was zoonotic in nature, with its essential host being the bat. Notwithstanding, the intermediary host through which it was transmitted to people is conditional. Transmission is known to have happened because of express contact with the contaminated animals or via their secretory liquids. Viral RNA has additionally been built up in canines and felines living with COVID-19 positive people. However, it has not been uncovered that these creatures can pass on the infection to individuals [21]. Also, the spread must happen predominantly through direct contact. Up till now, it has been standard that the spread of COVID-19 by direct individual-to-individual contact by means of coughing, sneezing, and inward breath of polluted vaporizers and contact spread through the oral, nasal, and ocular membranes [22].

With specific accentuation on the data lacking on the immune reaction incited by COVID-19 in people, it’s been difficult to set up the methods by which the immune system make a drawn out response that could fight the viral infection and deflect disease relapse. At last, group immunity is exploited if the populace procure a laid-back integration model. With this situation, everybody is defense-less against getting contaminated with COVID-19 bringing to the fore its inalienable indications. By and by, this plan of laidback blending will rely upon the preventive rules executed by every nation's administration overall which involves quarantine, seclusion, social separation, and fortified pre-emptive rules for in danger gatherings, for example, pregnant ladies, the old people, and youngsters [23]. Hypothetically, it is likely to achieve group immunity under the expressed speculations. In any case, by and by absence of intrinsic comprehension of the adaptive immune response and, in the non-existence of an appropriate vaccine, the moral limitations to achieve such immunity make this cure excessively deceptive to preliminary [24].

a) Creating herd immunity within populations

To make herd immunity inside a populace, the immunity achieved by immunization or natural infection must be turn away ahead spread of infections. For SARS-CoV-2, clinical signs are a helpless marker of transmissibility, as asymptomatic hosts can be exceptionally transmissible and add to the spread of an epidemic [25]. For microorganisms in which deep rooted immunity is prompted, similar to the case for measles immunization, herd immunity is significantly efficacious and can turn away microbe spread inside a populace. Nonetheless, this circumstance is sensibly atypical, as immunity for some infectious diseases, for example, pertussis and rotavirus, fade in the long haul [25]. Subsequently, herd immunity is less productive and discontinuous outbreaks can still surface. Eventually, if immunity is unpredictably spread in a populace, gatherings of disease-prone hosts that can frequently interact with one another may continue.

b) Herd immunity efficiency for COVID-19 transmission suppression

As indicated by the WHO, herd immunity is basically serviceable for communicable diseases [26]. For transferable infections, however, the immune-competent people must not exclusively be protected yet
there is the need of likewise decreasing disease among immununo-suppressed hosts through herd safeguard [27]. Attributable to herd protection, various maladies can be eradicated without 100% vaccination presentation. For a valid example Haemophilus influenzae type B vaccine introduction of under 70% in Gambia was adequate to eliminate Hib disease, with equal findings in Navajo populaces [28, 29].

For COVID-19, herd immunity, with its relatively inherent effect, probably won’t facilitate the infection as the outcomes rely upon the case and adequacy of the control measures and the ability to bring at the same time mounting outbreaks under instant control when required [30]. Antibodies for this infection are not yet accessible, however existing chemotherapeutic synergistic utilization, for example, azithromycin (AZT), hydroxychloroquine sulfate, chloroquine phosphate has been viewed as likely valuable. Regardless, their handiness and safety are built up in extra investigations for this novel malady [31, 32, 33].

III. Specifics of Herd Immunity and COVID-19

The current COVID-19 pandemic has produced over 81 million of clinically established cases and has claimed an absolute number of more than 1.7 million lives worldwide as of 31st December, 2020. A few exploratory preliminaries to evaluate novel immunization varieties and medication reconstituting approaches for the prevention and fixing of COVID-19 disease are directly in progress. Regardless, it is inconclusive whether these preliminaries will produce valuable cure, and it is dubious how broad these examinations will take albeit a positive assessment for any immunization preliminary is in any event 12 to year and a half. In the inaccessibility of an immunization, developing of COVID-19 herd immunity through common disease is still theoretically possible.

a) Epidemiological considerations for COVID-19 herd immunity

Inconsistency in method of spread between people may assume a key part in COVID-19 dispersal. Super-spreading occasions happen when conditions positive for high paces of transmission emerge. These occasions include a solitary index case infecting countless secondary contacts and are known to be significant in driving episodes of infectious diseases, including SARS, MERS, and measles [34]. Reports of COVID-19 super-spreading occasions have been recorded, recommending that heterogeneity in infectivity may essentially affect the elements of its transmission [35]. At long last, the variables that impact inter-individual heterogeneity in COVID-19 vulnerability, clinical pathology, and disease result are not surely known.

b) Immunological antibody responses to COVID-19

Following contamination with COVID-19, perceptible IgM and IgG antibodies create inside days to weeks of symptom onset beginning in most infected people [36, 37, 38]. Why a few patients appear not to build up a humoral immune reaction, as reflected by recognizable antibodies, is dubious. Adding to this vulnerability is the indistinct connection between antibody reaction and clinical improvement. The observations from a small investigation of 9 patients with COVID-19 found that more prominent clinical severity delivered higher antibody titers [36]. Be that as it may, antibody recognition and higher titers have not generally been found to correspond with clinical improvement in COVID-19 [37, 38]. Additionally, mild COVID-19 symptoms can resolve earlier detection by IgM and IgG antibodies, albeit distinguishable IgM and IgG antibodies have heralded decreases in COVID-19 viral loads [37, 38]. What appears more certain is that viral load regularly peaks from the get-go in infection, and afterward declines as antibodies sprang up and antibody titers ascend over the resulting 2 to 3 weeks [37, 38]. The stability of neutralizing antibodies (NAbs, principally IgG) against COVID-19 presently can’t seem to be characterized; ingenuity as long as 40 days from symptom onset has been depicted [36].

IV. Applications of Herd Immunity in the Current Covid-19 Pandemic

As per various models applied to infectious diseases, herd immunity is mathematically-connected with the spread and infection inclinations of the virus [39, 40] as displayed in the herd immunity network in Figure 1, which are the consequence of the relationship set up over a specific timeframe between the number of healthy subjects and those vulnerable to infection, the infected subjects that can no longer add to the transmission of the infection, infected subjects, and the normal or vaccine-immune subjects in a populace, mediated by the infectiousness of the virus, the incubation time frame, the transmissibility period, the virus limit between individuals, the elements of contact among the populace, and the term of the viral disease [41].
Herd immunity

Infected

Immune

Susceptible

Figure 1: Succinct outline of a herd immunity network. Infected: Persons who have contacted a disease likely because of the immunosuppressed nature of their immune framework. Immune: Persons whose immunological system is able to withstand infectious disease spread. Susceptible: Persons who are vulnerable to communicable disease spread.

Hardly, any investigations describe children as key cases in familial groups [42, 43], and plainly, children all the more regularly have asymptomatic diseases when contrasted and grown-ups [33, 43, 44]. It is of dire significance in demonstrating the pandemic to attempt cautious surveillance, including asymptomatic children and velocity of infection dependent on serology, to more readily describe disease in children and their part in transmission frameworks. Regardless of whether diverse control guidelines decelerate, in the end halting the local spread of COVID-19 infection, the effective containment of this infection actually leaves the populace in danger of resurgence because of deficient acquisition of immunity. In the event that herd immunity can be actuated, it would go about as a boundary to stop the spread of disease [45]. Additionally, vaccines, variable susceptibility and exposure to a great extent establish herd immunity.

Immunization of children to instigate herd immunity has demonstrated efficacy in forestalling the spread of numerous infectious diseases, where children have a critical part in transmission. An elevated level of immunity in one age gathering, who assume a function in transmission, can create herd immunity for others [46], and it is obvious that vaccine inoculation of children is more successful than immunization of old individuals, in specific circumstances, as exhibited in vaccination against flu [46, 47], pneumococcal infection [48], rotavirus [19] and numerous others. It is imperative that the overall function of various age cohorts in transmission must be considered. The backhanded advantages of COVID-19 vaccination in children may give or make some assured protection to more established, unvaccinated populations. At the point when children are immunized, it will be simpler to accomplish enough immunity required for general protection in a given populace.

Coronavirus mortality is emphatically age-ward, and Africa has a similarly more youthful populace than other continents [49]. Consequently, children may be a significant objective for mediations pointed toward decreasing transmission in nations with youthful populaces, particularly since access through school vaccination might be more direct than getting to grown-
ups. Despite the fact that the need for COVID-19 immunization would legitimately be for that at the most noteworthy danger of infection, for example, medical services labourers, and those at the most elevated danger of severe infection, for instance, older adults, vaccination of children might be another critical cohort for their own safeguard and to help herd immunity.

a) Herd immunity constraints in the current covid-19 pandemic

Herd immunity might be accomplished when the populace is exposed to the infection and develops a characteristic immune reaction and somatic defense system to the infection or when the populace is vaccinated against the COVID-19 malady to accomplish immunity along these lines, by vaccinating certain cohorts of the populace, the spread of the infection will go down. Without an immunization, building herd immunity against COVID-19 through natural infection is hypothetically conceivable. Be that as it may, there is no ethical way to arrive at this objective, as the social results of characteristic natural exposure might be overwhelming [42].

Without a doubt, current mathematical and epidemiological examinations propose that herd immunity through common methods may not be the response to stop the novel coronavirus; exposure to the viral infection ought to be maintained until either an immunization or viable pharmacological medicines are accessible. Hence, pharmacological Interventions (PIs, for example, the utilization of hydroxychloroquine, azithromycin, lopinavir, ritonavir, ribavirin, chloroquine phosphate, and arbidol, have been proposed to be conceivably compelling in fighting COVID-19 once the infection's hereditary arrangement and component of infection are unchangeable [50]. Notwithstanding, the adequacy and efficacy of these competitor drugs in the treatment of COVID-19 should be affirmed in further preclinical and clinical preliminaries, in spite of the in vitro examinations and non-clinical preliminaries previously available [51].

V. Group Immunity and Vaccination in Covid-19 Transmission Supression

Vaccine development could help halt the spread of the infection, particularly among the most susceptible populaces. This objective has become the procedure most seriously sought after by worldwide research facilities [52, 42]. Immunizations have generally been viewed as a type of a preventive mediation for immediate and aberrant protection for herd immunity in an comprehensive populace Reformist vaccination missions and enhancements in complete disinfection in metropolitan settings have assisted with improving herd immunity [53]. Note that vaccination is additionally balanced by vaccine type, the individual life form reaction, anticipation program adherence, and the age of administration [54].

Vaccines train the body to perceive and battle a particular microbe. The viral spread is confined when the immunization rate or the commonness of a high level of positive serological people in the populace truly limit the transmission of the infectious agent starting with one human then onto the next. Building up a "basic" populace immunity rate to control the expansion of COVID-19 is, with current logical information simply theoretical. Moreover, the mass immunization of billions of individuals could be one of the most significant worldwide challenges of the 21st century [15]. Presently, 25 vaccines are being created to battle COVID-19, with subsidizing chiefly originating from private drug foundations. Figure 2 shows the varying vaccine choices presently being investigated. Some research groups are utilizing inactivated viruses, however most investigations on vaccine varieties center around vaccinations dependent on viral proteins and nucleic acids.
As recently detailed, COVID-19 is particularly hazardous in individuals of cutting advanced age or with existing underlying conditions, for example, diabetes, coronary issues, malignancy, or a weakened immune system. Other natural elements, for example, diet quality, inactive way of life, and clinical medicines, may likewise restrict the immune reactions of most weak individuals before future vaccine administration against COVID-19. Thusly, herd immunity might be the most significant "present moment" technique to secure this segment of the population [55]. Until a safe vaccine is ultimately developed, research on definite novel treatments (or an efficient blend of existing treatments), together with action plans to contain the spread of the virus, seem to be the only substitute for protecting at-risk populations [56].

Discovering an efficient vaccine will not be without firm challenges to surmount, such as its resultant effects, price and ease of accessibility, limited secondary effects to vulnerable people, long-term immune response, and the keenness of the population to be vaccinated voluntarily [27]. There are certain limits to swaying people to engage in mass vaccination [57], even when direct immunization could safely extend indirect immunity to the most vulnerable populations [58, 59]. However, public communication plans on the importance of herd immunity and easily accessible vaccination campaigns will be necessary to increase observance to prevention programs in the fight against the COVID-19 infection [60].

VI. Conclusion

Among humans, the novel COVID-19 spread happens by direct person-to-person contact via coughing, sneezing, and inhalation of droplets/infected aerosols and contact spread with the oral and nasal membranes. Herd immunity has expansively been used for the containment of multiple diseases and presents the basis for vaccines and their relevancies. The communicable pathogen has been discovered, but the transitional host is still undecided. Additionally, there should be long-lasting immunity, which is still feasible for COVID-19. With no vaccine in view, cluster immunity is likely to be attained when about 70% of the people has been infected. Diverse forms of vaccines are in advance stages of formulation worldwide in order to curb the scourge of the virus. The COVID-19 pandemic could only end if a clinically-safe, tested and effective vaccine is confirmed, with the concept of herd immunization acquired and embraced. Finally, pending the availability of a vaccine, epidemic spread suppression via exceptionally-concentrated health criterions potentially shows to be the workable and secure plan, implementing quarantine and the application of numerous contact with wherewithal management to suppress the spread of the virus.
Authors’ Contributions: All authors contributed uniformly to this review article. The final review manuscript was read and endorsed by all authors before submission.

Conflict of Interest: None declared

References Références Referencias


57. Ahmed SF, Quadeer AA, McKay MR. Preliminary identification of potential vaccine targets for the COVID-19 coronavirus (SARS-CoV-2) based on


MEMBERSHIPS

FELLOWS/ASSOCIATES OF MEDICAL RESEARCH COUNCIL

FMRC/AMRC MEMBERSHIPS

INTRODUCTION

FMRC/AMRC is the most prestigious membership of Global Journals accredited by Open Association of Research Society, U.S.A (OARS). The credentials of Fellow and Associate designations signify that the researcher has gained the knowledge of the fundamental and high-level concepts, and is a subject matter expert, proficient in an expertise course covering the professional code of conduct, and follows recognized standards of practice. The credentials are designated only to the researchers, scientists, and professionals that have been selected by a rigorous process by our Editorial Board and Management Board.

Associates of FMRC/AMRC are scientists and researchers from around the world are working on projects/researches that have huge potentials. Members support Global Journals’ mission to advance technology for humanity and the profession.

FMRC

FELLOW OF MEDICAL RESEARCH COUNCIL

FELLOW OF MEDICAL RESEARCH COUNCIL is the most prestigious membership of Global Journals. It is an award and membership granted to individuals that the Open Association of Research Society judges to have made a substantial contribution to the improvement of computer science, technology, and electronics engineering.

The primary objective is to recognize the leaders in research and scientific fields of the current era with a global perspective and to create a channel between them and other researchers for better exposure and knowledge sharing. Members are most eminent scientists, engineers, and technologists from all across the world. Fellows are elected for life through a peer review process on the basis of excellence in the respective domain. There is no limit on the number of new nominations made in any year. Each year, the Open Association of Research Society elect up to 12 new Fellow Members.
Benefit

To the institution
Get letter of appreciation
Global Journals sends a letter of appreciation of author to the Dean or CEO of the University or Company of which author is a part, signed by editor in chief or chief author.

Exclusive Network
Get access to a closed network
A FMRC member gets access to a closed network of Tier 1 researchers and scientists with direct communication channel through our website. Fellows can reach out to other members or researchers directly. They should also be open to reaching out by other.

Certificate
Certificate, LoR and Laser-Momento
Fellows receive a printed copy of a certificate signed by our Chief Author that may be used for academic purposes and a personal recommendation letter to the dean of member’s university.

Designation
Get honored title of membership
Fellows can use the honored title of membership. The “FMRC” is an honored title which is accorded to a person’s name viz. Dr. John E. Hall, Ph.D., FMRC or William Walldroff, M.S., FMRC.

Recognition on the Platform
Better visibility and citation
All the Fellow members of FMRC get a badge of “Leading Member of Global Journals” on the Research Community that distinguishes them from others. Additionally, the profile is also partially maintained by our team for better visibility and citation. All fellows get a dedicated page on the website with their biography.

© Copyright by Global Journals | Guidelines Handbook
FUTURE WORK
GET DISCOUNTS ON THE FUTURE PUBLICATIONS
Fellows receive discounts on the future publications with Global Journals up to 60%. Through our recommendation programs, members also receive discounts on publications made with OARS affiliated organizations.

GJ INTERNAL ACCOUNT
UNLIMITED FORWARD OF EMAILS
Fellows get secure and fast GJ work emails with unlimited storage of emails that they may use them as their primary email. For example, john [AT] globaljournals [DOT] org.

PREMIUM TOOLS
ACCESS TO ALL THE PREMIUM TOOLS
To take future researches to the zenith, fellows receive access to all the premium tools that Global Journals have to offer along with the partnership with some of the best marketing leading tools out there.

CONFERENCES & EVENTS
ORGANIZE SEMINAR/CONFERENCE
Fellows are authorized to organize symposium/seminar/conference on behalf of Global Journal Incorporation (USA). They can also participate in the same organized by another institution as representative of Global Journal. In both the cases, it is mandatory for him to discuss with us and obtain our consent. Additionally, they get free research conferences (and others) alerts.

EARLY INVITATIONS
EARLY INVITATIONS TO ALL THE SYMPOSIUMS, SEMINARS, CONFERENCES
All fellows receive the early invitations to all the symposiums, seminars, conferences and webinars hosted by Global Journals in their subject.
PUBLISHING ARTICLES & BOOKS

EARN 60% OF SALES PROCEEDS
Fellows can publish articles (limited) without any fees. Also, they can earn up to 70% of sales proceeds from the sale of reference/review books/literature/publishing of research paper. The FMRC member can decide its price and we can help in making the right decision.

REVIEWS

GET A REMUNERATION OF 15% OF AUTHOR FEES
Fellow members are eligible to join as a paid peer reviewer at Global Journals Incorporation (USA) and can get a remuneration of 15% of author fees, taken from the author of a respective paper.

ACCESS TO EDITORIAL BOARD

BECOME A MEMBER OF THE EDITORIAL BOARD
Fellows and Associates may join as a member of the Editorial Board of Global Journals Incorporation (USA) after successful completion of three years as Fellow and as Peer Reviewer.

AND MUCH MORE

GET ACCESS TO SCIENTIFIC MUSEUMS AND OBSERVATORIES ACROSS THE GLOBE
All members get access to 5 selected scientific museums and observatories across the globe. All researches published with Global Journals will be kept under deep archival facilities across regions for future protections and disaster recovery. They get 10 GB free secure cloud access for storing research files.
ASSOCIATE OF MEDICAL RESEARCH COUNCIL

ASSOCIATE OF MEDICAL RESEARCH COUNCIL is the membership of Global Journals awarded to individuals that the Open Association of Research Society judges to have made a significant contribution to the improvement of computer science, technology, and electronics engineering.

The primary objective is to recognize the leaders in research and scientific fields of the current era with a global perspective and to create a channel between them and other researchers for better exposure and knowledge sharing. Members are most eminent scientists, engineers, and technologists from all across the world. Associate membership can later be promoted to Fellow Membership. Associates are elected for life through a peer review process on the basis of excellence in the respective domain. There is no limit on the number of new nominations made in any year. Each year, the Open Association of Research Society elect up to 12 new Associate Members.
Benefit

To the Institution

Get Letter of Appreciation
Global Journals sends a letter of appreciation of author to the Dean or CEO of the University or Company of which author is a part, signed by editor in chief or chief author.

Exclusive Network

Get Access to a Closed Network
A AMRC member gets access to a closed network of Tier 2 researchers and scientists with direct communication channel through our website. Associates can reach out to other members or researchers directly. They should also be open to reaching out by other.

Certificate

Certificate, LoR and Laser-Momento
Associates receive a printed copy of a certificate signed by our Chief Author that may be used for academic purposes and a personal recommendation letter to the dean of member's university.

Designation

Get Honored Title of Membership
Associates can use the honored title of membership. The “AMRC” is an honored title which is accorded to a person’s name viz. Dr. John E. Hall, Ph.D., AMRC or William Walldroff, M.S., AMRC.

Recognition on the Platform

Better Visibility and Citation
All the Associate members of AMRC get a badge of "Leading Member of Global Journals" on the Research Community that distinguishes them from others. Additionally, the profile is also partially maintained by our team for better visibility and citation.
FUTURE WORK
GET DISCOUNTS ON THE FUTURE PUBLICATIONS
Associates receive discounts on future publications with Global Journals up to 30%. Through our recommendation programs, members also receive discounts on publications made with OARS affiliated organizations.

GJ ACCOUNT
UNLIMITED FORWARD OF EMAILS
Associates get secure and fast GJ work emails with 5GB forward of emails that they may use them as their primary email. For example, john@globaljournals.org.

PREMIUM TOOLS
ACCESS TO ALL THE PREMIUM TOOLS
To take future researches to the zenith, fellows receive access to almost all the premium tools that Global Journals have to offer along with the partnership with some of the best marketing leading tools out there.

CONFERENCES & EVENTS
ORGANIZE SEMINAR/CONFERENCE
Associates are authorized to organize symposium/seminar/conference on behalf of Global Journal Incorporation (USA). They can also participate in the same organized by another institution as representative of Global Journal. In both the cases, it is mandatory for him to discuss with us and obtain our consent. Additionally, they get free research conferences (and others) alerts.

EARLY INVITATIONS
EARLY INVITATIONS TO ALL THE SYMPOSIUMS, SEMINARS, CONFERENCES
All associates receive the early invitations to all the symposiums, seminars, conferences and webinars hosted by Global Journals in their subject.

© Copyright by Global Journals | Guidelines Handbook
PUBLISHING ARTICLES & BOOKS

EARN 60% OF SALES PROCEEDS

Associates can publish articles (limited) without any fees. Also, they can earn up to 30-40% of sales proceeds from the sale of reference/review books/literature/publishing of research paper.

EXCLUSIVE

FINANCIAL

REVIEWERS

GET A REMUNERATION OF 15% OF AUTHOR FEES

Associate members are eligible to join as a paid peer reviewer at Global Journals Incorporation (USA) and can get a remuneration of 15% of author fees, taken from the author of a respective paper.

EXCLUSIVE

FINANCIAL

AND MUCH MORE

GET ACCESS TO SCIENTIFIC MUSEUMS AND OBSERVATORIES ACROSS THE GLOBE

All members get access to 2 selected scientific museums and observatories across the globe. All researches published with Global Journals will be kept under deep archival facilities across regions for future protections and disaster recovery. They get 5 GB free secure cloud access for storing research files.

EXCLUSIVE

FINANCIAL
<table>
<thead>
<tr>
<th>ASSOCIATE</th>
<th>FELLOW</th>
<th>RESEARCH GROUP</th>
<th>BASIC</th>
</tr>
</thead>
<tbody>
<tr>
<td>$4800 lifetime designation</td>
<td>$6800 lifetime designation</td>
<td>$12500.00 organizational</td>
<td>APC per article</td>
</tr>
<tr>
<td>Certificate, LoR and Momento 2 discounted publishing/year</td>
<td>Certificate, LoR and Momento</td>
<td>Certificates, LoRs and Momentos Unlimited free publishing/year</td>
<td>GJ Community Access</td>
</tr>
<tr>
<td>Gradation of Research 10 research contacts/day 1 GB Cloud Storage GJ Community Access</td>
<td>Unlimited discounted publishing/year</td>
<td>Unlimited free publishing/year</td>
<td></td>
</tr>
<tr>
<td>Gradation of Research Unlimited research contacts/day 5 GB Cloud Storage</td>
<td>Gradation of Research Unlimited research contacts/day</td>
<td>Teen Unlimited Cloud Storage Unlimited Cloud Storage</td>
<td></td>
</tr>
<tr>
<td>Unlimited research contacts/day</td>
<td>Online Presence Assistance GJ Community Access</td>
<td>Online Presence Assistance GJ Community Access</td>
<td></td>
</tr>
</tbody>
</table>
We accept the manuscript submissions in any standard (generic) format.

We typeset manuscripts using advanced typesetting tools like Adobe InDesign, CorelDraw, TeXnicCenter, and TeXStudio. We usually recommend authors submit their research using any standard format they are comfortable with, and let Global Journals do the rest.

Alternatively, you can download our basic template from https://globaljournals.org/Template

Authors should submit their complete paper/article, including text illustrations, graphics, conclusions, artwork, and tables. Authors who are not able to submit manuscript using the form above can email the manuscript department at submit@globaljournals.org or get in touch with chiefeditor@globaljournals.org if they wish to send the abstract before submission.

BEFORE AND DURING SUBMISSION

Authors must ensure the information provided during the submission of a paper is authentic. Please go through the following checklist before submitting:

1. Authors must go through the complete author guideline and understand and agree to Global Journals’ ethics and code of conduct, along with author responsibilities.
2. Authors must accept the privacy policy, terms, and conditions of Global Journals.
3. Ensure corresponding author’s email address and postal address are accurate and reachable.
4. Manuscript to be submitted must include keywords, an abstract, a paper title, co-author(s’) names and details (email address, name, phone number, and institution), figures and illustrations in vector format including appropriate captions, tables, including titles and footnotes, a conclusion, results, acknowledgments and references.
5. Authors should submit paper in a ZIP archive if any supplementary files are required along with the paper.
6. Proper permissions must be acquired for the use of any copyrighted material.
7. Manuscript submitted must not have been submitted or published elsewhere and all authors must be aware of the submission.

DECLARATION OF CONFLICTS OF INTEREST

It is required for authors to declare all financial, institutional, and personal relationships with other individuals and organizations that could influence (bias) their research.

POLICY ON PLAGIARISM

Plagiarism is not acceptable in Global Journals submissions at all.

Plagiarized content will not be considered for publication. We reserve the right to inform authors’ institutions about plagiarism detected either before or after publication. If plagiarism is identified, we will follow COPE guidelines:

Authors are solely responsible for all the plagiarism that is found. The author must not fabricate, falsify or plagiarize existing research data. The following, if copied, will be considered plagiarism:

- Words (language)
- Ideas
- Findings
- Writings
- Diagrams
- Graphs
- Illustrations
- Lectures

© Copyright by Global Journals | Guidelines Handbook
Authorship Policies

Global Journals follows the definition of authorship set up by the Open Association of Research Society, USA. According to its guidelines, authorship criteria must be based on:

1. Substantial contributions to the conception and acquisition of data, analysis, and interpretation of findings.
2. Drafting the paper and revising it critically regarding important academic content.
3. Final approval of the version of the paper to be published.

Changes in Authorship

The corresponding author should mention the name and complete details of all co-authors during submission and in manuscript. We support addition, rearrangement, manipulation, and deletions in authors list till the early view publication of the journal. We expect that corresponding author will notify all co-authors of submission. We follow COPE guidelines for changes in authorship.

Copyright

During submission of the manuscript, the author is confirming an exclusive license agreement with Global Journals which gives Global Journals the authority to reproduce, reuse, and republish authors’ research. We also believe in flexible copyright terms where copyright may remain with authors/employers/institutions as well. Contact your editor after acceptance to choose your copyright policy. You may follow this form for copyright transfers.

Appealing Decisions

Unless specified in the notification, the Editorial Board’s decision on publication of the paper is final and cannot be appealed before making the major change in the manuscript.

Acknowledgments

Contributors to the research other than authors credited should be mentioned in Acknowledgments. The source of funding for the research can be included. Suppliers of resources may be mentioned along with their addresses.

Declaration of funding sources

Global Journals is in partnership with various universities, laboratories, and other institutions worldwide in the research domain. Authors are requested to disclose their source of funding during every stage of their research, such as making analysis, performing laboratory operations, computing data, and using institutional resources, from writing an article to its submission. This will also help authors to get reimbursements by requesting an open access publication letter from Global Journals and submitting to the respective funding source.

Preparing your Manuscript

Authors can submit papers and articles in an acceptable file format: MS Word (doc, docx), LaTeX (.tex, .zip or .rar including all of your files), Adobe PDF (.pdf), rich text format (.rtf), simple text document (.txt), Open Document Text (.odt), and Apple Pages (.pages). Our professional layout editors will format the entire paper according to our official guidelines. This is one of the highlights of publishing with Global Journals—authors should not be concerned about the formatting of their paper. Global Journals accepts articles and manuscripts in every major language, be it Spanish, Chinese, Japanese, Portuguese, Russian, French, German, Dutch, Italian, Greek, or any other national language, but the title, subtitle, and abstract should be in English. This will facilitate indexing and the pre-peer review process.

The following is the official style and template developed for publication of a research paper. Authors are not required to follow this style during the submission of the paper. It is just for reference purposes.
Manuscript Style Instruction (Optional)

- Microsoft Word Document Setting Instructions.
- Font type of all text should be Swis721 Lt BT.
- Page size: 8.27” x 11””, left margin: 0.65, right margin: 0.65, bottom margin: 0.75.
- Paper title should be in one column of font size 24.
- Author name in font size of 11 in one column.
- Abstract: font size 9 with the word “Abstract” in bold italics.
- Main text: font size 10 with two justified columns.
- Two columns with equal column width of 3.38 and spacing of 0.2.
- First character must be three lines drop-capped.
- The paragraph before spacing of 1 pt and after of 0 pt.
- Line spacing of 1 pt.
- Large images must be in one column.
- The names of first main headings (Heading 1) must be in Roman font, capital letters, and font size of 10.
- The names of second main headings (Heading 2) must not include numbers and must be in italics with a font size of 10.

Structure and Format of Manuscript

The recommended size of an original research paper is under 15,000 words and review papers under 7,000 words. Research articles should be less than 10,000 words. Research papers are usually longer than review papers. Review papers are reports of significant research (typically less than 7,000 words, including tables, figures, and references)

A research paper must include:

a) A title which should be relevant to the theme of the paper.
b) A summary, known as an abstract (less than 150 words), containing the major results and conclusions.
c) Up to 10 keywords that precisely identify the paper’s subject, purpose, and focus.
d) An introduction, giving fundamental background objectives.
e) Resources and techniques with sufficient complete experimental details (wherever possible by reference) to permit repetition, sources of information must be given, and numerical methods must be specified by reference.
f) Results which should be presented concisely by well-designed tables and figures.
g) Suitable statistical data should also be given.
h) All data must have been gathered with attention to numerical detail in the planning stage.

Design has been recognized to be essential to experiments for a considerable time, and the editor has decided that any paper that appears not to have adequate numerical treatments of the data will be returned unrefereed.

i) Discussion should cover implications and consequences and not just recapitulate the results; conclusions should also be summarized.
j) There should be brief acknowledgments.
k) There ought to be references in the conventional format. Global Journals recommends APA format.

Authors should carefully consider the preparation of papers to ensure that they communicate effectively. Papers are much more likely to be accepted if they are carefully designed and laid out, contain few or no errors, are summarizing, and follow instructions. They will also be published with much fewer delays than those that require much technical and editorial correction.

The Editorial Board reserves the right to make literary corrections and suggestions to improve brevity.
**Format Structure**

It is necessary that authors take care in submitting a manuscript that is written in simple language and adheres to published guidelines.

All manuscripts submitted to Global Journals should include:

**Title**

The title page must carry an informative title that reflects the content, a running title (less than 45 characters together with spaces), names of the authors and co-authors, and the place(s) where the work was carried out.

**Author details**

The full postal address of any related author(s) must be specified.

**Abstract**

The abstract is the foundation of the research paper. It should be clear and concise and must contain the objective of the paper and inferences drawn. It is advised to not include big mathematical equations or complicated jargon.

Many researchers searching for information online will use search engines such as Google, Yahoo or others. By optimizing your paper for search engines, you will amplify the chance of someone finding it. In turn, this will make it more likely to be viewed and cited in further works. Global Journals has compiled these guidelines to facilitate you to maximize the web-friendliness of the most public part of your paper.

**Keywords**

A major lynchpin of research work for the writing of research papers is the keyword search, which one will employ to find both library and internet resources. Up to eleven keywords or very brief phrases have to be given to help data retrieval, mining, and indexing.

One must be persistent and creative in using keywords. An effective keyword search requires a strategy: planning of a list of possible keywords and phrases to try.

Choice of the main keywords is the first tool of writing a research paper. Research paper writing is an art. Keyword search should be as strategic as possible.

One should start brainstorming lists of potential keywords before even beginning searching. Think about the most important concepts related to research work. Ask, “What words would a source have to include to be truly valuable in a research paper?” Then consider synonyms for the important words.

It may take the discovery of only one important paper to steer in the right keyword direction because, in most databases, the keywords under which a research paper is abstracted are listed with the paper.

**Numerical Methods**

Numerical methods used should be transparent and, where appropriate, supported by references.

**Abbreviations**

Authors must list all the abbreviations used in the paper at the end of the paper or in a separate table before using them.

**Formulas and equations**

Authors are advised to submit any mathematical equation using either MathJax, KaTeX, or LaTeX, or in a very high-quality image.

**Tables, Figures, and Figure Legends**

Tables: Tables should be cautiously designed, uncrowned, and include only essential data. Each must have an Arabic number, e.g., Table 4, a self-explanatory caption, and be on a separate sheet. Authors must submit tables in an editable format and not as images. References to these tables (if any) must be mentioned accurately.
Figures

Figures are supposed to be submitted as separate files. Always include a citation in the text for each figure using Arabic numbers, e.g., Fig. 4. Artwork must be submitted online in vector electronic form or by emailing it.

Preparation of Electronic Figures for Publication

Although low-quality images are sufficient for review purposes, print publication requires high-quality images to prevent the final product being blurred or fuzzy. Submit (possibly by e-mail) EPS (line art) or TIFF (halftone/photos) files only. MS PowerPoint and Word Graphics are unsuitable for printed pictures. Avoid using pixel-oriented software. Scans (TIFF only) should have a resolution of at least 350 dpi (halftone) or 700 to 1100 dpi (line drawings). Please give the data for figures in black and white or submit a Color Work Agreement form. EPS files must be saved with fonts embedded (and with a TIFF preview, if possible).

For scanned images, the scanning resolution at final image size ought to be as follows to ensure good reproduction: line art: >650 dpi; halftones (including gel photographs): >350 dpi; figures containing both halftone and line images: >650 dpi.

Color charges: Authors are advised to pay the full cost for the reproduction of their color artwork. Hence, please note that if there is color artwork in your manuscript when it is accepted for publication, we would require you to complete and return a Color Work Agreement form before your paper can be published. Also, you can email your editor to remove the color fee after acceptance of the paper.

Tips for Writing a Good Quality Medical Research Paper

1. Choosing the topic: In most cases, the topic is selected by the interests of the author, but it can also be suggested by the guides. You can have several topics, and then judge which you are most comfortable with. This may be done by asking several questions of yourself, like "Will I be able to carry out a search in this area? Will I find all necessary resources to accomplish the search? Will I be able to find all information in this field area?" If the answer to this type of question is "yes," then you ought to choose that topic. In most cases, you may have to conduct surveys and visit several places. Also, you might have to do a lot of work to find all the rises and falls of the various data on that subject. Sometimes, detailed information plays a vital role, instead of short information. Evaluators are human: The first thing to remember is that evaluators are also human beings. They are not only meant for rejecting a paper. They are here to evaluate your paper. So present your best aspect.

2. Think like evaluators: If you are in confusion or getting demotivated because your paper may not be accepted by the evaluators, then think, and try to evaluate your paper like an evaluator. Try to understand what an evaluator wants in your research paper, and you will automatically have your answer. Make blueprints of paper: The outline is the plan or framework that will help you to arrange your thoughts. It will make your paper logical. But remember that all points of your outline must be related to the topic you have chosen.

3. Ask your guides: If you are having any difficulty with your research, then do not hesitate to share your difficulty with your guide (if you have one). They will surely help you out and resolve your doubts. If you can't clarify what exactly you require for your work, then ask your supervisor to help you with an alternative. He or she might also provide you with a list of essential readings.

4. Use of computer is recommended: As you are doing research in the field of medical research then this point is quite obvious. Use right software: Always use good quality software packages. If you are not capable of judging good software, then you can lose the quality of your paper unknowingly. There are various programs available to help you which you can get through the internet.

5. Use the internet for help: An excellent start for your paper is using Google. It is a wondrous search engine, where you can have your doubts resolved. You may also read some answers for the frequent question of how to write your research paper or find a model research paper. You can download books from the internet. If you have all the required books, place importance on reading, selecting, and analyzing the specified information. Then sketch out your research paper. Use big pictures: You may use encyclopedias like Wikipedia to get pictures with the best resolution. At Global Journals, you should strictly follow here.
6. Bookmarks are useful: When you read any book or magazine, you generally use bookmarks, right? It is a good habit which helps to not lose your continuity. You should always use bookmarks while searching on the internet also, which will make your search easier.

7. Revise what you wrote: When you write anything, always read it, summarize it, and then finalize it.

8. Make every effort: Make every effort to mention what you are going to write in your paper. That means always have a good start. Try to mention everything in the introduction—what is the need for a particular research paper. Polish your work with good writing skills and always give an evaluator what he wants. Make backups: When you are going to do any important thing like making a research paper, you should always have backup copies of it either on your computer or on paper. This protects you from losing any portion of your important data.

9. Produce good diagrams of your own: Always try to include good charts or diagrams in your paper to improve quality. Using several unnecessary diagrams will degrade the quality of your paper by creating a hodgepodge. So always try to include diagrams which were made by you to improve the readability of your paper. Use of direct quotes: When you do research relevant to literature, history, or current affairs, then use of quotes becomes essential, but if the study is relevant to science, use of quotes is not preferable.

10. Use proper verb tense: Use proper verb tenses in your paper. Use past tense to present those events that have happened. Use present tense to indicate events that are going on. Use future tense to indicate events that will happen in the future. Use of wrong tenses will confuse the evaluator. Avoid sentences that are incomplete.

11. Pick a good study spot: Always try to pick a spot for your research which is quiet. Not every spot is good for studying.

12. Know what you know: Always try to know what you know by making objectives, otherwise you will be confused and unable to achieve your target.

13. Use good grammar: Always use good grammar and words that will have a positive impact on the evaluator; use of good vocabulary does not mean using tough words which the evaluator has to find in a dictionary. Do not fragment sentences. Eliminate one-word sentences. Do not ever use a big word when a smaller one would suffice.

Verbs have to be in agreement with their subjects. In a research paper, do not start sentences with conjunctions or finish them with prepositions. When writing formally, it is advisable to never split an infinitive because someone will (wrongly) complain. Avoid clichés like a disease. Always shun irritating alliteration. Use language which is simple and straightforward. Put together a neat summary.

14. Arrangement of information: Each section of the main body should start with an opening sentence, and there should be a changeover at the end of the section. Give only valid and powerful arguments for your topic. You may also maintain your arguments with records.

15. Never start at the last minute: Always allow enough time for research work. Leaving everything to the last minute will degrade your paper and spoil your work.

16. Multitasking in research is not good: Doing several things at the same time is a bad habit in the case of research activity. Research is an area where everything has a particular time slot. Divide your research work into parts, and do a particular part in a particular time slot.

17. Never copy others' work: Never copy others' work and give it your name because if the evaluator has seen it anywhere, you will be in trouble. Take proper rest and food: No matter how many hours you spend on your research activity, if you are not taking care of your health, then all your efforts will have been in vain. For quality research, take proper rest and food.

18. Go to seminars: Attend seminars if the topic is relevant to your research area. Utilize all your resources.

19. Refresh your mind after intervals: Try to give your mind a rest by listening to soft music or sleeping in intervals. This will also improve your memory. Acquire colleagues: Always try to acquire colleagues. No matter how sharp you are, if you acquire colleagues, they can give you ideas which will be helpful to your research.

© Copyright by Global Journals | Guidelines Handbook
20. **Think technically:** Always think technically. If anything happens, search for its reasons, benefits, and demerits. Think and then print: When you go to print your paper, check that tables are not split, headings are not detached from their descriptions, and page sequence is maintained.

21. **Adding unnecessary information:** Do not add unnecessary information like "I have used MS Excel to draw graphs." Irrelevant and inappropriate material is superfluous. Foreign terminology and phrases are not apropos. One should never take a broad view. Analogy is like feathers on a snake. Use words properly, regardless of how others use them. Remove quotations. Puns are for kids, not grunt readers. Never oversimplify: When adding material to your research paper, never go for oversimplification; this will definitely irritate the evaluator. Be specific. Never use rhythmic redundancies. Contractions shouldn’t be used in a research paper. Comparisons are as terrible as clichés. Give up ampersands, abbreviations, and so on. Remove commas that are not necessary. Parenthetical words should be between brackets or commas. Understatement is always the best way to put forward earth-shaking thoughts. Give a detailed literary review.

22. **Report concluded results:** Use concluded results. From raw data, filter the results, and then conclude your studies based on measurements and observations taken. An appropriate number of decimal places should be used. Parenthetical remarks are prohibited here. Proofread carefully at the final stage. At the end, give an outline to your arguments. Spot perspectives of further study of the subject. Justify your conclusion at the bottom sufficiently, which will probably include examples.

23. **Upon conclusion:** Once you have concluded your research, the next most important step is to present your findings. Presentation is extremely important as it is the definite medium through which your research is going to be in print for the rest of the crowd. Care should be taken to categorize your thoughts well and present them in a logical and neat manner. A good quality research paper format is essential because it serves to highlight your research paper and bring to light all necessary aspects of your research.

In **Informal Guidelines of Research Paper Writing**

**Key points to remember:**
- Submit all work in its final form.
- Write your paper in the form which is presented in the guidelines using the template.
- Please note the criteria peer reviewers will use for grading the final paper.

**Final points:**

One purpose of organizing a research paper is to let people interpret your efforts selectively. The journal requires the following sections, submitted in the order listed, with each section starting on a new page:

**The introduction:** This will be compiled from reference matter and reflect the design processes or outline of basis that directed you to make a study. As you carry out the process of study, the method and process section will be constructed like that. The results segment will show related statistics in nearly sequential order and direct reviewers to similar intellectual paths throughout the data that you gathered to carry out your study.

**The discussion section:**

This will provide understanding of the data and projections as to the implications of the results. The use of good quality references throughout the paper will give the effort trustworthiness by representing an alertness to prior workings.

Writing a research paper is not an easy job, no matter how trouble-free the actual research or concept. Practice, excellent preparation, and controlled record-keeping are the only means to make straightforward progression.

**General style:**

Specific editorial column necessities for compliance of a manuscript will always take over from directions in these general guidelines.

**To make a paper clear:** Adhere to recommended page limits.

© Copyright by Global Journals | Guidelines Handbook
Mistakes to avoid:

- Insertion of a title at the foot of a page with subsequent text on the next page.
- Separating a table, chart, or figure—confine each to a single page.
- Submitting a manuscript with pages out of sequence.
- In every section of your document, use standard writing style, including articles ("a" and "the").
- Keep paying attention to the topic of the paper.
- Use paragraphs to split each significant point (excluding the abstract).
- Align the primary line of each section.
- Present your points in sound order.
- Use present tense to report well-accepted matters.
- Use past tense to describe specific results.
- Do not use familiar wording; don't address the reviewer directly. Don't use slang or superlatives.
- Avoid use of extra pictures—include only those figures essential to presenting results.

Title page:
Choose a revealing title. It should be short and include the name(s) and address(es) of all authors. It should not have acronyms or abbreviations or exceed two printed lines.

Abstract: This summary should be two hundred words or less. It should clearly and briefly explain the key findings reported in the manuscript and must have precise statistics. It should not have acronyms or abbreviations. It should be logical in itself. Do not cite references at this point.

An abstract is a brief, distinct paragraph summary of finished work or work in development. In a minute or less, a reviewer can be taught the foundation behind the study, common approaches to the problem, relevant results, and significant conclusions or new questions.

Write your summary when your paper is completed because how can you write the summary of anything which is not yet written? Wealth of terminology is very essential in abstract. Use comprehensive sentences, and do not sacrifice readability for brevity; you can maintain it succinctly by phrasing sentences so that they provide more than a lone rationale. The author can at this moment go straight to shortening the outcome. Sum up the study with the subsequent elements in any summary. Try to limit the initial two items to no more than one line each.

Reason for writing the article—theory, overall issue, purpose.

- Fundamental goal.
- To-the-point depiction of the research.
- Consequences, including definite statistics—if the consequences are quantitative in nature, account for this; results of any numerical analysis should be reported. Significant conclusions or questions that emerge from the research.

Approach:

- Single section and succinct.
- An outline of the job done is always written in past tense.
- Concentrate on shortening results—limit background information to a verdict or two.
- Exact spelling, clarity of sentences and phrases, and appropriate reporting of quantities (proper units, important statistics) are just as significant in an abstract as they are anywhere else.

Introduction:
The introduction should "introduce" the manuscript. The reviewer should be presented with sufficient background information to be capable of comprehending and calculating the purpose of your study without having to refer to other works. The basis for the study should be offered. Give the most important references, but avoid making a comprehensive appraisal of the topic. Describe the problem visibly. If the problem is not acknowledged in a logical, reasonable way, the reviewer will give no attention to your results. Speak in common terms about techniques used to explain the problem, if needed, but do not present any particulars about the protocols here.
The following approach can create a valuable beginning:

- Explain the value (significance) of the study.
- Defend the model—why did you employ this particular system or method? What is its compensation? Remark upon its appropriateness from an abstract point of view as well as pointing out sensible reasons for using it.
- Present a justification. State your particular theory(ies) or aim(s), and describe the logic that led you to choose them.
- Briefly explain the study's tentative purpose and how it meets the declared objectives.

**Approach:**

Use past tense except for when referring to recognized facts. After all, the manuscript will be submitted after the entire job is done. Sort out your thoughts; manufacture one key point for every section. If you make the four points listed above, you will need at least four paragraphs. Present surrounding information only when it is necessary to support a situation. The reviewer does not desire to read everything you know about a topic. Shape the theory specifically—do not take a broad view.

As always, give awareness to spelling, simplicity, and correctness of sentences and phrases.

**Procedures (methods and materials):**

This part is supposed to be the easiest to carve if you have good skills. A soundly written procedures segment allows a capable scientist to replicate your results. Present precise information about your supplies. The suppliers and clarity of reagents can be helpful bits of information. Present methods in sequential order, but linked methodologies can be grouped as a segment. Be concise when relating the protocols. Attempt to give the least amount of information that would permit another capable scientist to replicate your outcome, but be cautious that vital information is integrated. The use of subheadings is suggested and ought to be synchronized with the results section.

When a technique is used that has been well-described in another section, mention the specific item describing the way, but draw the basic principle while stating the situation. The purpose is to show all particular resources and broad procedures so that another person may use some or all of the methods in one more study or referee the scientific value of your work. It is not to be a step-by-step report of the whole thing you did, nor is a methods section a set of orders.

**Materials:**

*Materials may be reported in part of a section or else they may be recognized along with your measures.*

**Methods:**

- Report the method and not the particulars of each process that engaged the same methodology.
- Describe the method entirely.
- To be succinct, present methods under headings dedicated to specific dealings or groups of measures.
- Simplify—detail how procedures were completed, not how they were performed on a particular day.
- If well-known procedures were used, account for the procedure by name, possibly with a reference, and that's all.

**Approach:**

It is embarrassing to use vigorous voice when documenting methods without using first person, which would focus the reviewer's interest on the researcher rather than the job. As a result, when writing up the methods, most authors use third person passive voice.

Use standard style in this and every other part of the paper—avoid familiar lists, and use full sentences.

**What to keep away from:**

- Resources and methods are not a set of information.
- Skip all descriptive information and surroundings—save it for the argument.
- Leave out information that is immaterial to a third party.
Results:
The principle of a results segment is to present and demonstrate your conclusion. Create this part as entirely objective details of the outcome, and save all understanding for the discussion.

The page length of this segment is set by the sum and types of data to be reported. Use statistics and tables, if suitable, to present consequences most efficiently.

You must clearly differentiate material which would usually be incorporated in a study editorial from any unprocessed data or additional appendix matter that would not be available. In fact, such matters should not be submitted at all except if requested by the instructor.

Content:
- Sum up your conclusions in text and demonstrate them, if suitable, with figures and tables.
- In the manuscript, explain each of your consequences, and point the reader to remarks that are most appropriate.
- Present a background, such as by describing the question that was addressed by creation of an exacting study.
- Explain results of control experiments and give remarks that are not accessible in a prescribed figure or table, if appropriate.
- Examine your data, then prepare the analyzed (transformed) data in the form of a figure (graph), table, or manuscript.

What to stay away from:
- Do not discuss or infer your outcome, report surrounding information, or try to explain anything.
- Do not include raw data or intermediate calculations in a research manuscript.
- Do not present similar data more than once.
- A manuscript should complement any figures or tables, not duplicate information.
- Never confuse figures with tables—there is a difference.

Approach:
As always, use past tense when you submit your results, and put the whole thing in a reasonable order.

Put figures and tables, appropriately numbered, in order at the end of the report.

If you desire, you may place your figures and tables properly within the text of your results section.

Figures and tables:
If you put figures and tables at the end of some details, make certain that they are visibly distinguished from any attached appendix materials, such as raw facts. Whatever the position, each table must be titled, numbered one after the other, and include a heading. All figures and tables must be divided from the text.

Discussion:
The discussion is expected to be the trickiest segment to write. A lot of papers submitted to the journal are discarded based on problems with the discussion. There is no rule for how long an argument should be.

Position your understanding of the outcome visibly to lead the reviewer through your conclusions, and then finish the paper with a summing up of the implications of the study. The purpose here is to offer an understanding of your results and support all of your conclusions, using facts from your research and generally accepted information, if suitable. The implication of results should be fully described.

Infer your data in the conversation in suitable depth. This means that when you clarify an observable fact, you must explain mechanisms that may account for the observation. If your results vary from your prospect, make clear why that may have happened. If your results agree, then explain the theory that the proof supported. It is never suitable to just state that the data approved the prospect, and let it drop at that. Make a decision as to whether each premise is supported or discarded or if you cannot make a conclusion with assurance. Do not just dismiss a study or part of a study as "uncertain."
Research papers are not acknowledged if the work is imperfect. Draw what conclusions you can based upon the results that you have, and take care of the study as a finished work.

- You may propose future guidelines, such as how an experiment might be personalized to accomplish a new idea.
- Give details of all of your remarks as much as possible, focusing on mechanisms.
- Make a decision as to whether the tentative design sufficiently addressed the theory and whether or not it was correctly restricted. Try to present substitute explanations if they are sensible alternatives.
- One piece of research will not counter an overall question, so maintain the large picture in mind. Where do you go next? The best studies unlock new avenues of study. What questions remain?
- Recommendations for detailed papers will offer supplementary suggestions.

**Approach:**

When you refer to information, differentiate data generated by your own studies from other available information. Present work done by specific persons (including you) in past tense.

Describe generally acknowledged facts and main beliefs in present tense.

---

**The Administration Rules**

**Administration Rules to Be Strictly Followed before Submitting Your Research Paper to Global Journals Inc.**

*Please read the following rules and regulations carefully before submitting your research paper to Global Journals Inc. to avoid rejection.*

**Segment draft and final research paper:** You have to strictly follow the template of a research paper, failing which your paper may get rejected. You are expected to write each part of the paper wholly on your own. The peer reviewers need to identify your own perspective of the concepts in your own terms. Please do not extract straight from any other source, and do not rephrase someone else's analysis. Do not allow anyone else to proofread your manuscript.

**Written material:** You may discuss this with your guides and key sources. Do not copy anyone else's paper, even if this is only imitation, otherwise it will be rejected on the grounds of plagiarism, which is illegal. Various methods to avoid plagiarism are strictly applied by us to every paper, and, if found guilty, you may be blacklisted, which could affect your career adversely. To guard yourself and others from possible illegal use, please do not permit anyone to use or even read your paper and file.
Please note that following table is only a Grading of "Paper Compilation" and not on "Performed/Stated Research" whose grading solely depends on Individual Assigned Peer Reviewer and Editorial Board Member. These can be available only on request and after decision of Paper. This report will be the property of Global Journals.

<table>
<thead>
<tr>
<th>Topics</th>
<th>Grades</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A-B</td>
</tr>
<tr>
<td><strong>Abstract</strong></td>
<td>Clear and concise with appropriate content, Correct format. 200 words or below</td>
</tr>
<tr>
<td></td>
<td>Containing all background details with clear goal and appropriate details, flow specification, no grammar and spelling mistake, well organized sentence and paragraph, reference cited</td>
</tr>
<tr>
<td><strong>Introduction</strong></td>
<td>Clear and to the point with well arranged paragraph, precision and accuracy of facts and figures, well organized subheads</td>
</tr>
<tr>
<td><strong>Methods and Procedures</strong></td>
<td>Well organized, Clear and specific, Correct units with precision, correct data, well structuring of paragraph, no grammar and spelling mistake</td>
</tr>
<tr>
<td><strong>Result</strong></td>
<td>Well organized, meaningful specification, sound conclusion, logical and concise explanation, highly structured paragraph reference cited</td>
</tr>
<tr>
<td><strong>Discussion</strong></td>
<td>Complete and correct format, well organized</td>
</tr>
<tr>
<td><strong>References</strong></td>
<td>Complete and correct format, well organized</td>
</tr>
</tbody>
</table>
INDEX

A

Abundant · 3, 6
Accentuation · 41
Ailments · 40
Alleviate · 34

C

Conductive · 2

E

Embryonic · 1, 2, 3, 6, 7, 8, 12
Endorsed · 40, 47
Enumeration · 18
Epigenetic · 7, 8, 10
Epithelium · 3, 4, 5, 6, 7, 11, 12, 14, 15
Eradicate · 40

I

Inalienable · 41
Incessant · 33
Inferable · 40
Inoculation · 18, 20, 22, 44

P

Presumptive · 18, 20, 21, 23, 26, 27, 28, 30, 31, 32
Primitive · 5
Progenitor · 3, 5, 6, 8, 10, 11, 12, 13, 15
Proliferation · 3, 14
Propagation · 1, 2, 48

S

Surfactant · 5, 6, 8, 9, 12
Susceptible · 33, 35, 37, 38, 45