

African Journal of Microbiology Research

Volume 9 Number 34, 26 August, 2015
ISSN 1996-0808



*Academic
Journals*

ABOUT AJMR

The **African Journal of Microbiology Research (AJMR)** (ISSN 1996-0808) is published Weekly (one volume per year) by Academic Journals.

African Journal of Microbiology Research (AJMR) provides rapid publication (weekly) of articles in all areas of Microbiology such as: Environmental Microbiology, Clinical Microbiology, Immunology, Virology, Bacteriology, Phycology, Mycology and Parasitology, Protozoology, Microbial Ecology, Probiotics and Prebiotics, Molecular Microbiology, Biotechnology, Food Microbiology, Industrial Microbiology, Cell Physiology, Environmental Biotechnology, Genetics, Enzymology, Molecular and Cellular Biology, Plant Pathology, Entomology, Biomedical Sciences, Botany and Plant Sciences, Soil and Environmental Sciences, Zoology, Endocrinology, Toxicology. The Journal welcomes the submission of manuscripts that meet the general criteria of significance and scientific excellence. Papers will be published shortly after acceptance. All articles are peer-reviewed.

Submission of Manuscript

Please read the **Instructions for Authors** before submitting your manuscript. The manuscript files should be given the last name of the first author

[Click here to Submit manuscripts online](#)

If you have any difficulty using the online submission system, kindly submit via this email ajmr@academicjournals.org.

With questions or concerns, please contact the Editorial Office at ajmr@academicjournals.org.

Editors

Prof. Dr. Stefan Schmidt,

*Applied and Environmental Microbiology
School of Biochemistry, Genetics and Microbiology
University of KwaZulu-Natal
Private Bag X01
Scottsville, Pietermaritzburg 3209
South Africa.*

Prof. Fukai Bao

*Department of Microbiology and Immunology
Kunming Medical University
Kunming 650031,
China*

Dr. Jianfeng Wu

*Dept. of Environmental Health Sciences,
School of Public Health,
University of Michigan
USA*

Dr. Ahmet Yilmaz Coban

*OMU Medical School,
Department of Medical Microbiology,
Samsun,
Turkey*

Dr. Seyed Davar Siadat

*Pasteur Institute of Iran,
Pasteur Square, Pasteur Avenue,
Tehran,
Iran.*

Dr. J. Stefan Rokem

*The Hebrew University of Jerusalem
Department of Microbiology and Molecular Genetics,
P.O.B. 12272, IL-91120 Jerusalem,
Israel*

Prof. Long-Liu Lin

*National Chiayi University
300 Syuefu Road,
Chiayi,
Taiwan*

N. John Tonukari, Ph.D

*Department of Biochemistry
Delta State University
PMB 1
Abraka, Nigeria*

Dr. Thaddeus Ezeji

*Assistant Professor
Fermentation and Biotechnology Unit
Department of Animal Sciences
The Ohio State University
1680 Madison Avenue
USA.*

Associate Editors

Dr. Mamadou Gueye

*MIRCEN/ Laboratoire commun de microbiologie
IRD-ISRA-UCAD, BP 1386,
DAKAR, Senegal.*

Dr. Caroline Mary Knox

*Department of Biochemistry, Microbiology and
Biotechnology
Rhodes University
Grahamstown 6140
South Africa.*

Dr. Hesham Elsayed Mostafa

*Genetic Engineering and Biotechnology Research
Institute (GEBRI)
Mubarak City For Scientific Research,
Research Area, New Borg El-Arab City,
Post Code 21934, Alexandria, Egypt.*

Dr. Wael Abbas El-Naggar

*Head of Microbiology Department,
Faculty of Pharmacy,
Mansoura University,
Mansoura 35516, Egypt.*

Dr. Abdel Nasser A. El-Moghazy

*Microbiology, Molecular Biology, Genetics Engineering
and Biotechnology
Dept of Microbiology and Immunology
Faculty of Pharmacy
Al-Azhar University
Nasr city,
Cairo, Egypt*

Editorial Board

Dr. Barakat S.M. Mahmoud

*Food Safety/Microbiology
Experimental Seafood Processing Laboratory
Costal Research and Extension Center
Mississippi State University
3411 Frederic Street
Pascagoula, MS 39567
USA*

Prof. Mohamed Mahrous Amer

*Poultry Disease (Viral Diseases of poultry)
Faculty of Veterinary Medicine,
Department of Poultry Diseases
Cairo university
Giza, Egypt*

Dr. Xiaohui Zhou

*Molecular Microbiology, Industrial Microbiology,
Environmental Microbiology, Pathogenesis, Antibiotic
resistance, Microbial Ecology
Washington State University
Bustad Hall 402 Department of Veterinary
Microbiology and Pathology, Pullman,
USA*

Dr. R. Balaji Raja

*Department of Biotechnology,
School of Bioengineering,
SRM University,
Chennai
India*

Dr. Aly E Abo-Amer

*Division of Microbiology, Botany Department, Faculty
of Science, Sohag University.
Egypt.*

Dr. Haoyu Mao

*Department of Molecular Genetics and Microbiology
College of Medicine
University of Florida
Florida, Gainesville
USA.*

Dr. Rachna Chandra

*Environmental Impact Assessment Division
Environmental Sciences
Sálim Ali Center for Ornithology and Natural History
(SACON),
Anaikatty (PO), Coimbatore-641108, India*

Dr. Yongxu Sun

*Department of Medicinal Chemistry and
Biomacromolecules
Qiqihar Medical University, Qiqihar 161006
Heilongjiang Province
P.R. China*

Dr. Ramesh Chand Kasana

*Institute of Himalayan Bioresource Technology
Palampur, Distt. Kangra (HP),
India*

Dr. S. Meena Kumari

*Department of Biosciences
Faculty of Science
University of Mauritius
Reduit*

Dr. T. Ramesh

*Assistant Professor
Marine Microbiology
CAS in Marine Biology
Faculty of Marine Sciences
Annamalai University
Parangipettai - 608 502
Cuddalore Dist. Tamilnadu,
India*

Dr. Pagano Marcela Claudia

*Post doctoral fellowship at Department of Biology,
Federal University of Ceará - UFC,
Brazil.*

Dr. EL-Sayed E. Habib

*Associate Professor,
Dept. of Microbiology,
Faculty of Pharmacy,
Mansoura University,
Egypt.*

Dr. Pongsak Rattanachaikunsopon

*Department of Biological Science,
Faculty of Science,
Ubon Ratchathani University,
Warin Chamrap, Ubon Ratchathani 34190,
Thailand*

Dr. Gokul Shankar Sabesan

*Microbiology Unit, Faculty of Medicine,
AIMST University
Jalan Bedong, Semeling 08100,
Kedah,
Malaysia*

Dr. Kwang Young Song

*Department of Biological Engineering,
School of Biological and Chemical Engineering,
Yanbian University of Science and Technology,
Yanji,
China.*

Dr. Kamel Belhamel

*Faculty of Technology,
University of Bejaia
Algeria*

Dr. Sladjana Jevremovic

*Institute for Biological Research
Sinisa Stankovic,
Belgrade,
Serbia*

Dr. Tamer Edirne

*Dept. of Family Medicine, Univ. of Pamukkale
Turkey*

Dr. R. Balaji Raja M.Tech (Ph.D)

*Assistant Professor,
Department of Biotechnology,
School of Bioengineering,
SRM University,
Chennai.
India*

Dr. Minglei Wang

University of Illinois at Urbana-Champaign, USA

Dr. Mohd Fuat ABD Razak

*Institute for Medical Research
Malaysia*

Dr. Davide Pacifico

*Istituto di Virologia Vegetale – CNR
Italy*

Prof. Dr. Akrum Hamdy

*Faculty of Agriculture, Minia University, Egypt
Egypt*

Dr. Ntobeko A. B. Ntusi

*Cardiac Clinic, Department of Medicine,
University of Cape Town and
Department of Cardiovascular Medicine,
University of Oxford
South Africa and
United Kingdom*

Prof. N. S. Alzoreky

*Food Science & Nutrition Department,
College of Agricultural Sciences & Food,
King Faisal University,
Saudi Arabia*

Dr. Chen Ding

*College of Material Science and Engineering,
Hunan University,
China*

Dr Svetlana Nikolić

*Faculty of Technology and Metallurgy,
University of Belgrade,
Serbia*

Dr. Sivakumar Swaminathan

*Department of Agronomy,
College of Agriculture and Life Sciences,
Iowa State University,
Ames, Iowa 50011
USA*

Dr. Alfredo J. Anceno

*School of Environment, Resources and Development
(SERD),
Asian Institute of Technology,
Thailand*

Dr. Iqbal Ahmad

*Aligarh Muslim University,
Alighrah
India*

Dr. Josephine Nketsia-Tabiri

*Ghana Atomic Energy Commission
Ghana*

Dr. Juliane Elisa Welke

*UFRGS – Universidade Federal do Rio
Grande do Sul
Brazil*

Dr. Mohammad Nazrul Islam

*NIMR; IPH-Bangalore & NIUM
Bangladesh*

Dr. Okonko, Iheanyi Omezuruike

*Department of Virology,
Faculty of Basic Medical Sciences,
College of Medicine,
University of Ibadan,
University College Hospital,
Ibadan,
Nigeria*

Dr. Giuliana Noratto

*Texas A&M University
USA*

Dr. Phanikanth Venkata Turlapati

*Washington State University
USA*

Dr. Khaleel I. Z. Jawasreh

*National Centre for Agricultural Research and
Extension, NCARE
Jordan*

Dr. Babak Mostafazadeh, MD

*Shaheed Beheshti University of Medical Sciences
Iran*

Dr. S. Meena Kumari

*Department of Biosciences
Faculty of Science
University of Mauritius
Reduit
Mauritius*

Dr. S. Anju

*Department of Biotechnology,
SRM University, Chennai-603203
India*

Dr. Mustafa Maroufpor

Iran

Prof. Dong Zhichun

*Professor, Department of Animal Sciences and
Veterinary Medicine,
Yunnan Agriculture University,
China*

Dr. Mehdi Azami

*Parasitology & Mycology Dept,
Baghaeei Lab.,
Shams Abadi St.
Isfahan
Iran*

Dr. Anderson de Souza Sant'Ana

*University of São Paulo.
Brazil.*

Dr. Juliane Elisa Welke

*UFRGS – Universidade Federal do Rio Grande do Sul
Brazil*

Dr. Paul Shapshak

*USF Health,
Depts. Medicine (Div. Infect. Disease & Internat Med)
and Psychiatry & Beh Med.
USA*

Dr. Jorge Reinheimer

*Universidad Nacional del Litoral (Santa Fe)
Argentina*

Dr. Qin Liu

*East China University of Science
and Technology
China*

Dr. Xiao-Qing Hu

*State Key Lab of Food Science and Technology
Jiangnan University
P. R. China*

Prof. Branislava Kocic

*Specialist of Microbiology and Parasitology
University of Nis, School of Medicine Institute
for Public Health Nis, Bul. Z. Djindjica 50, 18000 Nis
Serbia*

Dr. Rafel Socias

*CITA de Aragón,
Spain*

Prof. Kamal I. Mohamed

*State University of New York at Oswego
USA*

Dr. Adriano Cruz

*Faculty of Food Engineering-FEA
University of Campinas (UNICAMP)
Brazil*

Dr. Mike Agenbag (Michael Hermanus Albertus)

*Manager Municipal Health Services,
Joe Gqabi District Municipality
South Africa*

Dr. D. V. L. Sarada

*Department of Biotechnology,
SRM University, Chennai-603203
India.*

Dr. Samuel K Ameyaw

*Civista Medical Center
United States of America*

Prof. Huaizhi Wang

*Institute of Hepatopancreatobiliary
Surgery of PLA Southwest Hospital,
Third Military Medical University
Chongqing400038
P. R. China*

Prof. Bakhiet AO

*College of Veterinary Medicine, Sudan
University of Science and Technology
Sudan*

Dr. Saba F. Hussain

*Community, Orthodontics and Paediatric Dentistry
Department
Faculty of Dentistry
Universiti Teknologi MARA
40450 Shah Alam, Selangor
Malaysia*

Prof. Dr. Zohair I.F.Rahemo

*State Key Lab of Food Science and Technology
Jiangnan University
P. R. China*

Dr. Afework Kassu

*University of Gondar
Ethiopia*

Prof. Isidro A. T. Savillo

*ISCOF
Philippines*

Dr. How-Yee Lai

*Taylor's University College
Malaysia*

Dr. Nidheesh Dadheech

*MS. University of Baroda, Vadodara, Gujarat, India.
India*

Dr. Omitoyin Siyanbola

*Bowen University,
Iwo
Nigeria*

Dr. Franco Mutinelli

*Istituto Zooprofilattico Sperimentale delle Venezie
Italy*

Dr. Chanpen Chanchao

*Department of Biology,
Faculty of Science,
Chulalongkorn University
Thailand*

Dr. Tsuyoshi Kasama

*Division of Rheumatology,
Showa University
Japan*

Dr. Kuender D. Yang, MD.

*Chang Gung Memorial Hospital
Taiwan*

Dr. Liane Raluca Stan

*University Politehnica of Bucharest,
Department of Organic Chemistry "C.Nenitzescu"
Romania*

Dr. Muhamed Osman

*Senior Lecturer of Pathology & Consultant
Immunopathologist
Department of Pathology,
Faculty of Medicine,
Universiti Teknologi MARA,
40450 Shah Alam, Selangor
Malaysia*

Dr. Mohammad Feizabadi

*Tehran University of medical Sciences
Iran*

Prof. Ahmed H Mitwalli

*State Key Lab of Food Science and Technology
Jiangnan University
P. R. China*

Dr. Mazyar Yazdani

*Department of Biology,
University of Oslo,
Blindern,
Oslo,
Norway*

Dr. Ms. Jemimah Gesare Onsare

*Ministry of Higher, Education
Science and Technology
Kenya*

Dr. Babak Khalili Hadad

*Department of Biological Sciences,
Roudehen Branch,
Islamic Azad University,
Roudehen
Iran*

Dr. Ehsan Sari

*Department of Plant Pathology,
Iranian Research Institute of Plant Protection,
Tehran,
Iran.*

Dr. Snjezana Zidovec Lepej

*University Hospital for Infectious Diseases
Zagreb,
Croatia*

Dr. Dilshad Ahmad

*King Saud University
Saudi Arabia*

Dr. Adriano Gomes da Cruz

*University of Campinas (UNICAMP)
Brazil*

Dr. Hsin-Mei Ku

*Agronomy Dept. NCHU 250 Kuo
Kuang Rd, Taichung,
Taiwan*

Dr. Fereshteh Naderi

*Physical chemist,
Islamic Azad University,
Shahre Ghods Branch
Iran*

Dr. Adibe Maxwell Ogochukwu

*Department of Clinical Pharmacy and Pharmacy
Management,
University of Nigeria,
Nsukka.
Nigeria*

Dr. William M. Shafer

*Emory University School of Medicine
USA*

Dr. Michelle Bull

*CSIRO Food and Nutritional Sciences
Australia*

Prof. Dr. Márcio Garcia Ribeiro (DVM, PhD)

*School of Veterinary Medicine and Animal Science-
UNESP,
Dept. Veterinary Hygiene and Public Health,
State of Sao Paulo
Brazil*

Prof. Dr. Sheila Nathan

*National University of Malaysia (UKM)
Malaysia*

Prof. Ebiamadon Andi Brisibe

*University of Calabar,
Calabar,
Nigeria*

Dr. Julie Wang

*Burnet Institute
Australia*

Dr. Jean-Marc Chobert

*INRA- BIA, FIPL
France*

Dr. Zhilong Yang, PhD

*Laboratory of Viral Diseases
National Institute of Allergy and Infectious Diseases,
National Institutes of Health*

Dr. Dele Raheem

*University of Helsinki
Finland*

Dr. Li Sun

*PLA Centre for the treatment of infectious diseases,
Tangdu Hospital,
Fourth Military Medical University
China*

Dr. Biljana Miljkovic-Selimovic

*School of Medicine,
University in Nis,
Serbia; Referent laboratory for Campylobacter and
Helicobacter,
Center for Microbiology,
Institute for Public Health, Nis
Serbia*

Dr. Xinan Jiao

*Yangzhou University
China*

Dr. Endang Sri Lestari, MD.

*Department of Clinical Microbiology,
Medical Faculty,
Diponegoro University/Dr. Kariadi Teaching Hospital,
Semarang
Indonesia*

Dr. Hojin Shin

*Pusan National University Hospital
South Korea*

Dr. Yi Wang

*Center for Vector Biology, 180 Jones Avenue
Rutgers University, New Brunswick, NJ 08901-8536
USA*

Dr. Heping Zhang

*The Key Laboratory of Dairy Biotechnology and
Engineering,
Ministry of Education,
Inner Mongolia Agricultural University.
China*

Prof. Natasha Potgieter

*University of Venda
South Africa*

Dr. Alemzadeh

*Sharif University
Iran*

Dr. Sonia Arriaga

*Instituto Potosino de Investigación Científica y
Tecnológica/División de Ciencias Ambientales
Mexico*

Dr. Armando Gonzalez-Sanchez

*Universidad Autonoma Metropolitana Cuajimalpa
Mexico*

Dr. Pradeep Parihar

*Lovely Professional University, Phagwara, Punjab.
India*

Dr. William H Roldán

*Department of Medical Microbiology,
Faculty of Medicine,
Peru*

Dr. Kanzaki, L I B

*Laboratory of Bioprospection. University of Brasilia
Brazil*

Prof. Philippe Dorchie

*Laboratory of Bioprospection. University of Brasilia
Brazil*

Dr. C. Ganesh Kumar

*Indian Institute of Chemical Technology,
Hyderabad
India*

Dr. Farid Che Ghazali

*Universiti Sains Malaysia (USM)
Malaysia*

Dr. Samira Bouhdid

*Abdelmalek Essaadi University,
Tetouan,
Morocco*

Dr. Zainab Z. Ismail

*Department of Environmental Engineering, University
of Baghdad.
Iraq*

Dr. Ary Fernandes Junior

*Universidade Estadual Paulista (UNESP)
Brasil*

Dr. Papaevangelou Vassiliki

*Athens University Medical School
Greece*

Dr. Fangyou Yu

*The first Affiliated Hospital of Wenzhou Medical
College
China*

Dr. Galba Maria de Campos Takaki

*Catholic University of Pernambuco
Brazil*

Dr. Kwabena Ofori-Kwakye

*Department of Pharmaceutics,
Kwame Nkrumah University of Science & Technology,
KUMASI
Ghana*

Prof. Dr. Liesel Brenda Gende

*Arthropods Laboratory, School of Natural and Exact
Sciences, National University of Mar del Plata
Buenos Aires,
Argentina.*

Dr. Adeshina Gbonjubola

*Ahmadu Bello University,
Zaria.
Nigeria*

Prof. Dr. Stylianos Chatzipanagiotou

*University of Athens – Medical School
Greece*

Dr. Dongqing BAI

*Department of Fishery Science,
Tianjin Agricultural College,
Tianjin 300384
P. R. China*

Dr. Dingqiang Lu

*Nanjing University of Technology
P.R. China*

Dr. L. B. Sukla

*Scientist –G & Head, Biominerals Department,
IMMT, Bhubaneswar
India*

Dr. Hakan Parlakpınar

*MD. Inonu University, Medical Faculty, Department
of Pharmacology, Malatya
Turkey*

Dr Pak-Lam Yu

*Massey University
New Zealand*

Dr Percy Chimwamurombe

*University of Namibia
Namibia*

Dr. Euclésio Simionatto

*State University of Mato Grosso do Sul-UEMS
Brazil*

Dr. Hans-Jürg Monstein

*Clinical Microbiology, Molecular Biology Laboratory,
University Hospital, Faculty of Health Sciences, S-581
85 Linköping
Sweden*

Dr. Ajith, T. A

*Associate Professor Biochemistry, Amala Institute of
Medical Sciences, Amala Nagar, Thrissur, Kerala-680
555
India*

Dr. Feng-Chia Hsieh

*Biopesticides Division, Taiwan Agricultural Chemicals
and Toxic Substances Research Institute, Council of
Agriculture
Taiwan*

Prof. Dra. Suzan Pantaroto de Vasconcellos

*Universidade Federal de São Paulo
Rua Prof. Artur Riedel, 275 Jd. Eldorado, Diadema, SP
CEP 09972-270
Brasil*

Dr. Maria Leonor Ribeiro Casimiro Lopes Assad

*Universidade Federal de São Carlos - Centro de
Ciências Agrárias - CCA/UFSCar
Departamento de Recursos Naturais e Proteção
Ambiental
Rodovia Anhanguera, km 174 - SP-330
Araras - São Paulo
Brasil*

Dr. Pierangeli G. Vital

*Institute of Biology, College of Science, University of
the Philippines
Philippines*

Prof. Roland Ndip

*University of Fort Hare, Alice
South Africa*

Dr. Shawn Carraher

*University of Fort Hare, Alice
South Africa*

Dr. José Eduardo Marques Pessanha

*Observatório de Saúde Urbana de Belo
Horizonte/Faculdade de Medicina da Universidade
Federal de Minas Gerais
Brasil*

Dr. Yuanshu Qian

*Department of Pharmacology, Shantou University
Medical College
China*

Dr. Helen Treichel

*URI-Campus de Erechim
Brazil*

Dr. Xiao-Qing Hu

*State Key Lab of Food Science and Technology
Jiangnan University
P. R. China*

Dr. Olli H. Tuovinen

*Ohio State University, Columbus, Ohio
USA*

Prof. Stoyan Groudev

*University of Mining and Geology "Saint Ivan Rilski"
Sofia
Bulgaria*

Dr. G. Thirumurugan

*Research lab, GIET School of Pharmacy, NH-5,
Chaitanya nagar, Rajahmundry-533294.
India*

Dr. Charu Gomber

*Thapar University
India*

Dr. Jan Kuever

*Bremen Institute for Materials Testing,
Department of Microbiology,
Paul-Feller-Str. 1, 28199 Bremen
Germany*

Dr. Nicola S. Flanagan

*Universidad Javeriana, Cali
Colombia*

Dr. André Luiz C. M. de A. Santiago

*Universidade Federal Rural de Pernambuco
Brazil*

Dr. Dhruva Kumar Jha

*Microbial Ecology Laboratory,
Department of Botany,
Gauhati University,
Guwahati 781 014, Assam
India*

Dr. N Saleem Basha

*M. Pharm (Pharmaceutical Biotechnology)
Eritrea (North East Africa)*

Prof. Dr. João Lúcio de Azevedo

*Dept. Genetics-University of São Paulo-Faculty of
Agriculture- Piracicaba, 13400-970
Brasil*

Dr. Julia Inés Fariña

*PROIMI-CONICET
Argentina*

Dr. Yutaka Ito

*Kyoto University
Japan*

Dr. Cheruiyot K. Ronald

*Biomedical Laboratory Technologist
Kenya*

Prof. Dr. Ata Akcil

*S. D. University
Turkey*

Dr. Adhar Manna

*The University of South Dakota
USA*

Dr. Cícero Flávio Soares Aragão

*Federal University of Rio Grande do Norte
Brazil*

Dr. Gunnar Dahlen

*Institute of odontology, Sahlgrenska Academy at
University of Gothenburg
Sweden*

Dr. Pankaj Kumar Mishra

*Vivekananda Institute of Hill Agriculture, (I.C.A.R.),
ALMORA-263601, Uttarakhand
India*

Dr. Benjamas W. Thanomsub

*Srinakharinwirot University
Thailand*

Dr. Maria José Borrego

*National Institute of Health – Department of Infectious
Diseases
Portugal*

Dr. Catherine Carrillo

*Health Canada, Bureau of Microbial Hazards
Canada*

Dr. Marcotty Tanguy

*Institute of Tropical Medicine
Belgium*

Dr. Han-Bo Zhang

*Laboratory of Conservation and Utilization for Bio-
resources*

*Key Laboratory for Microbial Resources of the
Ministry of Education,
Yunnan University, Kunming 650091.
School of Life Science,
Yunnan University, Kunming,
Yunnan Province 650091.
China*

Dr. Ali Mohammed Somily

*King Saud University
Saudi Arabia*

Dr. Nicole Wolter

*National Institute for Communicable Diseases and
University of the Witwatersrand,
Johannesburg
South Africa*

Dr. Marco Antonio Nogueira

*Universidade Estadual de Londrina
CCB/Depto. De microbiologia
Laboratório de Microbiologia Ambiental
Caixa Postal 6001
86051-980 Londrina.
Brazil*

Dr. Bruno Pavoni

*Department of Environmental Sciences University of
Venice
Italy*

Dr. Shih-Chieh Lee

*Da-Yeh University
Taiwan*

Dr. Satoru Shimizu

*Horonobe Research Institute for the Subsurface
Environment,
Northern Advancement Center for Science &
Technology
Japan*

Dr. Tang Ming

*College of Forestry, Northwest A&F University,
Yangling
China*

Dr. Olga Gortzi

*Department of Food Technology, T.E.I. of Larissa
Greece*

Dr. Mark Tarnopolsky

*Mcmaster University
Canada*

Dr. Sami A. Zabin

*Al Baha University
Saudi Arabia*

Dr. Julia W. Pridgeon

*Aquatic Animal Health Research Unit, USDA, ARS
USA*

Dr. Lim Yau Yan

*Monash University Sunway Campus
Malaysia*

Prof. Rosemeire C. L. R. Pietro

*Faculdade de Ciências Farmacêuticas de Araraquara,
Univ Estadual Paulista, UNESP
Brazil*

Dr. Nazime Mercan Dogan

*PAU Faculty of Arts and Science, Denizli
Turkey*

Dr Ian Edwin Cock

*Biomolecular and Physical Sciences
Griffith University
Australia*

Prof. N K Dubey

*Banaras Hindu University
India*

Dr. S. Hemalatha

*Department of Pharmaceutics, Institute of
Technology,
Banaras Hindu University, Varanasi. 221005
India*

Dr. J. Santos Garcia A.

*Universidad A. de Nuevo Leon
Mexico India*

Dr. Somboon Tanasupawat

*Department of Biochemistry and Microbiology,
Faculty of Pharmaceutical Sciences,
Chulalongkorn University,
Bangkok 10330
Thailand*

Dr. Vivekananda Mandal

*Post Graduate Department of Botany,
Darjeeling Government College,
Darjeeling – 734101.
India*

Dr. Shihua Wang

*College of Life Sciences,
Fujian Agriculture and Forestry University
China*

Dr. Victor Manuel Fernandes Galhano

*CITAB-Centre for Research and Technology of Agro-
Environment and Biological Sciences, Integrative
Biology and Quality Research Group,
University of Trás-os-Montes and Alto Douro,
Apartado 1013, 5001-801 Vila Real
Portugal*

Dr. Maria Cristina Maldonado

*Instituto de Biotecnología. Universidad Nacional de
Tucuman
Argentina*

Dr. Alex Soltermann

*Institute for Surgical Pathology,
University Hospital Zürich
Switzerland*

Dr. Dagmara Sirova

*Department of Ecosystem Biology, Faculty Of Science,
University of South Bohemia,
Branisovska 37, Ceske Budejovice, 37001
Czech Republic*

Dr. E. O Igbinosa

*Department of Microbiology,
Ambrose Alli University,
Ekpoma, Edo State,
Nigeria.*

Dr. Hodaka Suzuki

*National Institute of Health Sciences
Japan*

Dr. Mick Bosilevac

*US Meat Animal Research Center
USA*

Dr. Nora Lía Padola

*Imunoquímica y Biotecnología- Fac Cs Vet-UNCPBA
Argentina*

Dr. Maria Madalena Vieira-Pinto

*Universidade de Trás-os-Montes e Alto Douro
Portugal*

Dr. Stefano Morandi

*CNR-Istituto di Scienze delle Produzioni Alimentari
(ISPA), Sez. Milano
Italy*

Dr Line Thorsen

*Copenhagen University, Faculty of Life Sciences
Denmark*

Dr. Ana Lucia Falavigna-Guilherme

*Universidade Estadual de Maringá
Brazil*

Dr. Baoqiang Liao

*Dept. of Chem. Eng., Lakehead University, 955 Oliver
Road, Thunder Bay, Ontario
Canada*

Dr. Ouyang Jinping

*Patho-Physiology department,
Faculty of Medicine of Wuhan University
China*

Dr. John Sorensen

*University of Manitoba
Canada*

Dr. Andrew Williams

*University of Oxford
United Kingdom*

Dr. Chi-Chiang Yang

*Chung Shan Medical University
Taiwan, R.O.C.*

Dr. Quanming Zou

*Department of Clinical Microbiology and Immunology,
College of Medical Laboratory,
Third Military Medical University
China*

Prof. Ashok Kumar

*School of Biotechnology,
Banaras Hindu University, Varanasi
India*

Dr. Chung-Ming Chen

*Department of Pediatrics, Taipei Medical University
Hospital, Taipei
Taiwan*

Dr. Jennifer Furin

*Harvard Medical School
USA*

Dr. Julia W. Pridgeon

*Aquatic Animal Health Research Unit, USDA, ARS
USA*

Dr. Alireza Seidavi

*Islamic Azad University, Rasht Branch
Iran*

Dr. Thore Rohwerder

*Helmholtz Centre for Environmental Research UFZ
Germany*

Dr. Daniela Billi

*University of Rome Tor Vergata
Italy*

Dr. Ivana Karabegovic

*Faculty of Technology, Leskovac, University of Nis
Serbia*

Dr. Flaviana Andrade Faria

*IBILCE/UNESP
Brazil*

Prof. Margareth Linde Athayde

*Federal University of Santa Maria
Brazil*

Dr. Guadalupe Virginia Nevarez Moorillon

*Universidad Autonoma de Chihuahua
Mexico*

Dr. Tatiana de Sousa Fiuza

*Federal University of Goias
Brazil*

Dr. Indrani B. Das Sarma

*Jhulelal Institute of Technology, Nagpur
India*

Dr. Guanghua Wang

*Northeast Institute of Geography and Agroecology,
Chinese Academy of Sciences
China*

Dr. Renata Vadkertiova

*Institute of Chemistry, Slovak Academy of Science
Slovakia*

Dr. Charles Hocart

*The Australian National University
Australia*

Dr. Guoqiang Zhu

*University of Yangzhou College of Veterinary Medicine
China*

Dr. Guilherme Augusto Marietto Gonçalves

*São Paulo State University
Brazil*

Dr. Mohammad Ali Faramarzi

*Tehran University of Medical Sciences
Iran*

Dr. Suppasil Maneerat

*Department of Industrial Biotechnology, Faculty of
Agro-Industry, Prince of Songkla University, Hat Yai
90112
Thailand*

Dr. Francisco Javier Las heras Vazquez

*Almeria University
Spain*

Dr. Cheng-Hsun Chiu

*Chang Gung memorial Hospital, Chang Gung
University
Taiwan*

Dr. Ajay Singh

*DDU Gorakhpur University, Gorakhpur-273009 (U.P.)
India*

Dr. Karabo Shale

*Central University of Technology, Free State
South Africa*

Dr. Lourdes Zélia Zanoni

*Department of Pediatrics, School of Medicine, Federal
University of Mato Grosso do Sul, Campo Grande,
Mato Grosso do Sul
Brazil*

Dr. Tulin Askun

*Balikesir University
Turkey*

Dr. Marija Stankovic

*Institute of Molecular Genetics and Genetic
Engineering
Republic of Serbia*

Dr. Scott Weese

*University of Guelph
Dept of Pathobiology, Ontario Veterinary College,
University of Guelph,
Guelph, Ontario, N1G2W1,
Canada*

Dr. Sabiha Essack

*School of Health Sciences
South African Committee of Health Sciences
University of KwaZulu-Natal
Private Bag X54001
Durban 4000
South Africa*

Dr. Hare Krishna

*Central Institute for Arid Horticulture,
Beechwal, Bikaner-334 006, Rajasthan,
India*

Dr. Anna Mensuali

*Dept. of Life Science,
Scuola Superiore
Sant'Anna*

Dr. Ghada Sameh Hafez Hassan

*Pharmaceutical Chemistry Department,
Faculty of Pharmacy, Mansoura University,
Egypt*

Dr. Kátia Flávia Fernandes

*Biochemistry and Molecular Biology
Universidade Federal de Goiás
Brasil*

Dr. Abdel-Hady El-Gilany

*Public Health & Community Medicine
Faculty of Medicine,
Mansoura University
Egypt*

Dr. Hongxiong Guo

*STD and HIV/AIDS Control and Prevention,
Jiangsu provincial CDC,
China*

Dr. Konstantina Tsaousi

*Life and Health Sciences,
School of Biomedical Sciences,
University of Ulster*

Dr. Bhavnaben Gowan Gordhan

*DST/NRF Centre of Excellence for Biomedical TB
Research
University of the Witwatersrand and National Health
Laboratory Service
P.O. Box 1038, Johannesburg 2000,
South Africa*

Dr. Ernest Kuchar

*Pediatric Infectious Diseases,
Wroclaw Medical University,
Wroclaw Teaching Hospital,
Poland*

Dr. Hongxiong Guo

*STD and HIV/AIDS Control and Prevention,
Jiangsu provincial CDC,
China*

Dr. Mar Rodriguez Jovita

*Food Hygiene and Safety, Faculty of Veterinary
Science.
University of Extremadura,
Spain*

Dr. Jes Gitz Holler

*Hospital Pharmacy,
Aalesund. Central Norway Pharmaceutical Trust
Professor Brochs gt. 6. 7030 Trondheim,
Norway*

Prof. Chengxiang FANG

*College of Life Sciences,
Wuhan University
Wuhan 430072, P.R.China*

Dr. Anchalee Tungtrongchitr

*Siriraj Dust Mite Center for Services and Research
Department of Parasitology,
Faculty of Medicine Siriraj Hospital,
Mahidol University
2 Prannok Road, Bangkok Noi,
Bangkok, 10700, Thailand*

Instructions for Author

Electronic submission of manuscripts is strongly encouraged, provided that the text, tables, and figures are included in a single Microsoft Word file (preferably in Arial font).

The **cover letter** should include the corresponding author's full address and telephone/fax numbers and should be in an e-mail message sent to the Editor, with the file, whose name should begin with the first author's surname, as an attachment.

Article Types

Three types of manuscripts may be submitted:

Regular articles: These should describe new and carefully confirmed findings, and experimental procedures should be given in sufficient detail for others to verify the work. The length of a full paper should be the minimum required to describe and interpret the work clearly.

Short Communications: A Short Communication is suitable for recording the results of complete small investigations or giving details of new models or hypotheses, innovative methods, techniques or apparatus. The style of main sections need not conform to that of full-length papers. Short communications are 2 to 4 printed pages (about 6 to 12 manuscript pages) in length.

Reviews: Submissions of reviews and perspectives covering topics of current interest are welcome and encouraged. Reviews should be concise and no longer than 4-6 printed pages (about 12 to 18 manuscript pages). Reviews are also peer-reviewed.

Review Process

All manuscripts are reviewed by an editor and members of the Editorial Board or qualified outside reviewers. Authors cannot nominate reviewers. Only reviewers randomly selected from our database with specialization in the subject area will be contacted to evaluate the manuscripts. The process will be blind review.

Decisions will be made as rapidly as possible, and the Journal strives to return reviewers' comments to authors as fast as possible. The editorial board will re-review manuscripts that are accepted pending revision. It is the goal of the AJMR to publish manuscripts within weeks after submission.

Regular articles

All portions of the manuscript must be typed double-spaced and all pages numbered starting from the title page.

The Title should be a brief phrase describing the contents of the paper. The Title Page should include the authors' full names and affiliations, the name of the corresponding author along with phone, fax and E-mail information. Present addresses of authors should appear as a footnote.

The Abstract should be informative and completely self-explanatory, briefly present the topic, state the scope of the experiments, indicate significant data, and point out major findings and conclusions. The Abstract should be 100 to 200 words in length. Complete sentences, active verbs, and the third person should be used, and the abstract should be written in the past tense. Standard nomenclature should be used and abbreviations should be avoided. No literature should be cited.

Following the abstract, about 3 to 10 key words that will provide indexing references should be listed.

A list of non-standard **Abbreviations** should be added. In general, non-standard abbreviations should be used only when the full term is very long and used often. Each abbreviation should be spelled out and introduced in parentheses the first time it is used in the text. Only recommended SI units should be used. Authors should use the solidus presentation (mg/ml). Standard abbreviations (such as ATP and DNA) need not be defined.

The Introduction should provide a clear statement of the problem, the relevant literature on the subject, and the proposed approach or solution. It should be understandable to colleagues from a broad range of scientific disciplines.

Materials and methods should be complete enough to allow experiments to be reproduced. However, only truly new procedures should be described in detail; previously published procedures should be cited, and important modifications of published procedures should be mentioned briefly. Capitalize trade names and include the manufacturer's name and address. Subheadings should be used. Methods in general use need not be described in detail.

Results should be presented with clarity and precision. The results should be written in the past tense when describing findings in the authors' experiments. Previously published findings should be written in the present tense. Results should be explained, but largely without referring to the literature. Discussion, speculation and detailed interpretation of data should not be included in the Results but should be put into the Discussion section.

The Discussion should interpret the findings in view of the results obtained in this and in past studies on this topic. State the conclusions in a few sentences at the end of the paper. The Results and Discussion sections can include subheadings, and when appropriate, both sections can be combined.

The Acknowledgments of people, grants, funds, etc should be brief.

Tables should be kept to a minimum and be designed to be as simple as possible. Tables are to be typed double-spaced throughout, including headings and footnotes. Each table should be on a separate page, numbered consecutively in Arabic numerals and supplied with a heading and a legend. Tables should be self-explanatory without reference to the text. The details of the methods used in the experiments should preferably be described in the legend instead of in the text. The same data should not be presented in both table and graph form or repeated in the text.

Figure legends should be typed in numerical order on a separate sheet. Graphics should be prepared using applications capable of generating high resolution GIF, TIFF, JPEG or Powerpoint before pasting in the Microsoft Word manuscript file. Tables should be prepared in Microsoft Word. Use Arabic numerals to designate figures and upper case letters for their parts (Figure 1). Begin each legend with a title and include sufficient description so that the figure is understandable without reading the text of the manuscript. Information given in legends should not be repeated in the text.

References: In the text, a reference identified by means of an author's name should be followed by the date of the reference in parentheses. When there are more than two authors, only the first author's name should be mentioned, followed by 'et al'. In the event that an author cited has had two or more works published during the same year, the reference, both in the text and in the reference list, should be identified by a lower case letter like 'a' and 'b' after the date to distinguish the works.

Examples:

Abayomi (2000), Agindotan et al. (2003), (Kelebeni, 1983), (Usman and Smith, 1992), (Chege, 1998;

1987a,b; Tijani, 1993,1995), (Kumasi et al., 2001) References should be listed at the end of the paper in alphabetical order. Articles in preparation or articles submitted for publication, unpublished observations, personal communications, etc. should not be included in the reference list but should only be mentioned in the article text (e.g., A. Kingori, University of Nairobi, Kenya, personal communication). Journal names are abbreviated according to Chemical Abstracts. Authors are fully responsible for the accuracy of the references.

Examples:

Chikere CB, Omoni VT and Chikere BO (2008). Distribution of potential nosocomial pathogens in a hospital environment. *Afr. J. Biotechnol.* 7: 3535-3539.

Moran GJ, Amii RN, Abrahamian FM, Talan DA (2005). Methicillinresistant *Staphylococcus aureus* in community-acquired skin infections. *Emerg. Infect. Dis.* 11: 928-930.

Pitout JDD, Church DL, Gregson DB, Chow BL, McCracken M, Mulvey M, Laupland KB (2007). Molecular epidemiology of CTXM-producing *Escherichia coli* in the Calgary Health Region: emergence of CTX-M-15-producing isolates. *Antimicrob. Agents Chemother.* 51: 1281-1286.

Pelczar JR, Harley JP, Klein DA (1993). *Microbiology: Concepts and Applications.* McGraw-Hill Inc., New York, pp. 591-603.

Short Communications

Short Communications are limited to a maximum of two figures and one table. They should present a complete study that is more limited in scope than is found in full-length papers. The items of manuscript preparation listed above apply to Short Communications with the following differences: (1) Abstracts are limited to 100 words; (2) instead of a separate Materials and Methods section, experimental procedures may be incorporated into Figure Legends and Table footnotes; (3) Results and Discussion should be combined into a single section.

Proofs and Reprints: Electronic proofs will be sent (e-mail attachment) to the corresponding author as a PDF file. Page proofs are considered to be the final version of the manuscript. With the exception of typographical or minor clerical errors, no changes will be made in the manuscript at the proof stage.

Fees and Charges: Authors are required to pay a \$550 handling fee. Publication of an article in the African Journal of Microbiology Research is not contingent upon the author's ability to pay the charges. Neither is acceptance to pay the handling fee a guarantee that the paper will be accepted for publication. Authors may still request (in advance) that the editorial office waive some of the handling fee under special circumstances

Copyright: © 2015, Academic Journals.

All rights Reserved. In accessing this journal, you agree that you will access the contents for your own personal use but not for any commercial use. Any use and or copies of this Journal in whole or in part must include the customary bibliographic citation, including author attribution, date and article title.

Submission of a manuscript implies: that the work described has not been published before (except in the form of an abstract or as part of a published lecture, or thesis) that it is not under consideration for publication elsewhere; that if and when the manuscript is accepted for publication, the authors agree to automatic transfer of the copyright to the publisher.

Disclaimer of Warranties

In no event shall Academic Journals be liable for any special, incidental, indirect, or consequential damages of any kind arising out of or in connection with the use of the articles or other material derived from the AJMR, whether or not advised of the possibility of damage, and on any theory of liability.

This publication is provided "as is" without warranty of any kind, either expressed or implied, including, but not limited to, the implied warranties of merchantability, fitness for a particular purpose, or non-infringement. Descriptions of, or references to, products or publications does not imply endorsement of that product or publication. While every effort is made by Academic Journals to see that no inaccurate or misleading data, opinion or statements appear in this publication, they wish to make it clear that the data and opinions appearing in the articles and advertisements herein are the responsibility of the contributor or advertiser concerned. Academic Journals makes no warranty of any kind, either express or implied, regarding the quality, accuracy, availability, or validity of the data or information in this publication or of any other publication to which it may be linked.

African Journal of Microbiology Research

Table of Content: Volume 9 Number 34, 26 August, 2015

ARTICLES

Detection of extended-spectrum beta-lactamases (ESBLs) in clinical isolates of *Klebsiella pneumoniae* using the ESBL NDP test and flow cytometric assay in comparison to the standard disc diffusion

Samaa Taha, Nahed Youssef, Amany Elkazaz and Hazem Ramadan

Effects of lithium compounds on the growth of white-rot fungi

Mateus D. Nunes, Willian L. Cardoso, José M. R. Luz and Maria C. M. Kasuya

Microbiological load of yoghurt sold in Omoku schools, Rivers State, Nigeria

Chimezie Gabriel Dirisu, Gloria Lily and Ebere Igwe

Full Length Research Paper

Detection of extended-spectrum beta-lactamases (ESBLs) in clinical isolates of *Klebsiella pneumoniae* using the ESBL NDP test and flow cytometric assay in comparison to the standard disc diffusion

Samaa Taha^{1,2*}, Nahed Youssef³, Amany Elkazaz⁴ and Hazem Ramadan⁵

¹Department of Microbiology and Medical Immunology, Faculty of Medicine, Suez- Canal University, Ismailia 41522, Egypt.

²Department of Microbiology, College of Medicine, Taif University, Al-Taif, Saudi Arabia.

³Department of Clinical Pathology, Faculty of Medicine, Suez- Canal University, Ismailia 41522, Egypt.

⁴Department of Medical Biochemistry and Molecular Biology, Faculty of Medicine, Suez- Canal University, Ismailia 41522, Egypt.

⁵Department of Hygiene and Zoonoses, Faculty of Veterinary Medicine, Mansoura University, Mansoura 35516, Egypt.

Received 2 August, 2015; Accepted 17 August, 2015

This study was undertaken to evaluate the comparison among three different assays: extended-spectrum beta-lactamases (ESBL) Nordmann/ Dortet/ Poirel (NDP) test, flow cytometric assay and disc diffusion method for the detection of ESBL production. Sixty clinical isolates of *Klebsiella pneumoniae* were isolated from patients' clinical samples admitted to Suez-Canal University Hospital, Ismailia Governorate. The percentages of ESBLs producing *Klebsiella pneumoniae* ranged from 70 to 80% by ESBL NDP and flow cytometric assays, respectively in comparison to 76.6% by disc diffusion method. The sensitivity and specificity of the three assays were evaluated and the sensitivity by ESBL NDP and disc diffusion method was 100%, while by the flow cytometric assay, it was 91.3%. The specificity of disc diffusion method in detection of ESBLs was 100%, followed by the ESBL NDP test (85.7%) and flow cytometric assay (77.8%). Kappa testing showed perfect agreement between the ESBL NDP test and disc diffusion method (kappa=0.9), while flow cytometric assay showed substantial agreement (kappa=0.7). The ESBL NDP test offers an applicable tool for rapid detection of ESBL-production. Although, flow cytometric assay is a promising method that might be used in the clinical microbiology laboratory but there is a need for the experienced personnel along with the device.

Key words: Extended-spectrum beta-lactamases (ESBLs), ESBL NDP test, flow cytometry.

INTRODUCTION

Extended-spectrum beta-lactamases (ESBLs) produced by Gram-negative bacteria are considered one of the largest and rapidly evolving group of plasmid-mediated enzymes that confer resistance to oxyimino-cephalosporins and monobactams (Pitout,

2010). *Escherichia coli* and *Klebsiella pneumoniae*, being the major source of community- and hospital-acquired infections are mostly ESBL producers (Pitout and Laupland, 2008).

ESBL recognition has an important clinical impact as

inappropriate treatment can lead to therapeutic failures and consequently to adverse clinical outcomes (Schwaber and Carmeli, 2007). A variety of ESBLs have been reported in *Enterobacteriaceae*, being mostly of the CTX-M-, TEM- and SHV-types (Bush and Jacoby, 2010; Poirel et al., 2012). ESBL detection is necessary to screen patients, improve hospital infection control practices and to curb inappropriate antibiotic use that prolonged the efficacy of the currently available antibiotics (Schwaber et al., 2006; Zahar et al., 2009).

Current techniques for detecting ESBL producers are based on the determination of susceptibility to expanded-spectrum cephalosporins followed by the inhibition of the ESBL activity, mostly by clavulanic acid or tazobactam (Drieux et al., 2008). Sensitivities and specificities of the double disk test and of the E-test proposed for that purpose are good, ranging from 80 to 95% (Gazin et al., 2012). The automated methods used in the detection of ESBL producing organisms had a much higher sensitivity (80 to 99%) than specificity (50 to 80%). However, those tests require overnight growths consuming 24-48 h before ESBL production is detected with a subsequent delay in the initiation of appropriate antibiotic therapy (Schwaber et al., 2006; Drieux et al., 2008; Gazin et al., 2012).

Molecular detection of ESBL genes (PCR and sequencing) is an interesting alternative but remains costly and requires a certain degree of expertise (Drieux et al., 2008; Gazin et al., 2012) since recently, real time PCR and DNA microarray (Check-Points) are commercially available to detect ESBL gene variants (Cuzon et al., 2012). However, those PCR-based techniques require isolation of bacteria from clinical samples prior to susceptibility testing and phenotypic identifications and hence; those results can be obtained at least 48 h after obtaining the clinical samples. Also, they are usually not performed in a routine laboratory but restricted to epidemiological purposes. Therefore a simple and efficient technique for ESBL producers is required (Nordmann et al., 2012).

The ESBL NDP test is a novel test, based on the hydrolysis of the β -lactam ring of a cephalosporin (cefotaxime), which generates a carboxyl group, by acidifying a culture medium. It uses 96-well microtiter plates or a single tube and the acidity resulted from this hydrolysis is identified by the color change using a pH indicator (red phenol) while, inhibition of ESBL activity is evidenced by adding tazobactam in a complementary well (Cuzon et al., 2012).

A rapid, powerful high-throughput technology allowing analysis of several thousand cells per second and providing quantitative and statistically significant data is the flow cytometry (FC) (Shapiro, 2001). Bacterial cells

are incubated with cephalosporins (ceftazidime or cefotaxime) in the presence and absence of clavulanic acid; subsequently, cells are stained with the fluorescent dye Bis-(1, 3-dibutylbarbituric acid) trimethine oxonol [DiBAC4 (3)] which is able to diffuse across depolarized membranes. Susceptible isolates display increased fluorescence after 1 h of incubation; conversely, the increase of the depolarized population was only observed after incubation with clavulanic acid associated with ceftazidime or cefotaxime in ESBL producers (Ramos et al., 2012).

In the present study, we assessed two new methods (a flow cytometric assay and the ESBL NDP test) for detection of ESBLs in clinical isolates of *Klebsiella pneumoniae* in comparison with the standard disc diffusion method.

MATERIALS AND METHODS

Bacterial strains

A total of 60 clinical isolates of *K. pneumoniae* were isolated from patients (24 males and 36 females) with different clinical infections (12 sputum, 26 urine, 12 pus and 10 blood samples) admitted to Suez-Canal University Hospital, Ismailia Governorate from January to August 2014. The samples were collected from various clinical origins. Blood samples were inoculated into blood culture bottles (Egyptian Diagnostic Media, Egypt) then incubated at 37°C for 7-14 days. Subcultures were done every 48 h on blood agar and MacConkey's agar (Oxoid, UK) plates. Other samples were cultured on nutrient agar (Oxoid, UK) blood agar and MacConkey's agar. Gram negative bacilli giving non-lactose fermenting colonies on MacConkey's agar were taken for biochemical tests including mannitol motility, triple sugar iron, indole, citrate, MR, VP and carbohydrate utilization tests for identification (Birgul, 2010). *K. pneumoniae* ATCC 700603 and *E. coli* ATCC 25922 were used as ESBL- positive and negative, respectively (CLSI, 2014). All isolates were kept in soft agar at -20°C till the time for ESBL detection.

Antimicrobial drugs and ESBL phenotypic detection

For the disc diffusion method, antibiotic discs of ceftazidime (CAZ, 30 μ g), cefotaxime (CTX, 30 μ g), Cefotaxime- clavulanic acid (30/10 μ g) (CTC 40 μ g) and Ceftazidime- clavulanic acid (30/10 μ g) (CZC 40 μ g) were purchased from Bioanalyse Chemical Co Ltd, Turkey. Cefotaxime sodium salt, tazobactam (TZB) and clavulanic acid (CLA) were purchased from Sigma-Aldrich, Saint-Quentin-Fallavier, France for the ESBL NDP test. For flow cytometric assay, bis-(1, 3-dibutylbarbituric acid) trimethine oxonol [DiBAC4 (3)], a fluorescent probe that binds to membranes and to intracellular proteins of depolarized cells, was purchased from Invitrogen/Life technologies, Carlsbad, USA; a stock solution (1 mg/ml) was prepared in dimethyl sulphoxide (DMSO).

The disc diffusion method

Stored isolates were subcultured on MacConkey's agar and the

*Corresponding author. E-mail: samaa_taha@yahoo.com. Tel: 00201001652144.

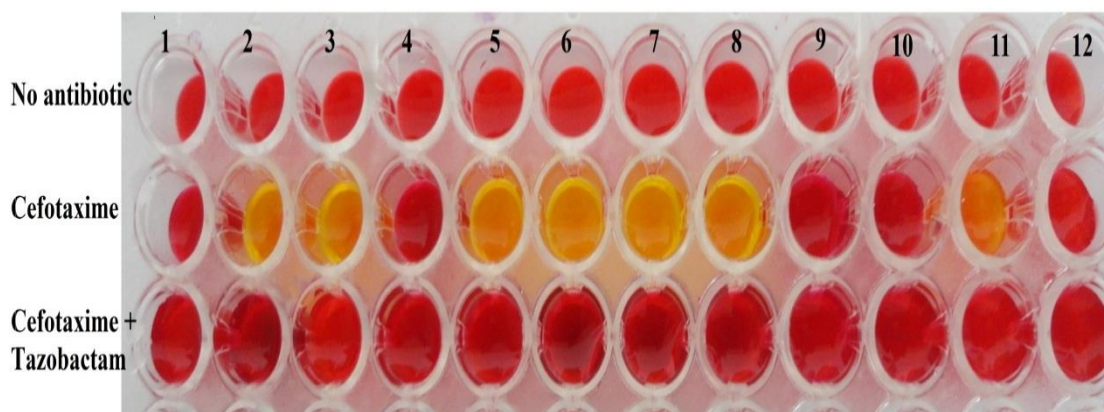


Figure 1. Representative results of the ESBL NDP test. Strains 1 and 2 are negative and positive controls, respectively; strains 3, 5, 6, 7, 8 and 11 are ESBL producers; strains 4, 9, 10 and 12 are non-ESBL producers.

pure isolated colonies of identified bacteria was adjusted to 0.5 McFarland turbidity standards in 0.85% saline and lawn culture was spread using sterile swabs on Muller Hinton Agar media (Hi-media). All the strains were screened for ESBL production using CTX (30 µg) and CAZ (30 µg). Strains showing zone of inhibition of ≤ 27 mm for CTX and ≤ 22 mm for CAZ were selected for ESBL combined disc conformation test. Combined discs of CTC (40 µg) and CZC (40 µg) were used in the confirmation test according to the CLSI M2-A10 protocol (CLSI, 2009).

The ESBL NDP (Nordmann/ Dortet/ Poirel) test

Strains were isolated on MacConkey's agar and incubated at 37°C for 24 h before performing the NDP rapid ESBL test as described by Nordmann et al. (2012). Briefly, one calibrated loop inoculum (10 µl) of the tested strain was resuspended in 150 µl of 20 mM Tris-HCl lysis buffer in eppendorf tubes containing microbeads. Then, microbead tubes were vortexed for 30 min at room temperature for the mechanical lysis of bacteria. After centrifugation, 30 µl of the supernatant was mixed in a well of a 96-well tray with 100 µl of a 1 ml solution made of 3 mg of purified cefotaxime sodium salt in a pH 7.8 phenol red solution. The pH value was then adjusted to a 7.8 value by the addition of drops from 1 N NaOH solution. Mixture of the phenol red solution and the enzymatic suspension being tested was incubated at 37°C for 30 min. Similarly, culture extracts were analyzed in wells containing cefotaxime and tazobactam (4 mg/ml). A test was considered as positive when the well containing cefotaxime alone turned from red to yellow/orange and the well containing cefotaxime supplemented with tazobactam remained red (ESBL producer).

Flow cytometric analysis

Bacterial isolates from fresh agar plates were inoculated in trypticase soy broth and incubated at 37°C with shaking until the log phase was reached (about 1 h and 15 min). Subsequently, a suspension containing 5×10^6 cells/ml in fresh medium was prepared and the bacterial cells were exposed either to 4 mg/L of CTX, or 16 mg/L of CAZ, alone or with 4 mg/L of CLA, for 60 and 120 min. In parallel, after incubation, the cells were centrifuged and washed in PBS. The dye DiBAC4 (3) was added in a concentration of 1 µg/ml for 30 min, at room temperature and protected from

light. The flow cytometric assay was used according to Ramos et al. (2012). It was performed on a FACSCalibur flow cytometer (BD, Sparks, USA). Nearly, 10,000–30,000 events of each sample were measured with the Software Cell Quest. The acquisition settings were defined using non-treated, non-stained cells (autofluorescence) and after adjusting the photomultiplier tubes' voltage to the first logarithmic (log) decade. The fluorescence intensity at 530/30 nm (FL1) was registered after incubation with antimicrobials and staining with 1 µg/ml DiBAC4 (3).

Statistical analysis

Sensitivity, specificity, positive and negative predictive values were assessed for the ESBL NDP test and the flow cytometric assay considering the standard disc diffusion method as a gold standard. The kappa values were calculated to evaluate the agreement between each of the ESBL NDP test and the flow cytometric assay and the disc diffusion method (Viera and Garrett, 2005).

RESULTS

The disc diffusion method had classified the 60 tested strains into 46 (76.6%) ESBL producers and 14 (23.3%) non-ESBL producers. Using the disc diffusion method, an ESBL producer isolates showed resistance to CTX and CAZ then the susceptibility increased (≥ 5 mm increase in zone diameter) to combined discs CTC and CZC while non-ESBL producer isolates were resistant to CTX and CAZ with no increase in the susceptibility to combined discs CTC and CZC.

Using the ESBL NDP test, 80% (n= 48) of the tested isolates produced ESBLs as the color of the wells turned from red to yellow in presence of cefotaxime and remained red when tazobactam was added (Figure 1) and 20% (n=12) tested negative for ESBL production. The sensitivity and specificity of the test were 100 and 85.7%, respectively in comparison with the standard disc diffusion method whereas the positive and negative

Table 1. Results of the disc diffusion method, the ESBL NDP test and the flow cytometric assay for detection of ESBLs in clinical isolates of *Klebsiella pneumoniae*.

Test result	The disc diffusion method	The ESBL NDP test	Flow cytometric assay
ESBL producers	46	48	42
Non- ESBL producers	14	12	18
Total	60	60	60
Sensitivity	100%	100%	91.3%
Specificity	100%	85.7%	77.8%
Positive predictive value		95.8%	91.3%
Negative predictive value		100%	77.8%

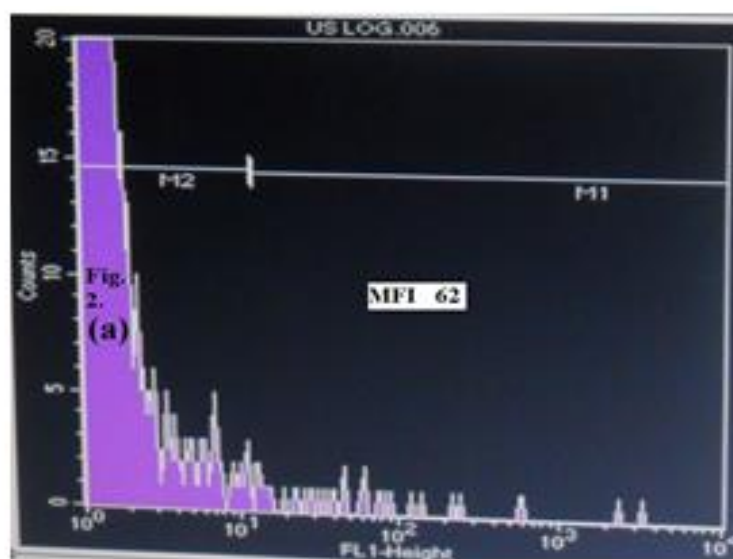


Figure 2a. Flow cytometric histogram representing the emitted fluorescence at FL1 (green- 530 nm) of non-treated and non-stained cells (autofluorescence). The mean fluorescence intensity (MFI) was 62.

predictive values of this test were 95.8 and 100%, respectively. Kappa testing showed an almost perfect agreement between the ESBL NDP test and disc diffusion method in detecting ESBLs ($\kappa=0.9$).

Out of the 60 tested isolates, 42 (70%) and 18 (30%) were ESBL and non- ESBL producers, respectively with the flow cytometric assay method. The sensitivity and specificity of the test were 91.3 and 77.8% whereas the positive and negative predictive values of this test were 91.3 and 77.8%, respectively in comparison with the standard disc diffusion method (Table 1). The intrinsic autofluorescence signal of bacterial cells was detected at the first decade of the logarithmic scale [the mean fluorescence intensity (MFI) was 62]. This corresponds to very low fluorescence intensity without interference with the assessment of membrane depolarization using DIBAC4 (3) as a voltage sensor probe (Figure 2a).

Higher intensity of green fluorescence (530/30 nm - FL1) was obtained with dead cells compared with viable cells; consequently, two distinct regions were defined, respectively, for depolarized and polarized cells after staining with DIBAC4 (3). Considering the ESBL-positive clinical isolates, the MFI was 293 after treatment with CTX for 60 min, and then drastically increased to 1541 following simultaneous incubation with both CTX and CLA for 60 min (Figure 2b, c). For non- ESBL producer isolates, the MFI was 72 after treatment with CTX (4 mg/L) for 60 min and remained around value 73 after treatment with both CTX and CLA for 60 min (Figure 3a,b).

For evaluation of agreement between the flow cytometric assay and disc diffusion method, Kappa testing showed substantial agreement between both tests ($\kappa=0.7$).

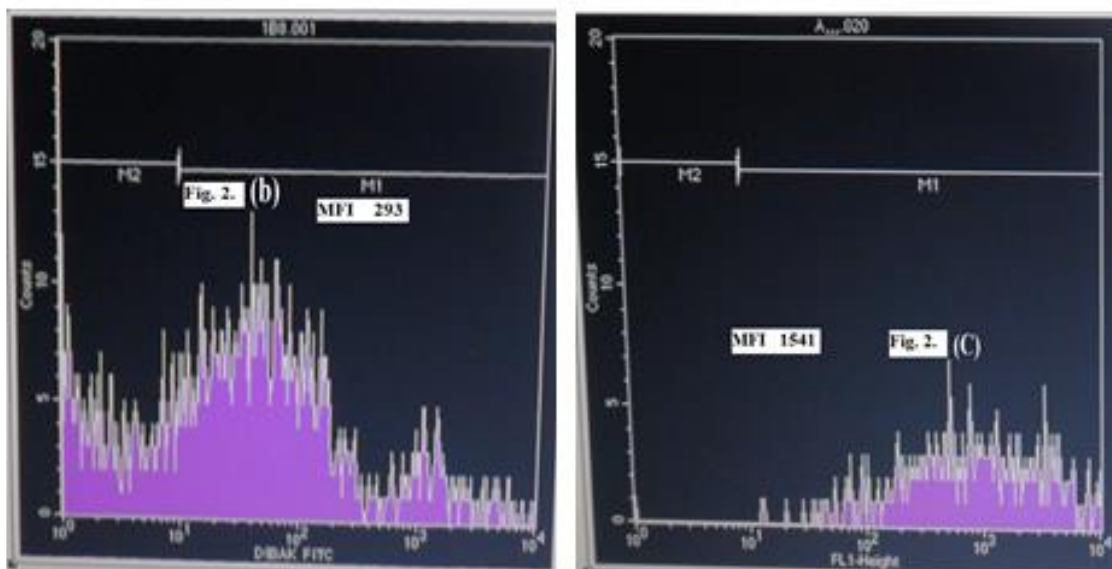


Figure 2b, c. Flow cytometric histogram representing the emitted fluorescence at FL1 (green- 530 nm) of an example of ESBL producer isolate. (B) After treatment with CTX (4 mg/L) for 60 min; the MFI was 293. c) After treatment with CTX (4 mg/L) and CLA (4 mg/L) for 60 min: the MFI was 1541.

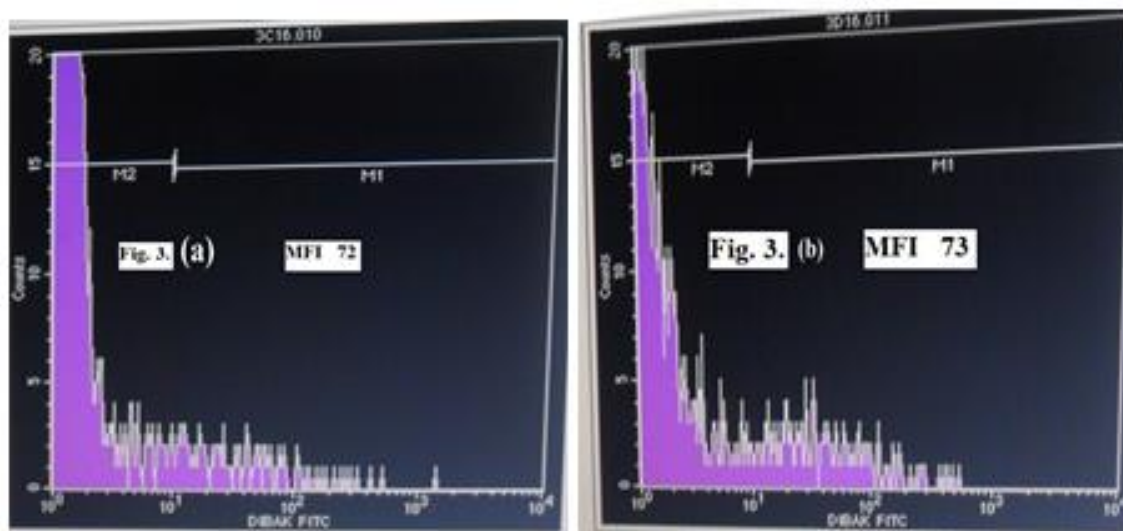


Figure 3a,b. Flow cytometric histogram representing the emitted fluorescence at FL1 (green- 530 nm) of an example of a non-ESBL producer isolate. (a) After treatment with CTX (4 mg/L) for 60 min; the MFI was 72. b) After treatment with CTX (4 mg/L) and CLA (4 mg/L) for 60 min: the MFI was 73.

DISCUSSION

ESBLs are the main cause of resistance to beta-lactam antibiotics which are among the safest and most frequently prescribed antimicrobial agents all over the world. As their occurrence has been increasing, it becomes essential to evaluate their occurrence in *E. coli* and *K. pneumoniae* which are mostly ESBL producers

(Pitout and Laupland, 2008; Sahu et al., 2011).

The incidence of ESBL-producing *K. pneumoniae* varies from country to another depending upon various factors, like antibiotic policy, the carriage rate among hospital personnel, and the type of disinfection used especially in the ICU (Sarojamma and Ramakrishna, 2011). It is recognized that Egypt has an extremely high rate of ESBL producers, with up to 70% of isolates

producing the enzyme (Borg et al., 2006). In the present study, 76.6% (n= 46) of the 60 tested strains were ESBL producers and 23.3% (n=14) were non- ESBL producers. This could be attributed to the empirical usage of 3rd generation cephalosporins in treatment of nosocomial infections in our hospitals.

Although molecular methods brought speed and accuracy, they are costly and not suitable for low income developing countries (Gazin et al., 2012). In this work, we assessed two phenotypic methods; the ESBL NDP test and the flow cytometric assay for detection of ESBLs in *K. pneumoniae* clinical isolates in comparison with the standard disc diffusion method. The ESBL NDP test was able to detect all ESBL-producing isolates that hydrolyze cefotaxime (color change from red to yellow in the first well), while the second well that contained tazobactam remained red (inhibition of hydrolysis), thus corresponding to a positive test. The sensitivity and positive predictive value of the test were 100 and 95.8%, respectively. This result was higher than that of Nordmann et al. (2012) who evaluated the ESBL NDP test retrospectively on a collection of 255 strains (from various clinical and geographical origins and previously characterized at the molecular level). In their published study, the sensitivity of the test was 92.6%. Also, our results are higher than those of Dortet et al. (2014) who applied the ESBL NDP test on 500 ESBL producing Enterobacteriaceae recovered from urine samples. They reported that the sensitivity of the ESBL NDP test was 98% and the positive predictive value was 98% which is higher than ours. The discrepancy of the results may be attributed to the different geographical origins and the large number of tested isolates in comparison with our study. Two false positive isolates were detected by the ESBL NDP test as some isolates could contain combined ESBL and AmpC-overproducing enzymes giving a positive result, if the corresponding AmpC hydrolyses cefotaxime at high level.

The specificity and the negative predictive value of the ESBL NDP test in our study were 85.7% and 100%, respectively. These results are lower than those of Nordmann et al. (2012) and Dortet et al. (2014) whereas, it was 100% in the first study and 99.8% in the second one. This could be explained by the inability of the test in detecting non-CTX-M ESBL producers and strains which had MIC values of cefotaxime lower than the resistance breakpoint for that molecule (>8 µg/ml).

Our results show an almost perfect agreement between the ESBL NDP test and disc diffusion method in detecting ESBLs (kappa =0.9) which agrees with those of Dortet et al. (2014) who observed a perfect correlation between cefotaxime resistance and positivity of the ESBL NDP test.

Compared to the standard disc diffusion method, flow cytometric assay yielded a sensitivity of (91.3%) while the specificity was 77.8%. It correctly detected 42 isolates out of the 46 ESBL positive isolates previously catalogued by

the standard disc diffusion method. Only 4 strains tested false negative result which might be obtained whenever complex mutant or rare ESBL types are present as isolates expressing these enzymes confer resistance to cephalosporins but are partially inhibited or not inhibited by CLA acid, respectively (Canton et al., 2008; Drawz and Bonomo, 2010).

Our results are in concordance with those of Ramos et al. (2012) who tested 20 ESBL-negative and 41 ESBL-positive isolates phenotypically catalogued by the standard disc diffusion method and molecular typing. In their study flow cytometric analysis correctly detected all the 41 ESBL-positive isolates. It showed an excellent correlation either with phenotypic analysis or molecular typing however, in our study flow cytometric analysis showed substantial agreement with the standard disc diffusion method (kappa= 0.7).

The ESBL NDP test offers a simple and rapid test with an almost perfect agreement with the standard disc diffusion method in detecting ESBLs which could significantly help in guiding first-line antibiotic therapy and improve the outcome of infected patients. Flow cytometric assay is a promising method that might be used in the clinical microbiology laboratory provided that the availability of the device and a trained personnel. Although, the standard method remains the best one because of its low price for the lab and the patient.

Conflict of interests

The author(s) did not declare any conflict of interest.

REFERENCES

- Birgul K (2010). Investigation of extended spectrum beta lactamase production of bacteria by direct urine inoculation. *Afr. J. Microbiol. Res.* 4: 1087-1090.
- Borg MA, Scicluna E, de Kraker M, van de Sande-Bruinsma N, Tiemersma E, Gur D (2006). Antibiotic resistance in the southeastern Mediterranean preliminary results from the ARMed project. *Euro. Surveill.* 11:164-7.
- Bush K, Jacoby GA (2010). Updated functional classification of β -lactamases. *Antimicrob. Agents Chemother.* 54:969-976.
- Canton R, Morosini MI, de la Maza OM, de la Pedrosa EG (2008). IRT and CMT beta-lactamases and inhibitor resistance. *Clin. Microbiol. Infect.* 14: 53-62.
- CLSI-Clinical and Laboratory Standards Institute (2009). Performance standard for antimicrobial disk susceptibility tests. M2-A10. Wayne.
- CLSI-Clinical and Laboratory Standards Institute (2014). M100-S24 Performance Standards for Antimicrobial Susceptibility Testing; Twenty-Fourth Informational Supplement. CLSI, Wayne, PA.
- Cuzon G, Naas T, Bogaerts P, Glupczynski Y, Nordmann P (2012). Evaluation of a DNA microarray for the rapid detection of extended-spectrum β -lactamases (TEM, SHV and CTX-M), plasmid-mediated cephalosporinases (CMY-2-like, DHA, FOX, ACC-1, ACT/MIR and CMY-1-like/MOX) and carbapenemases (KPC, OXA-48, VIM, IMP and NDM). *J. Antimicrob. Chemother.* 67(8): 1865-1869.
- Dortet L, Poirel L, Nordmann P (2014). Rapid Detection of Extended-Spectrum- β -Lactamase-Producing Enterobacteriaceae from Urine Samples by Use of the ESBL NDP Test. *J. Clin. Microbiol.* 52(10): 3701-3706.

- Drawz SM, Bonomo RA (2010). Three Decades of Beta-Lactamase Inhibitors. *Clin. Microbiol. Rev.* 23(1):160-201.
- Drieux L, Brossier F, Sougakoff W, Jarlier V (2008). Phenotypic detection of extended-spectrum β -lactamase production in *Enterobacteriaceae*; review and bench guide. *Clin. Microbiol. Infect.* 14 (Suppl. 1): 90-103.
- Gazin M, Paasch F, Goosens H, Malhotra-Kumar S (2012). Current trends in culture-based and molecular detection of extended-spectrum β -lactamase-harboring and carbapenem-resistant *Enterobacteriaceae*. *J. Clin. Microbiol.* 50(4): 1140-1146.
- Nordmann P, Dortet L, Poirel L (2012). Rapid detection of extended-spectrum-beta-lactamase-producing *Enterobacteriaceae*. *J. Clin. Microbiol.* 50(9): 3016-3022.
- Pitout JD (2010). Infections with extended-spectrum β -lactamase-producing *Enterobacteriaceae*: changing epidemiology and drug treatment choices. *Drugs* 70(3):313-333.
- Pitout JD, Laupland KB (2008). Extended-spectrum β -lactamase-producing *Enterobacteriaceae*: an emerging public-health concern. *Lancet Infect. Dis.* 8:159-166.
- Poirel L, Bonnin R, Nordmann P (2012). Genetic support and diversity of acquired extended-spectrum β -lactamases in Gram negative rods. *Infect. Genet. Evol.* 12:883-93.
- Ramos F, Espinar MJ, Rocha R, Santos-Antunes J, Rodrigues AG, Canto'n R, Pina-Vaz C (2012). A novel flow cytometric assay for rapid detection of extended-spectrum beta-lactamases. *Clin. Microbiol. Infect.* 19: E8-E15.
- Sahu SK, Dalal AS, Bansal G (2011). Detection of extended-spectrum β -lactamases in clinical isolates of *E. coli* and *Klebsiella* species from Udaipur Rajasthan. *Biomed. Res.* 22(3): 367-373.
- Sarojamma V, Ramakrishna V (2011). Prevalence of ESBL-producing *Klebsiella pneumoniae* isolates in tertiary care hospital. *Int. Scholarly Res. Network Microbiol.* 2011. Article ID 318348. doi: 10.5402/2011/318348.
- Schwaber MJ, Carmeli Y (2007). Mortality and delay in effective therapy associated with extended-spectrum beta-lactamase production in *Enterobacteriaceae* bacteraemia: a systematic review and meta-analysis. *J. Antimicrob. Chemother.* 60: 913-920.
- Schwaber MJ, Navon-Venezia S, Kaye KS, Ben-Ami R, Schwartz D, Carmeli Y (2006). Clinical and economic impact of bacteremia with extended-spectrum β lactamase-producing *Enterobacteriaceae*. *Antimicrob. Agents Chemother.* 50:1257-62.
- Shapiro HM (2001). Multiparameter flow cytometry of bacteria: implications for diagnostics and therapeutics. *Cytometry* 43: 223-226.
- Viera AJ, Garrett JM (2005). Understanding interobserver agreement: the kappa statistic. *Fam. Med.* 37(5): 360-363.
- Zahar JR, Lortholary O, Martin C, Potel G, Plésiat P, Nordmann P (2009). Addressing the challenge of extended-spectrum β -lactamases. *Curr. Opin. Investig. Drugs* 10(2):172-180.

Full Length Research Paper

Effects of lithium compounds on the growth of white-rot fungi

Mateus D. Nunes, Willian L. Cardoso, José M. R. Luz and Maria C. M. Kasuya*

BIOAGRO, Department of Microbiology, Universidade Federal de Viçosa, Campus Universitário, 36570-000, Viçosa, MG, Brazil.

Received 10 April, 2015; Accepted 17 August, 2015

Identifying the most suitable lithium compounds for fungal growth is important for the lithium (Li) enrichment of mushrooms. However, enrichment thus far has been carried out using LiCl. In an effort to identify an alternative source of lithium, we investigated the effect of five lithium compounds on lag phase, growth rate and biomass of ten species of white-rot fungi. The objective of this work was to make a screening of promising lithium compounds for further studies of Li enrichment of mushrooms. The fungal growth was affected depending on the species and lithium form tested. Lithium sulfate and lithium chloride were found to be the most promising candidate compounds for the Li enrichment of mushrooms.

Key words: Li-enriched fungi, mycelial growth, Lithium compounds.

INTRODUCTION

Lithium is not an essential mineral for humans (Schrauzer, 2002), but studies have suggested that its uptake can influence human behavior (Dawson et al., 1970; Schrauzer and Shrestha, 1990; Severus et al., 2009). Indeed, lithium salts are commonly used to treat bipolar disorder, but the side effects caused by ingestion of these salts are numerous and severe (Kjølholt et al., 2003; Aral and Vecchio-Sadus, 2008; Ghaemi, 2010), so much so that their sale in the USA was prohibited in 1949 by the Food and Drug Administration and not resumed until 1970.

The main sources of lithium for human consumption are vegetables and grains (Schrauzer, 2002). Lithium level in mushrooms varies depending lithium availability and ability of fungi to accumulate lithium. In the

environmental, this element has been found at average concentration of 0.189 ppm in mushrooms (Vetter, 2005). According to Vetter (2005) due to the low levels of lithium, the mushrooms are not suitable source of lithium for humans. However, mushrooms can be enriched with lithium (de Assunção et al., 2012).

Li-enriched mushrooms have been shown by de Assunção et al. (2012) to be a promising alternative source of lithium due to the higher solubility in water of the lithium found in the mushrooms than lithium carbonate, which is interesting as solubility in water is one of the factors that affect the absorption of compounds in the intestine. Therefore, the lithium found in the mushrooms can be more bioavailable to humans than lithium carbonate. As the control of lithium

*Corresponding author. E-mail: mkasuya@ufv.br. Tel: +55-031-3899-2970. Fax: +55-031-3899-2573.

Table 1. Concentration of lithium in culture medium according to fungi specie.

Fungi	Lithium level in Li-medium (mg L ⁻¹) ^a
<i>Hericium erinaceum</i> (HE 01)	50
<i>Lyophyllum shimeji</i> (LY 01)	50
<i>Lentinula edodes</i> (UFV 73)	50
<i>Ganoderma subamboinense</i> (GR 117)	100
<i>Grifolla frondosa</i> (GF)	100
<i>Pleurotus ostreatus</i> (PLO 06)	150
<i>Pleurotus ostreatus</i> (P.98)	150
<i>Pleurotus eryngii</i> (PE 04)	150
<i>Pholiota nameko</i> (PHOLI)	200
<i>Pleurotus djamor</i> (PLO 13)	270

^a The above lithium levels were used for all lithium compounds tested and based on a previous experiment (Nunes et al., 2014).

consumed by direct consumption of Li-enriched mushrooms is difficult, development of medicines based on the lithium found in the mushrooms seems to be a good approach to provide new lithium compounds for humans and perhaps, decrease the side-effects currently observed.

Only the mushroom *Pleurotus ostreatus* was shown to be capable of Li enrichment. Nunes et al. (2014) screened 12 white-rot fungi with the goal of identifying other mushrooms suitable for Li-enrichment and found that *Pleurotus djamor* and *Pholiota nameko* were more resistant to LiCl than *P. ostreatus*, making these species promising candidates.

However, both studies use LiCl for enrichment (de Assunção et al., 2012; Nunes et al., 2014). Chlorine is known to have antimicrobial properties (Wilson et al., 2005). Therefore, other lithium compounds may be more suitable for use in Li enrichment. Furthermore, the accessibility and price of different lithium compounds vary between regions. Therefore, knowing which lithium compounds can be used effectively is useful information.

To make a screening of promising lithium compounds for further studies of Li enrichment of mushrooms, we tested the effect of five lithium compounds on development of nine white-rot species that produce commercially available edible mushrooms.

MATERIALS AND METHODS

Microorganism

The fungi tested were all currently available commercial species able to produce mushrooms on non-composted substrate, which is easier and less expensive to use than composted substrates. The fungi used were *Ganoderma subamboinense* var. *laevisporum* Bazzalo and Wright (GR 117), *Grifola frondosa* (Dicks.) Gray (GF), *Hericium erinaceus* (Bulls.) Pers. (HE), *Lentinula edodes* (Berk.) Pegler (UFV 73), *Lyophyllum shimeji* (Kawam.) Hongo (LY), *Pleurotus eryngii* (DC.) Qué. (PLE 04), *Pleurotus ostreatus* (Jacq.: Fr.) Kummer (PLO 06), *P. ostreatus* (Jacq.: Fr.) Kummer (P.98),

Pleurotus djamor (Rumph. ex Fr.) Boedijn (PLO 13) and *Pholiota nameko* (T. Itô) Ito and Imai (PHOLI). These fungi belong to the collection of the Laboratório de Associações Micorrízicas / Departamento de Microbiologia / BIOAGRO / UFV. The fungi were grown in Potato Dextrose Agar (PDA, Fluka Analytical, St. Louis, Missouri, USA) at 22 ± 1 °C for seven days. Two different isolates of *P. ostreatus* that presented different commercially important characteristics were included.

Culture media and cultivation conditions

Fungi were grown on PDA containing one of the following lithium compounds: lithium acetate, lithium chloride, lithium hydroxide, lithium sulfate, or lithium carbonate. The pH of all media was adjusted to 5.5. The concentrations chosen for enrichment of the PDA medium were determined by the lithium content of each compound (Table 1) and the concentration of LiCl that allowed mycelial growth for each fungus in previous experiments (Nunes et al., 2014). It should be noted that lithium concentrations used were different for each fungus, but lithium molar concentrations were equal among different compounds. The culture medium was then autoclaved at 121°C for 20 min. PDA plugs of inoculum 5 mm in diameter containing active mycelium were cut from the board of the colony. Inoculum plugs were firmly placed with the mycelium side down in the centers of Petri dishes. Four replicate plates were prepared for each lithium salt and fungus and were incubated at 22 ± 1°C with room moisture.

Lag phase and growth rate

After incubation, colonies were observed daily to determine the start of mycelial growth. The fungal growth rate was determined by measuring each colony's diameter in two orthogonal directions. Measurements were made for 45 d or until maximum Petri dish colonization. Measurements were taken every 48 h.

Biomass

To determine the mycelial dry mass, the entire contents of the Petri dish (mycelium + culture media) were placed in a bottle with approximately 200 mL of distilled water and heated in a water bath for 1–5 min to dissolve all culture medium (da Silva et al., 2013). The solution was then filtered, and retained mycelium was dried in an oven at 80 °C until a constant weight was reached.

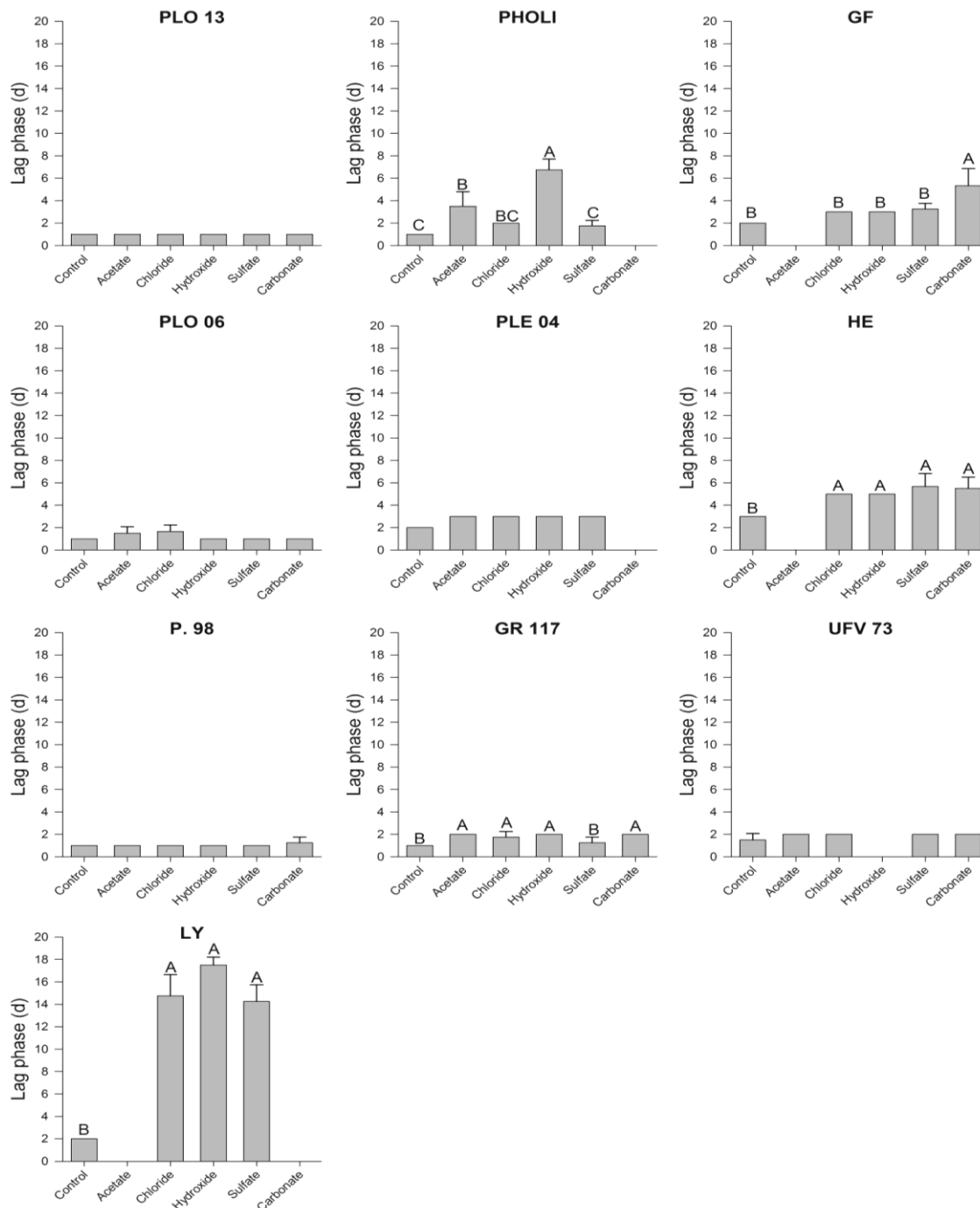


Figure 1. Lag phase of white rot fungi in culture media supplemented with different lithium compounds. Mean growth of 4 replicates. Missing bars indicates fungal growth inhibition. Letters above columns compare individual fungi by lithium compounds level (Tukey's test; $p \leq 0.05$).

Statistical analysis

Experiments used a randomized design. The data were subjected to analysis of variance (ANOVA), and the averages were compared by Tukey's test ($P < 0.05$) using Minitab statistical software (Version 16.0).

RESULTS AND DISCUSSION

The lag phase of five fungi increased due to the addition of lithium compounds to the culture medium (Figure 1). One-way ANOVA revealed significant effects of lithium

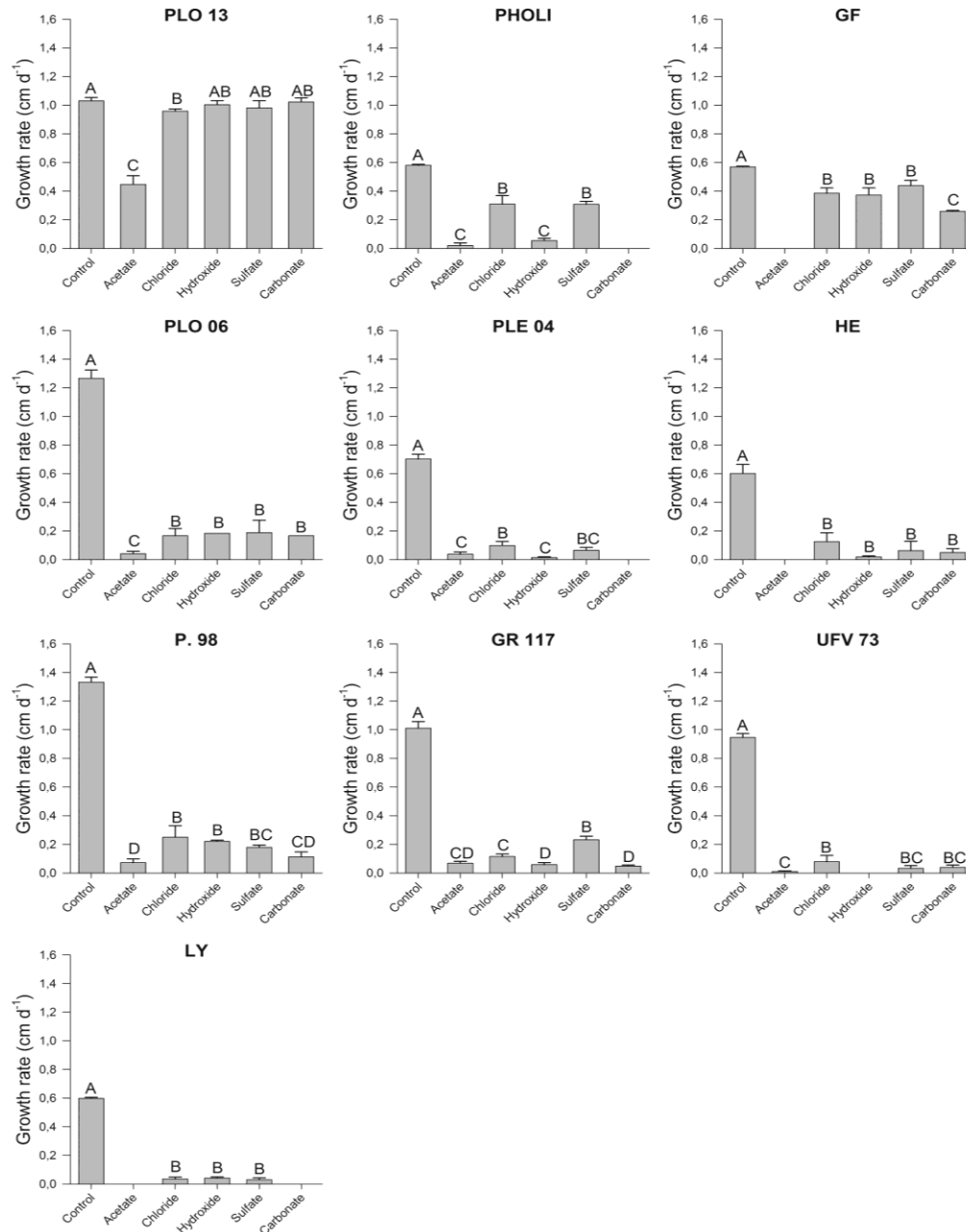


Figure 2. Mycelial growth rate of white rot fungi in culture media supplemented with different lithium compounds. Mean growth of 4 replicates. Missing bars indicate fungal growth inhibition. Letters above columns compare individual fungi by lithium compounds level (Tukey's test; $p \leq 0.05$).

compound addition on the lag phase of *H. erinaceus* (HE 01; $F_{(4;15)} = 10.80$; $p < 0.001$), *G. frondosa* (GF; $F_{(4;15)} = 12.76$; $p < 0.001$), *G. subamboinense* var. *laevisporum* (GR 117; $F_{(5;18)} = 18.68$; $p < 0.001$), *P. nameko* (PHOLI; $F_{(4;15)} = 32.65$, $p < 0.001$) and *L. shimeji* (LY 01; $F_{(3;12)} = 93.56$; $p < 0.001$), showing a *shimeji* increase in lag phase (Figure 1). Moreover, we observed that different lithium

compounds had a similar negative influence on the lag phase (Figure 1), which suggest that the lithium is the main factor influencing this fungal growth phase.

We observed a significant effect ($P < 0.05$) of lithium compounds on the growth rate and biomass of all fungi tested (Figures 2 and 3). Richter et al. (2008) noted that the radial growth rate may not represent fungal biomass reduction. Indeed, biomass evaluation has been shown to

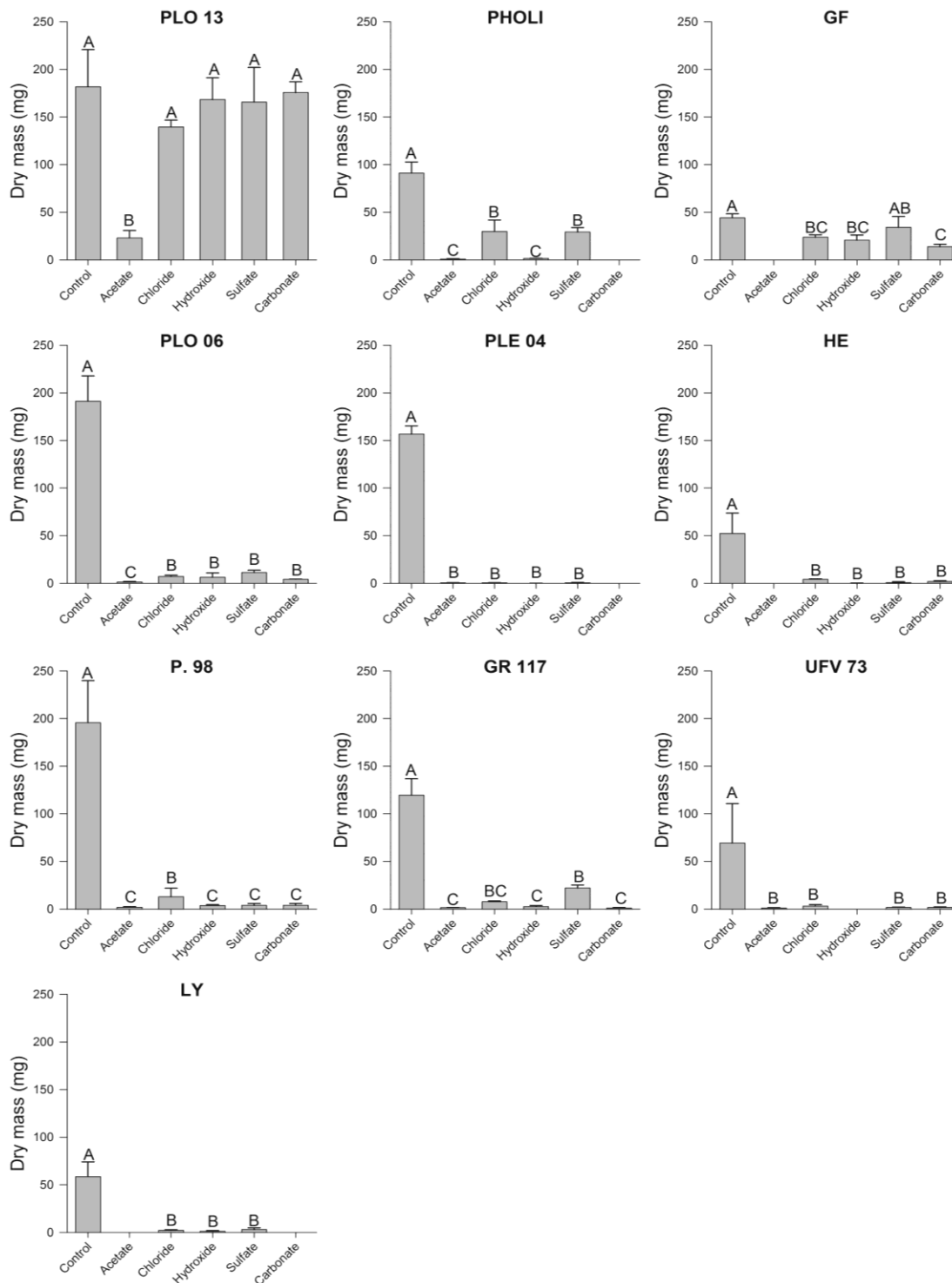


Figure 3. Mycelial dry mass of white rot fungi in culture media supplemented with different lithium compounds. Mean growth of 4 replicates. Missing bars indicates fungal growth inhibition. Letters above columns compare individual fungi by lithium compounds level (Tukey's test; $p \leq 0.05$).

be a more sensitive method (Nunes et al., 2014). The profiles of biomass and growth rate data observed clearly show that lithium acetate and lithium carbonate were the

most toxic compounds (Figures 2 and 3). Acetate is an organic acid that is able to cross the plasmatic membrane and affect cytoplasmic pH, negatively affecting many

metabolic pathways (Cheung et al., 2010). Carbonate has been shown to strongly inhibit Nce103, an enzyme that participates in many physiological processes in eukaryotes (Innocenti et al., 2008). The fungal growth inhibition observed (Figure 2) suggests that these phenomena may be occurring in the fungal cell. Thus, these compounds are not recommended for use in fungal Li enrichment.

The effect of lithium hydroxide on growth rate and biomass varies among the fungal strains tested (Figures 2 and 3). For some fungi, lithium hydroxide results were similar ($P > 0.05$) to those obtained for lithium sulfate and lithium chloride (Figures 1 and 3). In contrast, the addition of lithium hydroxide decreased ($P < 0.05$) the growth rate and biomass of *P. nameko* (PHOLI), *G. subamboinense* var. *laevisporum* (GR 117), *P. eryngii* (PLE 04) and *L. edodes* (UFV 73), clearly showing that this lithium compound was more toxic for these fungi. Furthermore, lithium hydroxide increased ($P < 0.05$) the lag phase of *P. nameko* (PHOLI). In addition, Xu (1997) showed that the activity of laccase, an important factor for white-rot fungus growth, was affected by hydroxide. Furthermore, it is not known how the effects of lithium hydroxide may vary with variation among fungal strains. Therefore, we do not recommend using lithium hydroxide for the Li enrichment of fungi.

The similar profile for lag phase, growth rate and dry mass observed in this study shows that lithium sulfate and lithium chloride were the less toxic compounds to fungi (Figures 1 and 3). As observed by de Assunção et al. (2012), Li is incorporated in the fungus when LiCl is added to the substrate used for mycelial growth. Thus, we can assume that lithium sulfate and chloride are the most promising for the Li enrichment of mushrooms. However, the effect of these lithium compounds on important parameters of mushroom cultivation and bioaccumulation of Li should be investigated.

Conflict of interests

The author(s) did not declare any conflict of interest.

ACKNOWLEDGEMENTS

The authors are very grateful to Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG) for the financial support.

REFERENCES

- Aral H, Vecchio-Sadus A (2008). Toxicity of lithium to humans and the environment-A literature review. *Ecotoxicol. Environ. Saf.* 70(3): 349 - 356.
- Cheung HNB, Huang GH, Yu H (2010). Microbial-growth inhibition during composting of food waste: Effects of organic acids. *Bioresour. Technol.* 101(15): 5925 - 5934.
- da Silva MCS, Nunes MD, da Luz JMR, Kasuya MCM (2013). Mycelial growth of *Pleurotus* Spp in Se-enriched culture media. *Adv. Microbiol.* 3(8): 11 - 18.
- Dawson EB, Moore TD, McGanity WJ (1970). The mathematical relationship of drinking water lithium and rainfall to mental hospital admission. *Dis. Nerv. Syst.* 31811.
- de Assunção LS, da Luz JMR, da Silva MdCS, Vieira PAF, Bazzolli DMS, Vanetti MCD, Kasuya MCM (2012). Enrichment of mushrooms: An interesting strategy for the acquisition of lithium. *Food Chem.* 134(2): 1123-1127.
- Ghaemi SN (2010). From BALANCE to DSM-5: taking lithium seriously. *Bipolar Disorders* 12(7): 673 - 677.
- Innocenti A, Mühlischlegel FA, Hall RA, Steegborn C, Scozzafava A, Supuran CT (2008). Carbonic anhydrase inhibitors: Inhibition of the β -class enzymes from the fungal pathogens *Candida albicans* and *Cryptococcus neoformans* with simple anions. *Bioorg. Med. Chem. Lett.* 18(18): 5066 - 5070.
- Kjølholt J, Stuer-Lauridsen F, Mogensen AS, Havelund S (2003) The elements in the second rank - an environmental problem now or in the future? The Danish Environmental Protection Agency
- Nunes MD, Cardoso WL, Da Luz JMR, Kasuya MCM (2014). Lithium chloride affects mycelial growth of white rot fungi: Fungal screening for Li-enrichment. *Afr. J. Microbiol. Res.* 8(21): 2111 - 2123.
- Richter DL, Robinson SC, Beardslee MP, Habarth ML (2008). Differential sensitivity of fungi to lithium chloride in culture media. *Mycol. Res.* 112(6): 717 - 724.
- Schrauzer G, Shrestha K (1990). Lithium in drinking water and the incidences of crimes, suicides, and arrests related to drug addictions. *Biol. Trace Elem. Res.* 25(2): 105 - 113.
- Schrauzer GN (2002). Lithium: Occurrence, Dietary Intakes, Nutritional Essentiality. *J. Am. Coll. Nutr.* 21(1): 14 - 21.
- Severus WE, Kleindienst N, Evoniuk G, Bowden C, Möller HJ, Bohus M, Frangou S, Greil W, Calabrese JR (2009). Is the polarity of relapse/recurrence in bipolar-I disorder patients related to serum lithium levels? Results from an empirical study. *J. Affect. Disord.* 115(3): 466 - 470.
- Vetter J (2005). Lithium content of some common edible wild-growing mushrooms. *Food Chem.* 90(1-2): 31-37.
- Wilson SC, Wu C, Andriychuk LA, Martin JM, Brasel TL, Jumper CA, Straus DC (2005). Effect of Chlorine Dioxide Gas on Fungi and Mycotoxins Associated with Sick Building Syndrome. *Appl. Environ. Microbiol.* 71(9): 5399 - 5403.
- Xu F (1997). Effects of Redox Potential and Hydroxide Inhibition on the pH Activity Profile of Fungal Laccases. *J. Biol. Chem.* 272(2): 924 - 928.

Full Length Research Paper

Microbiological load of yoghurt sold in Omoku schools, Rivers State, Nigeria

Chimezie Gabriel Dirisu^{1*}, Gloria Lily² and Ebere Igwe²

¹Department of Integrated Science Education, Federal College of Education (Technical) Omoku, Rivers, Nigeria.

²Department of Home Economics Education, Federal College of Education (Technical) Omoku, Rivers, Nigeria.

Received 25 April, 2015; Accepted 17 August, 2015

Sachets of various brands of yoghurt were randomly purchased from different retail outlets within Omoku schools and its pH and microbiological quality were determined using standard method. Total bacterial count (TBC) and Coliform count were done using standard plate count method after making serial dilutions of yoghurt samples. Nutrient agar (NA) was used for enumeration of TBC. NA plates were incubated at 37°C for 48 h. Coliform count was carried out using MacConkey agar (MCA) incubated at 37°C for 48 h. Total fungi were determined by using potato dextrose agar (PDA) and the plates were incubated at room temperature for 5-7 days. The pH of yoghurt samples ranged from 2.38±0.81 to 3.2±0.08, TBC ranged from 3.1 x 10⁵ to 5.1x 10⁵ cfu/mL. Total fungi count ranged from 3.2 x 10³ to 4.9 x 10³ cfu/mL. Total coliform count ranged from 0 to 1.0 cfu/mL. There was no significant difference in TBC and total coliform counts (p>0.05), but there was significant differences in total fungi counts (p<0.05). Results indicate that yoghurt sold in Omoku schools is of poor microbiological quality and thus their production and sale should be closely monitored in order to protect students and pupils and the general public from food-borne infection.

Key words: Contaminated, total bacteria count, total coliform, total fungi, bacteria, pathogens, yoghurt.

INTRODUCTION

Yoghurt is a sour milk beverage made by blending fermented milk with various ingredients that provide flavour and colour. Although, it is a traditional beverage in the Balkans and Middle East (Ghandge et al., 2008), yoghurt is consumed by all people of all nations. Yoghurt is produced by symbiotic actions of two lactic acid bacteria, namely *Streptococcus thermophilus* and *Lactobacillus bulgaricus* which ferment lactose to lactic acid, which gives it its sour taste (Steinkraus, 1997;

Tamine and Robinson, 2004; Kumar and Mishra, 2004; WDC, 2014). Yoghurt can serve as food and plays an important role in human nutrition, health maintaining, therapeutic and dietetic functions (Younus et al., 2002; Khan et al., 2008).

The nutritional quality of yoghurt has been reported and is known to contain high-quality protein, calcium and phosphorous. Its carbohydrate can be utilized easily by those intolerant to lactose (Younus et al., 2002; Alakali et

*Corresponding author. E-mail: chimeziedirisu@yahoo.com. Tel: +234 8103 3866 97.

al., 2008; Ghandge et al., 2008). It is also believed that yoghurt has valuable therapeutic properties and helps in curing gastrointestinal disorders (Athar, 1986; Wolinsky, 2000; Younus et al., 2002; Vasiljevic and Shah, 2008).

Yoghurt also serves as a medium for the growth of microorganisms due to its high nutritional content hence it is liable to contamination. Moulds and yeast are the primary contaminants in yoghurt. Fungi growing in yoghurt utilize some of the acid, which will invariably reduce the acidity and hence favour the growth of putrefactive bacteria (Oyeleke, 2009) or other pathogenic organisms such as *Staphylococcus aureus* (Ifeanyi et al., 2013; De et al., 2014; Makut et al., 2014). Evaluation of the bacterial quality of yoghurt is necessary due to the high risk associated with consuming sub-standard or unhygienic yoghurt containing pathogenic organisms. Although, there are reports of qualities of yoghurt sold in some parts of Nigeria, no research has been done for yoghurts sold in the oil producing community of Ogba/Egbema/Ndoni local government area (ONELGA). Analyzing yoghurt for its quality is therefore required in order to create awareness among ONELGA people about the existing situation and hence protect the consumers' health from food-borne epidemic.

MATERIALS AND METHODS

Sample collection and preparation

Eight sachets of four different brands of yoghurt samples (names withheld) were purchased randomly from retail outlets within schools in Omoku and transported to the Integrated Science Laboratory of Federal College of Education (Technical) Omoku for analyses.

pH analysis

The pH values of the samples were analyzed using a Jenway 3505 pH meter (Camlab, UK).

Total bacterial count

Total bacterial count was carried out by pour plate technique as described by Kawo et al. (2006). Briefly, one mL of 10^{-3} dilution series of yoghurt samples was plated on Nutrient agar (Oxoid) and incubated aerobically at 37°C for 24-48 h. Colonies that developed were counted and expressed in colony forming units per milliliter (cfu/mL).

Total coliform count

Coliform count was carried out using MacConkey agar (Oxoid). One millimeter of the diluted yoghurt sample was transferred into well labeled Petri-dishes. Fifteen millimeter of the sterile melted agar at 45°C was poured and then swirled to mix with the agar thoroughly. This was allowed to solidify before incubation at 37°C for 48 h. Colonies obtained were counted.

Total fungi count

Total fungi were determined by plating the diluted yoghurt samples

Table 1. pH of yoghurt

Sample	Mean \pm SD*
Y1	3.1 \pm 0.08
Y2	3.20 \pm 0.30
Y3	2.94 \pm 0.01
Y4	2.38 \pm 0.81

*SD, Standard deviation.

Table 2. Microbial load of yoghurt in Omoku Schools (cfu/mL).

Sample	Total bacteria	Total coliform	Total fungi
Y1	$3.1 \times 10^5 \pm 0.45$	1.0 \pm 0.50	$3.2 \times 10^3 \pm 0.26$
Y2	$4.1 \times 10^5 \pm 0.71$	1.0 \pm 0.50	$3.4 \times 10^3 \pm 0.59$
Y3	$4.9 \times 10^5 \pm 0.10$	0.0 \pm 0.0	$4.0 \times 10^3 \pm 1.25$
Y4	$5.1 \times 10^5 \pm 1.20$	1.0 \pm 0.0	$4.9 \times 10^3 \pm 1.24$

Values are presented as mean and standard deviation.

in potato dextrose agar (PDA) (Oxoid) to which 20 μ g chloramphenicol was added. The plates were incubated at room temperature for 5-7 days.

RESULTS AND DISCUSSION

The pH of yoghurt samples is shown in Table 1 and ranged from 2.38 \pm 0.81 to 3.2 \pm 0.08. The pH recorded is within the range of 2.35 \pm 3.18 reported by Makut et al. (2014) but lower than the ranges reported by Ifeanyi et al. (2013) which is from 3.93 \pm 4.50 and Digbabul et al. (2014) which is from 4.73 \pm 5.11. This low acidity tended to inhibit coliform and favour the growth of acidophilic yoghurt bacteria as well as yeast and moulds hence their presence in the product.

Total bacteria count in yoghurt sold in public schools in Omoku is shown in Table 2 and ranged from $3.1 \times 10^5 \pm 0.45$ to $5.1 \times 10^5 \pm 1.20$ cfu/mL. High bacteria count is expected because of the presence of starter cultures, which are mainly lactic acid bacteria. The count obtained is comparable to the values obtained in yoghurts from Abuja (Okpalugo et al., 2008), Onitsha (Ifeanyi et al., 2013) and Makurdi (Digbabul et al., 2014). The standard count is 106-107 cfu/mL (Codex *Alimentarius*, 2003; Rodrigues et al., 2010). Very high count however is used as an indication of post-pasteurization contamination (Tamine and Robinson, 2004).

Total fungi count ranged from $3.2 \times 10^3 \pm 0.26$ to $4.9 \times 10^3 \pm 1.24$ (Table 2). These values are above the limits stipulated (Codex *Alimentarius*, 2003; Tamine and Robinson, 2004). High counts of yeast and mould have also been reported in yoghurts (Okpalugo et al., 2008; Ifeanyi et al., 2013; De et al., 2014; Digbabul et al., 2014). The presence of yeast and mould is attributed to

Table 3. One-way ANOVA statistics.

Parameter	F-Statistic	p value	Remark
Total Bacteria Count	F=4.47	p=0.102	NS
Total Fungi	F=0.50	p=0.518	NS
Total yeast and mould	F=8.72	P=0.042	S
pH	F=2.16	P= 0.172	NS

NS, not significant; S, significant at $p=0.05$.

poor handling and production (Kawo et al., 2006; Oyeleke, 2009; Amakoromo et al., 2012; Ifeanyi et al., 2013).

Total coliform count ranged from 0.0 to $1.0 \times 10^5 \pm 0.50$ cfu/mL (Table 2). The low level is attributed to acidity of yoghurt and/or heat treatment (Jay, 1992). Coliform was reported in some yoghurts produced locally at Kampala (Mukisa and Kyoshabire, 2010) and Keffi (Makut et al., 2014) but not in yoghurt sold at Ibadan (Alli et al., 2010) and Makurdi (Digbabul et al., 2014), although they contained yeast. The presence of coliform bacteria indicates unhygienic practices during handling of the product (Montagana et al., 1998). *Escherichia coli* in particular indicates failure in general manufacturing practices (FAO, 1998; Tamine and Robinson, 2004). Singh and Prakash (2008) also noted that the presence of *E. coli* in a milk product indicates presence of other enteropathogenic microorganisms which constitute a public health hazard.

One-way analysis of variance indicated that there was no significant difference in pH, total bacteria and total coliform counts ($p>0.05$), but there was significant differences in total fungi counts ($p<0.05$) (Table 3). Similar results have been obtained for yoghurt samples sold in Onitsha in which no significant difference in coliform but significant difference was observed in total bacteria count (Ifeanyi et al., 2013).

Conclusion

Although no attempt was made to isolate any organisms in this study, related researches have shown that most yoghurt sold in Nigerian markets are contaminated with pathogenic bacteria such as *E. coli*, *Staphylococcus aureus*, *Bacillus* sp. and moulds, such as *Rhizopus* sp., *Aspergillus* sp. etc. The presence of coliform in the yoghurt samples in this study confirms the unhygienic standards under which it is produced as also observed by other researchers. The implication of the findings is that consumption of contaminated yoghurt may contribute to high prevalence of gastroenteritis in the area. The high fungi count is equally disturbing because some moulds have the potential to produce aflatoxins, which are known to cause food intoxication and some type of cancer.

Therefore, sanitary inspection of production premises as

well as consumer protection is hereby advocated.

Conflict of interests

The authors did not declare any conflict of interest.

REFERENCES

- Alakali JS, Okonkwo TM, Lordye EM (2008). Effect of stabilizers on the physicochemical and sensory attributes of thermized yoghurt. *Afr. J. Biotechnol.* 7(2):158-63.
- Alli JA, Oluwadun A, Okonko IO, Fagade OE, Kolade AF, Ogunleye VO (2010). Microbial Assessment and Microbiological Quality of Some Commercially Prepared Yoghurt Retailled in Ibadan, Oyo State, Southwestern Nigeria. *Br. J. Dairy Sci.* 1(2): 34-38.
- Amakoromo ER, Innocent-Adiele HC, Njoku HO (2012). Microbiological Quality of a Yoghurt-Like Product from African Yam Bean. *Nat. Sci.* 10(6): 6-9.
- Athar IH (1986). Preparation of Cheese and Yoghurt (Dahi) at Household Level. Pakistan Agricultural Research Council, Islamabad.
- Codex Alimentarius (2003). CODEX standard for fermented milks 242-2003. 2nd ed. Available at: www.codexalimentarius.net/download/standards/400/CXS_243e.pdf (accessed 12/8/2015).
- De N, Goodluck TM, Bobai M (2014). Microbiological quality assessment of bottled yogurt of different brands sold in Central Market, Kaduna Metropolis, Kaduna, Nigeria. *Int. J. Curr. Microbiol. Appl. Sci.* 3(2):20-27.
- Digbabul B, Shember J, Amove J (2014). Physicochemical, microbiological and sensory evaluation of yoghurt sold in Makurdi metropolis. *Afr. J. Food Sci. Technol.* 5(6):129-135
- FAO (Food and Agriculture Organization of the United Nations) (1998). Milk Processing Guide Series. Vol 4 FAO/TCP/KEN/6611. Training Programme for Small scale Dairy Sector and Dairy Training institute, Naivasha. Terminal Statement prepared for the Government of Kenya. FAO, Rome.
- Ghandge PN, Prasad K, Kadam PS (2008). Effect of fortification on the physicochemical and sensory properties of Buffalo milk yoghurt. *Electronic J. Environ. Agric. Food Chem.* 7(5):2890-2899
- Ifeanyi VO, Ihesiaba EO, Muomaife OM, Ikenga C (2013). Assessment of Microbiological Quality of Yogurt Sold By Street Vendors in Onitsha Metropolis, Anambra State, Nigeria. *Br. Microbiol. Res. J.* 3(2): 198-205.
- Kawo AH, Omole EM, Na'aliya J (2006). Quality assessment of some processed yoghurt products sold in Kano Metropolis, Kano, Nigeria. *BEST J.* 3(1): 96-99.
- Khan K, Rehman SU, Khan MA, Anwar F, Bhadar S (2008). Physical and chemical quality appraisal of commercial yoghurt brands sold at Lahore. *ARPN J. Agric. Biol. Sci.* 3(3):14 -21.
- Kumar P, Mishra HN (2004). Yoghurt Powder—A Review of Process Technology, Storage and Utilization. *Food Bioproducts Process.* 82 (2): 133-142.
- Makut D, Ogbonna AI, Dalami H (2014). An Assessment of the Bacteriological Quality of Different Brands of Yoghurt Sold in Keffi, Nasarawa State, Nigeria. *J. Nat. Sci. Res.* 4 (4):19-22.
- Montagana MT, Erroi R, Sanapo S, Caggiano G, Bagordo A, Donno A De (1998). Food products and fungal contamination Note 1. Preliminary investigation in commercial yogurt. *J. Prev. Med. Hyg.* 39: 68-70.
- Mukisa IM, Kyoshabire R (2010). Microbiological, physico-chemical and sensorial quality of small-scale produced stirred yoghurt on the market in Kampala city, Uganda. *Nutr. Food Sci.* 40 (4):409-418.
- Okpalugo J, Ibrahim K, Izebe KS, Inyang US (2008). Aspects of Microbial Quality of some Milk Products in Abuja, Nigeria. *Trop. J. Pharm. Res.* 7(4):1169 -1177.
- Oyeleke SB (2009). Microbial assessment of some commercially prepared yoghurt retailled in Minna, Niger State. *Afr. J. Microbiol. Res.* 3:245-248.

- Rodrigues LA, Ortolani MBT, Nero LA (2010). Microbiological quality of yogurt commercialized in Viçosa, Minas Gerais, Brazil. *Afr. J. Microbiol. Res.* 4:210-213.
- Singh P, Prakash A (2008). Isolation of *Escherichia coli*, *Staphylococcus aureus* and *Listeria monocytogenes* from milk products sold under market conditions at agra region. *Acta Agric. Slovenica* 92(1): 83–88.
- Steinkraus KH (1997). Classification of fermented foods: worldwide review of household fermentation techniques. *Food Control* 8:311 - 317.
- Tamine AY, Robinson K (2004). *Yoghurt. Science and Technology.* Published by Institute of Applied Science. pp. 32-56.
- Vasiljevic T, Shah NP (2008). Probiotics- from metchnikoff to bioactives. *Int. Dairy J.* 18:714-728.
- WDC (Watson Dairy Consulting) (2014). *Yogurt: manufacturing-making –production* [<http://www.dairyconsultant.co.uk/si-yoghurt.php>]- Accessed 2014
- Wolinsky I (ed) (2000). *Milk and milk product in the Diet. Handbook of Dairy food and nutrition.* 2nd ed. CRC Press, Boca Raton, Florida, USA.
- Younus S, Masud T, Aziz T (2002). Quality evaluation of market yoghurt/dahi. *Pak. J. Nutr.* 1(5): 226-230.

A microscopic view of various bacteria, including rod-shaped and spherical forms, rendered in shades of pink and purple. The background is dark, making the bacteria stand out.

African Journal of Microbiology Research

Related Journals Published by Academic Journals

- *African Journal of Biotechnology*
- *African Journal of Biochemistry Research*
- *Journal of Bacteriology Research*
- *Journal of Evolutionary Biology Research*
- *Journal of Yeast and Fungal Research*
- *Journal of Brewing and Distilling*

academicJournals