OPEN ACCESS

African Journal of Microbiology Research

March 2021
ISSN 1996-0808
DOI: 10.5897/AJMR
www.academicjournals.org

ACADEMIC JOURNALS
expand your knowledge

About AJMR

The African Journal of Microbiology Research (AJMR) is a peer reviewed open access journal. The journal commenced publication in May 2007. The journal covers all areas of microbiology such as environmental microbiology, clinical microbiology, immunology, virology, bacteriology, phycology, molecular and cellular biology, molecular microbiology, food microbiology, mycology and parasitology, microbial ecology, probiotics and prebiotics and industrial microbiology.

Indexing

CAB Abstracts, CABI's Global Health Database, Chemical Abstracts (CAS Source Index) Dimensions Database, Google Scholar, Matrix of Information for The Analysis of Journals (MIAR), Microsoft Academic, Research Gate

Open Access Policy

Open Access is a publication model that enables the dissemination of research articles to the global community without restriction through the internet. All articles published under open access can be accessed by anyone with internet connection.

The African Journal of Microbiology Research is an Open Access journal. Abstracts and full texts of all articles published in this journal are freely accessible to everyone immediately after publication without any form of restriction.

Article License

Article Copyright

When an article is published by in the African Journal of Microbiology Research, the author(s) of the article retain the copyright of article. Author(s) may republish the article as part of a book or other materials. When reusing a published article, author(s) should; Cite the original source of the publication when reusing the article. i.e. cite that the article was originally published in the African Journal of Microbiology Research. Include the article DOI, Accept that the article remains published by the African Journal of Microbiology Research (except in occasion of a retraction of the article).

A copyright statement is stated in the abstract page of each article. The following statement is an example of a copyright statement on an abstract page.

Self-Archiving Policy

The African Journal of Microbiology Research is a RoMEO green journal. This permits authors to archive any version of their article they find most suitable, including the published version on their institutional repository and any other suitable website.

Digital Archiving Policy

The African Journal of Microbiology Research is committed to the long-term preservation of its content. All articles published by the journal are preserved by Portico. In addition, the journal encourages authors to archive the published version of their articles on their institutional repositories and as well as other appropriate websites.
https://www.portico.org/publishers/ajournals/

Metadata Harvesting

The African Journal of Microbiology Research encourages metadata harvesting of all its content. The journal fully supports and implement the OAI version 2.0, which comes in a standard XML format. See Harvesting Parameter

Memberships and Standards

Academic Journals strongly supports the Open Access initiative. Abstracts and full texts of all articles published by Academic Journals are freely accessible to everyone immediately after publication.

All articles published by Academic Journals are licensed under the Creative Commons Attribution 4.0 International License (CC BY 4.0). This permits anyone to copy, redistribute, remix, transmit and adapt the work provided the original work and source is appropriately cited.

Crossref is an association of scholarly publishers that developed Digital Object Identification (DOI) system for the unique identification published materials. Academic Journals is a member of Crossref and uses the DOI system. All articles published by Academic Journals are issued DOI.

Similarity Check powered by iThenticate is an initiative started by CrossRef to help its members actively engage in efforts to prevent scholarly and professional plagiarism. Academic Journals is a member of Similarity Check.

CrossRef Cited-by Linking (formerly Forward Linking) is a service that allows you to discover how your publications are being cited and to incorporate that information into your online publication platform. Academic Journals is a member of CrossRef Cited-by.

Academic Journals is a member of the International Digital Publishing Forum (IDPF).
The IDPF is the global trade and standards organization dedicated to the development and promotion of electronic publishing and content consumption.

Contact

Editorial Office: ajmr@academicjournals.org

Help Desk: helpdesk@academicjournals.org

Website: http://www.academicjournals.org/journal/AJMR

Submit manuscript online http://ms.academicjournals.org

Academic Journals
73023 Victoria Island, Lagos, Nigeria
ICEA Building, 17th Floor,
Kenyatta Avenue, Nairobi, Kenya.

Editors

Editors

Table of Content

Vol. 15(3), pp. 120-124, March, 2021
DOI: 10.5897/AJMR2020.9473
Article Number: 331591266281
ISSN: 1996-0808

http://www.academicjournals.org/AJMR

ACADEMIC JOURNALS
expand your knowledge

African Journal of Microbiology Research

Full Length Research Paper

Rapidly growing mycobacterial infections associated with plastic surgery: An epidemiological description

Jessyca Rodrigues Braga[1], Marinésia Aparecida do Prado[2], Heliny Carneiro Cunha das Neves[2], Anaclara Ferreira Veiga Tipple[2], Dayane de Melo Costa[2], Sueli Lemes de Ávila Alves[3], Hélio Galdino-Júnior[2], Katiane Martins Mendonça[2] and Silvana de Lima Vieira dos Santos[2]*

[1]Clinics Hospital, Federal University of Goias, Goiânia, Brazil.
[2]Faculty of Nursing, Federal University of Goias, Goiânia, Brazil.
[3]Health Surveillance, Goiânia Municipal Health Department, Goiânia, Brazil.

Received 30 December, 2020; Accepted 19 February, 2021

Surgical site infections (SSI) caused by rapidly growing mycobacterial (RGM) have become increasingly frequent. The aim of this study is to describe the epidemiological, clinical aspects and factors associated with RGM infections related to plastic surgery. Notifications of SSI from 86 health care facilities of the capital of a Brazilian state within nine years, approximately, were assessed. RGM, predominantly *M. fortuitum* (39.0%), was isolated from 66 cases of infection folowing plastic surgery, mainly mammoplasty. All the cases were woman, with an average of 32.9 years. Amikacin/clarithomycin was the prevalent therapeutic regimen, and most of the isolates showed resistance to ciprofloxacin. There was an association of infection by *M. abscessus* and use of surgical instruments that were not exclusive to the institution (P=0.048). Thus, these findings emphasize the importance of SSI notifications and strict monitoring of surgical instruments reprocessing.

Key words: Surgical site infection, plastic surgery, non-tuberculous mycobacteria.

INTRODUCTION

Surgical site infections (SSI) caused by non-tuberculous rapidly growing mycobacteria (NTRGM) have been reported, including after plastic surgery, mainly liposuction and mammoplasty (Sharma et al., 2016; Romero et al., 2017). Outbreaks have occurred in geographically distant locations (Leão et al., 2010) in Brazil since 2000. The greatest occurred in the Southeastern region (172 cases), where 38 hospitals had cases confirmed by culture, with *Mycobacterium massiliense* isolates belonging to a single clone (BRA100), which showed

*Corresponding author. E-mail: silvanalvsantos@gmail.com. Tel: +55 (62) 99532 6465.

consistent tolerance to the 2% glutaraldehyde solution (Duarte et al., 2009).

Thus, health authorities concluded failures in the reprocessing of surgical instruments closely related to the inappropriate use of chemical sanitizers and, therefore, suspended sterilization in liquid media across the country in 2009 (Agência Nacional de Vigilância Sanitária, 2009). However, cases of post-surgical mycobacteriosis continued to be reported across the country. In the Goiás State, 44 cases were reported between 2014 to 2018 (Agência Nacional de Vigilância Sanitária, 2019). Given the complexity of SSIs, especially by mycobacteria, this study aimed to describe the epidemiology, clinical aspects and factors associated with NTRGM infections related to plastic surgery.

MATERIALS AND METHODS

After ethical approval (Protocol n. 1269485/2015), a retrospective study was performed on infection notifications of the official data base of the Public Health System (composed of 86 public and private hospitals) of a city in the Brazilian Midwest region. The data ranged from the first case and infection related to plastic surgery notified in 2007 (when the notification was due to spontaneous demand) until December 2015 (notifications became mandatory in 2008). Notifications of post-surgical mycobacterioses have become mandatory in Brazil since 2008. Before that, the municipal services used to investigate notifications of cases by spontaneous demand. National compulsory notifications are made through a computerized system called FormSUS, a form that interconnects the municipal, state and federal health departments. A case was considered: any patient who presented SSI, after a plastic surgery, notified with a positive culture result for NTRGM (from surgical wound or adjacent tissues). The resistance profile of the isolates was determined by minimum inhibitory concentration (MIC) (Clinical and Laboratory Standards Institute, 2011).

Data were collected by the reseachers following a check list, and analyzed using SPSS software version 24.0. NTRGM infections were considered as the outcome variable and as predictors: year of notification, type of institution, underlying disease, age, type of surgery, number of surgeries, surgical method, use of loaner surgical instruments/devices, reprocessing of surgical instruments in the healthcare facility, and surgical instruments exclusive of the healthcare facility. Poisson's bivariate analysis was performed, and statistical significance was established by Wald's chi-square test. To compare proportions, Fisher's exact test was used. Values of $P<0.05$ were considered statistically significant.

RESULTS

A total of 66 positive culture (95.7%; 95% CI: 88.0-98.5%) out of 69 notifications were identified, all in women.

The average of age was 32.9 years (SD: 8.6; median: 32; Minimum: 21; Maximum: 61). Half of them underwent more than one plastic procedure at the same day, and the majority (87.9%) underwent mammoplasty. Twenty hospitals were involved, with a predominance of private establishments (18/93.9%) (Table 1). Of these, two occurred in the same clinic. Regarding the clinical profile, the most common sign was the presence of secretion in the surgical wound (83.3%), followed by edema (48.5%) and hyperemia (42.2%) (Table 1). Surgical treatment with removal of prostheses was necessary in 37.8% of cases (Table 1). Two cases of physical sequelae were identified, resulting from infections with permanent scarring (breast and gluteus).

Of the 66 isolates, *M. fortuitum* (39%) predominated, followed by *M. abscessus* (18.0%), *M. peregrinum* (12.0%), *M. brisbanensis* (9.0%), and other species (*M. mageritense, M. senegalense, M. wolinsky, M. conceptionense* and *M. chelonae*). Five species were resistant to ciprofloxacin, but *M. abscessus* was sensible to this antimicrobial. *M. fortuitum* was the specie that showed resistance to the largest number of antimicrobials concomitantly, followed by *M. brisbanensis* and *M. abscessus* (Table 2). *M. abscessus* infection was associated with the use of surgical instruments non-exclusive to the healthcare facility (P=0.048).

DISCUSSION

M. fortuitum and *M. abscessus* are species frequently reported as etiologic agents in infections arising from plastic surgeries (Sharma et al., 2016; Romero et al., 2017). Resistance to ciprofloxacin was evidenced in NTRGM analyzed in different regions of Brazil (Duarte et al., 2009). SSIs caused by NTRGM are difficult to manage, since the diagnosis is late (Park et al., 2016) and the antimicrobial treatment is prolonged; it brings side effects to the patient, which also hinders the healing process of the infection (Sharma et al., 2016; Romero et al., 2017). In many cases, there is a need for surgical intervention. Studies have shown that the removal of breast implants after infections by *M. abscessus* and *M. fortuitum* resulted in a better prognosis for the patient (Sharma et al., 2016; Romero et al., 2017). On the other hand, surgical interventions for debridement and removal of prostheses due to infection have culminated in physical sequelae (Park et al., 2016).

It is known that exogenous factors to the patient, such as inadequate reprocessing of surgical instruments, increase the risk of SSI, and that the acquisition of loaner surgical instruments, as well as the use of products owned by the surgeon, are factors that interfere negatively in the quality of its reprocessing (Seavey, 2010). Although the healthcare facility has the responsibility to ensure the safety of the reusable medical devices (RMD), there are problems related to the management and reprocessing of these devices that can put patients and healthcare team at risk.

Table 1. Characterization of cases of rapidly growing mycobacterial infections after plastic surgery, according to notification forms, from a capital of the Brazilian Midwest region.

Variables	N¥	%
Number of surgeries in the same patient		
1	33	50.0
≥2	33	50.0
Type of surgery		
Abdominoplasty	14	21.2
Liposuction or liposculpture	21	31.8
Mammoplasty	58	87.9
Others	4	6.1
Surgery		
Video	1	1.5
Conventional	52	78.8
Use of loaner instrumentation		
Yes	7	10.6
No	29	43.9
Reprocessing of surgical instruments in the healthcare facility		
Yes	26	39.4
No	6	9.1
Surgical instruments not exclusive to the healthcare facility		
Yes	17	25.8
No	9	13.6
Notification by		
MCICHS*	31	47.0
Laboratories	25	37.9
HAICC**	10	15.2
Type of healthcare facility		
Private	62	93.9
Public	3	4.5
Year		
2007-2009	13	19.7
2010-2011	26	39.4
2012-2013	16	24.2
2014-2015	5	7.6
Sign/symptom		
Fever	17	25.8
Edema	32	48.5
Hiperthermia	28	42.4
Abscess	11	16.7
Nodule	8	12.1
Fistulization	5	7.6
Secretion	55	83.3
Others	38	57.6

Table 1. Contd.

Therapeutic scheme ***		
CLA+AMI	16	24.2
CLA+CIP	5	7.5
CLA	5	7.5
CLA+AMI+CIP	4	6.1
CLA+AMI+MIN	2	3.0
CIPRO	2	3.0
Others	14	21.2
Surgical treatment		
Prosthesis removal	25	37.8
Drainage of secretion from the surgical wound	6	9.1
Debridement	3	4.5
Graft	1	1.5

¥Valid data; * Municipal Coordination of Infection Control in Health Services; ** HICC: Healthcare Associated Infection Control Commission; *** CLA: Clarithromycin; AMI: Amikacin; CIP: Ciprofloxacin; MIN: Minocycline.

Table 2. Resistance and sensitivity profile of rapidly growing mycobacteria (N = 8) to antimicrobials isolated from patients with infection after plastic surgery in a capital of the Brazilian Midwest region.

Antimicrobial	Profile											
	***M. fortuitum* (n=2)**		***M. abscessus* (n=1)**		***M. peregrinum* (n=1)**		***M. brisbanensis* (n=2)**		***M. senegalense* (n=1)**		***M. conceptionense* (n=1)**	
	R	**S**	**R**	**S**	**R**	**S**	**R**	**S**	**R**	**S**	**R**	**S**
Isoniazid	1	-	1	-	-	-	1	-	-	-	-	-
Streptomycin	1	-	1	-	-	-	1	-	-	-	-	-
Ethambutol	1	-	1	-	-	-	1	-	1	-	-	-
Rifampicin	1	-	1	-	-	-	1	-	1	-	-	-
Rifabutin	1	-	1	-	-	-	1	-	-	-	-	-
Ciprofloxacino	1	1	-	1	1	-	1	1	1	-	1	-
Clofazimine	1	-	-	1	-	-	1	-	-	-	-	-
Clarithromycin	2	-	-	1	-	1	-	1	-	1	-	1
Ethionamide	1	-	1	-	-	-	1	-	-	-	-	-
Cycloserine	1	-	1	-	-	-	1	-	-	-	-	-
Doxycycline	2	-	1	-	-	1	1	1	-	-	-	1
Cefoxitin	1	-	-	-	-	1	-	1	-	-	-	1
Tobramycin	1	-	-	-	1	-	1	1	-	-	-	-
Sulfamethoxazole	1	-	-	-	1	-	1	1	1	-	1	1
Linezolid	-	-	-	-	-	-	-	-	-	-	-	1
Amikacin	-	2	-	1	-	1	-	2	-	1	-	1
Moxifloxacin	-	-	-	-	-	-	-	1	-	1	-	1

R: Resistant; S: Sensible.

The cleaning step, considered the most important in the reprocessing of RMD, is generally the most affected in the case of loaner instruments/devices, especially due to delays in the delivery of the surgical set to the establishment with the necessary advance for the fulfillment of all reprocessing steps. This can lead workers

to take “shortcuts” to meet the expected time for the surgical procedure. Additionally, the high-turnover of these sets in several healthcare services makes it impossible to guarantee the quality of the reprocessing steps in all of them (Seavey, 2010). An analysis of loaner instruments and orthopedic implants in clinical use revealed their contamination, including by biofilm, at the time of delivery to the hospital, as well as after the sterilization process, that is, ready-to-use (Costa et al., 2018).

Conclusion

In conclusion, SSIs by NTRGM following plastic surgery, predominantly by *M. fortuitum*, including species resistant to ciprofloxacin, occurred in young women, especially those that underwent the procedures in private clinics. These results highlight the importance of SSI notification through a robust surveillance system. The association of *M. abscessus* infection with the use of non-exclusive instruments points to the need for rigorous management and reprocessing of these devices, as well as the registration of these products both by the Sterilizing Service Unit and in the patients' records in order to allow its traceability.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

REFERENCES

Agência Nacional de Vigilância Sanitária (2009). Ministério da Saúde - Resolução nº 8 de 27 de fevereiro de 2009. Dispõe sobre as medidas para redução da ocorrência de infecções por Micobactérias de Crescimento Rápido - MCR em serviços de saúde – Brazil, Brasília. Available: https://www20.anvisa.gov.br/segurancadopaciente/index.php/legislacao/item/rdc-8-de-27-de-fevereiro-de-2009 Accessed 11 August 2015.

Agência Nacional de Vigilância Sanitária (2019). Ministério da Saúde - Boletim de Segurança do Paciente e Qualidade em Serviços de Saúde n°19, GVIMS/GGTES/Anvisa. Notificações de Casos de infecções por Micobactérias de Crescimento Rápido (MCR) de 2014-2018. Brazil, Brasília. Available: https://bit.ly/2GJkxFh Accessed 10 October 2019.

Clinical and Laboratory Standards Institute (2011). Susceptibility Testing of Micobacteria, Nocardiae, and Other Aerobic Actinomycetes; Approves Standard- Second Edition. CLSI document M24-A2. Wayne.

Costa DM, Lopes LK, Tipple AF, Johani K, Deva AK, Watanabe E, Vickery K (2018). Evaluation of stainless steel surgical instruments subjected to multiple use/processing. Infection, Disease and Health 23:3-9.

Duarte RS, Lourenço MC, Souza-Fonseca L, Leao SC, Efigenia de Lourdes TA, Rocha IL, Coelho FS, Viana-Niero C, Gomes KM, da Silva MG, Lorena NS, Pitombo MB, Ferreira RM, Garcia MH, de Oliveira GP, Lupi O, Vilaça BR, Serradas LR, Chebabo A, Marques EA, Teixeira LM, Dalcolmo M, Senna SG, Sampaio JL (2009). Epidemic of postsurgical infections caused by Mycobacterium massiliense. Journal of Clinical Microbiology 47(7):2149-55.

Leão SC, Viana-Niero C, Matsumoto CK, Lima KVB, Lopes ML, Palaci M, Hadad DJ, Vinhas S, Duarte RS, Lourenço MCS, Kipnis A, Neves ZC, Gabardo BMA, Ribeiro MO, Baethgen L, Assis DB, Madalosso G, Chimara E, Dalcolmo MP (2010). Epidemic of surgical-site infections by a single clone of rapidly growing mycobacteria in Brazil. Future Microbiology 5(6):971-80.

Park BY, Kwon JW, Kang SR, Hong SE (2016). Analysis of malpractice claims associated with surgical site infection in the field of plastic surgery. Journal of Korean Medical Science 31(12):1963-1968.

Romero FA, Powell EA, Babady NE, Kaltsas A, Figueroa CJ, Pulitzer M, Mehrara BJ, Glickman MS, Morjaria S (2017). Nontuberculous Mycobacterial Infections After Silicone Breast Implant Reconstruction Emphasize a Diversity of Infecting Mycobacteria. Open Forum Infectious Diseases 4(4):ofx189.

Seavey R (2010). Reducing the risks associated with loaner instrumentation and implants. AORN Journal 92(3):322-34.

Sharma P, Vazquez-Guillamet LJ, Miljkovic G (2016). Atypical Mycobacterial infection after abdominoplasty overseas: a case report and literature review. Case Reports in Infectious Diseases 2016:3642567.

Vol. 15(3), pp. 125-131, March, 2021
DOI: 10.5897/AJMR2020.9319
Article Number: 46B4BD666283
ISSN: 1996-0808

http://www.academicjournals.org/AJMR

ACADEMIC JOURNALS
expand your knowledge

African Journal of Microbiology Research

Full Length Research Paper

Evaluation of factors fuelling global antimicrobial resistance and its economic and clinical burden

Larry L. Mweetwa[1*], Paul Chukwuemeka Adiukwu[2], Kabo O. Tshiamo[3], Thatoyaone Kenaope[4], Tiroyaone Nthusang[5] and Emmanuel Tope Oluwabusola[1]

[1]Department of Chemistry, Marine Biodiscovery Centre, University of Aberdeen, United Kingdom.
[2]School of Pharmacy, University of Botswana, Plot 4775 Notwane Rd., Gaborone 00000, Botswana.
[3]Nathan and Family Pharmacy, P. O. Box 1299 ABG, Gaborone 00000, Botswana.
[4]Department of Pharmacy, Boitekanelo College, Plot 5824 Masetlheng Rd., Gaborone 00000, Botswana.
[5]Department of Pharmacy, School of Health Sciences, University of Zambia, Ridgeway Campus, P. O. Box 50110, Lusaka, Zambia.

Received 13 March, 2020; Accepted 14 August, 2020

The aim of the research is to assess the factors fuelling Antimicrobial Resistance (AMR) and its economic and clinical burden. For this purpose, a systematic review was conducted, which included an analysis of factors and economic and clinical burden of AMR. It was found that poorly treated sewage; discharges from antibiotic manufacturing plants, empirical prescribing, lack of antimicrobial stewardship, poor AMR monitoring; hospital-acquired infections and human-to-human contacts with environmental pathogens through food; and increase in global trade and travel are the factors behind the spread of AMR. Further, based on previous research, the study found a significant economic and clinical burden caused by AMR infections. It is recommended that well-organised antimicrobial stewardship be in place, AMR monitoring, limit access to antibiotics via over the counter dispensing without prescriptions, mandatory sensitivity tests for antimicrobial prescription be considered, proper hygiene in hospitals and medicine manufacturing sites must be ensured to reduce global AMR.

Key words: Antimicrobial Resistance, antimicrobial resistance (AMR), antibiotic resistance, economic burden, clinical burden.

INTRODUCTION

Over the last decade, antimicrobial resistance (AMR) has become a global menace. Its rise is due to the misuse of antimicrobial in food-producing humans and animals, and globalisation and suboptimal infection control facilitated its spread across the globe (Huttner et al., 2013). According to WHO (2017), AMR is a comprehensive term for resistance in several types of microorganism and involves resistance to antiviral, antiphrastic, antiviral and antibacterial drugs. Consequently, the effectiveness of standard treatment is lost, and infections continue to spread. AMR is observed as a global concern because of the existing and probable effect on global population health, Gross Domestic Product (GDP) and cost to healthcare, largely through inadequate treatment options (O'Neill, 2014).

Earlier reports suggest that figures of infections are

*Corresponding author. E-mail: l.mweetwa@abdn.ac.uk or larrymweetwa1@gmail.com.

increasing globally due to resistant microbes (ECDC, 2015). Generally, the societal (or economic) viewpoint includes the possible effect on the labour via lost productivity (Smith and Coast, 2012); however, it also comprises the burden on patient out-of-pocket expenditures and on careers (Tam and O'Brien, 2016; Naylor et al., 2018). According to Hwang and Gums (2016), the rise of AMR has placed significant societal concerns and economic burden on the healthcare systems. Various factors have resulted in the occurrence of AMR, including access and inappropriate use of antimicrobials, microbial adaptation via selective pressures and a lack of new antimicrobials in development. Recently, efforts have been made to reduce a few of these factors in outpatient and inpatient setting along with increasing public awareness and promoting education to clinicians. The programs such as antimicrobial stewardship offer a multi-intervention framework to control the inappropriate recommending of antimicrobials, offer best antimicrobial therapy, and avert the increase of AMR.

AMR in humans is impacted by environmental and bacterial factors, such as exposure to environmental waste and pollution, hospital-acquired infections, over the use of antibiotics in animal farming and food production (Allcock et al., 2017). Landers et al. (2012) concluded that antimicrobial use in clinical medicine has substantially reduced the problem of infectious diseases and supported multifaceted medical interventions, for example, advanced surgery and organ transplantation which routinely are susceptible to infections have had positive outcomes. Further, in clinical medicine, the overuse or misuse of antimicrobial is a key factor behind the development of AMR in the global population. Maragakis et al. (2008) assert that treatment factors such as delay in proper antimicrobial treatment and toxicity related to treatment contributed to negative impacts in patients infected with AMR organisms.

Antimicrobials have a significant role in diminishing mortality and morbidity from infective diseases. Nevertheless, these benefits have been threatened by the rise and spread of bacteria that resist antimicrobials (O'Neil, 2014; Smith and Coast, 2012). KPMG (2014) and Taylor et al. (2014) asserted that AMR in recent years had become a prevalent issue in different countries, despite their income level. It was found that in 2014, approximately 700,000 deaths were reported globally, and all of them were linked to infections that resulted from AMR organisms. The ratio of death is expected to reach 10 million annually in a few decades if no robust mitigation measures are put in place.

According to Ventola (2015), AMR infections are a significant economic and clinical burden to the healthcare system in the United States and also to patients and their families. Such infections are prevalent in hospitals, because of the presence of vulnerable patients, increased use of antibiotics and extensive use of invasive procedures in healthcare settings. Approximately 2 million Americans annually develop Health Care-Associated Infections (HAIs), largely because of AMR pathogens. It was estimated that the overall economic burden due to AMR infections on the US economy was around $35 billion annually lost in productivity and $20 billion in healthcare costs. Also, AMR infections cause a huge burden to communities and families because of healthcare costs and lost wages. The reasons behind AMR are complex and comprise human behaviour in different aspects of society, and major environmental factors are behind the spread of AMR infections. Endeavours have been made in the past to describe dissimilar features of AMR and the interventions required to address the challenge (Laxminarayan et al., 2013).

Nevertheless, there has been a lack of coordinated action primarily at the political, national and global level. Indeed, antibiotics have shown positive effects in societal and medical developments and at present are essential in healthcare settings. The developments in modern medicine, for example, organ transplantation, surgery, preterm babies' treatment and cancer chemotherapy would not be possible, which is taken for granted today without effective treatment for bacterial infections. It is most likely that in few years of time, that dire setback will have to be faced by humans economically, socially and medically, if unprecedented and real coordinated actions are not taken globally, we may revert to a pre-antibiotic era (Laxminarayan et al., 2013). Therefore, to emphasise more on this, the present study assesses what factors fuel antimicrobial resistance globally and how it is contributing to the economic and clinical burden. For this purpose, the research applies a systematic review technique to assess the factors and the associated burdens.

METHODOLOGY

Research approach and design

The study has used a qualitative research approach in order to investigate factors which contribute to the AMR crisis and how this crisis has resulted in increased economic and clinical costs. The qualitative research is deemed appropriate for the study as the objectives of the research do not include specific statistical measurement. Considering the nature of the qualitative approach, it helps the study to focus on drawing suppositions from different social meaning and settings. For this reason, the study performs a systematic review underlying its qualitative research approach.

Literature search

The systematic qualitative research is conducted through identifying articles which are related to the field of AMR explained as a crisis and its economic and clinical consequences. The articles which were searched for ensured to be between 2008 to 2019, from Google Scholar, International Healthcare Journals Online as well as other global healthcare official websites such as The Lancet Infectious Diseases Commission, Centres for Disease Control and

Prevention (CDC), and World Health Organisation (WHO). The articles and information published within these sites and journals were found to be relevant by searching for relevant keywords, such as; Antimicrobial Resistance, AMR crisis, Antibiotic Resistance, Factors of Antimicrobial Resistance, Causes of Antimicrobial Resistance, Consequences of AMR Infection, AMR Economic Cost, Economic Burden and Clinical Burden of AMR.

Inclusion criteria

Articles which included reviews and reported evidence of AMR crisis were included in the systematic qualitative review of the present research if they matched the following predefined study sample criteria:

(i) Reported trends of growing AMR infection among individuals around the globe
(ii) Were published at and after 2008.
(iii) Studied AMR from a global, economic, social and political point of view rather than just biological.
(iv) The research process and technique, sample size, conclusion and literature review were clearly reported in them

Data extraction and analysis

With the purpose to select the relevant research material having high quality for the current study, the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) approach was undertaken to extract the data. PRISMA approach assists the researcher to filter out research articles having inappropriate quality and information (Figure 1).

Initially, after a thorough search, the researchers were able to extract 545 citations, from which 152 duplicates were excluded. In the second step, the researcher reviewed 393 non-duplicate abstracts and excluded 351 abstracts due to lack of relevance, and the required period of study (criteria 2). Finally, the researchers reviewed 42 full-text papers; however, based on the aforementioned criteria (1, 3 and 4) and due to limited information related to the research problem, the sample size of the paper was reduced to 14 only, which were considered for analysis. The papers meeting the criteria were analysed and evaluated on the basis of their year of publication, location of study, method of testing and the findings. The data was then analysed using descriptive analysis technique and was discussed based on findings derived from the studies using a systematic review of the selected papers (Tables 1 and 2).

DISCUSSION

The table exhibits the list of studies which were reviewed to evaluate the factors that cause global antimicrobial resistance and the impact of these factors on the economic and clinical burden. For this reason, the systematic theme-based review is divided into two parts; namely the factors and the second one being the economic and clinical burdens.

The rapidly growing issue of antimicrobial resistance has become a major concern for governments around the world and authorities which are responsible for Global public healthcare. The major factor which has a substantial level of impact on an individual's health and causes infectious diseases is the environment that the individual resides in or is exposed to. Consequently, many professionals and researchers under this domain have identified environmental components to be a significant factor associated with antibiotic resistance. For example, the study of Sahoo (2012) highlighted the impact of the environment as a key driver in spreading AMR. Previous research suggests that aside from natural and physical environmental factors that influence antibiotic resistance development, behavioural and social factors also play a role (Pruden et al., 2012). Moreover, the factor of geographical variation is also important in antibiotic resistance. According to Parveen (2006), similar geographical variation in antibiotic resistance and agricultural factors is an environment to host antimicrobial-resistant bacteria. It is further explained that in many regions especially developing countries, easy access to first-line and second-line antibiotics and dispensing of antibiotics without prescriptions is a key driver and also limited options (Schwartz et al., 2018). Healthcare professionals are accustomed to using cheaper, ineffective and toxic antibiotics (referred to as biological substances before being healing compounds), and are considered toxic for clinical use like the antiparasitic or antiviral agent, (Rolain and Baquero, 2016), on patients hence increasing the antibiotic resistance of bacteria (Ventola, 2015).

Furthermore, other factors which contribute to the increasing crisis of antimicrobial resistance include the population levels in different geographical regions and human behaviours on various societal levels (Allcock et al., 2017). Similarly, as discussed in the table, the research of Aslam et al (2018) also highlighted factors similar to those documented in Parveen et al. (2006)'s study. The study drew conclusions upon the basis of unavailability of newer and advanced drugs due to regulatory requirements and a decrease in financial funding from the nations on research and development of new antibiotics with novel chemical structures. Hence these factors together fuel antimicrobial resistance thereby, increasing the global crisis.

Overall, the common factor which was found to be propelling the AMR crisis is the lack of knowledge of ways in which environment can affect health and antibodies. Further, agricultural and climate change is also the factor behind the growth of such bacteria (Laxminarayan et al., 2013; Hwang and Gums, 2016; Huttner et al., 2013). From the conclusion of these studies, it can be implied that the issue can be addressed by multidisciplinary approaches across health care institutions and environmental and agricultural sectors. Hence, governments and public healthcare regulatory bodies can implement these findings to minimise the factors and make people aware of the scourge. Besides, the recognition and identification of these factors can help to examine different approaches which will combat the rising problem of antimicrobial resistance, from social and

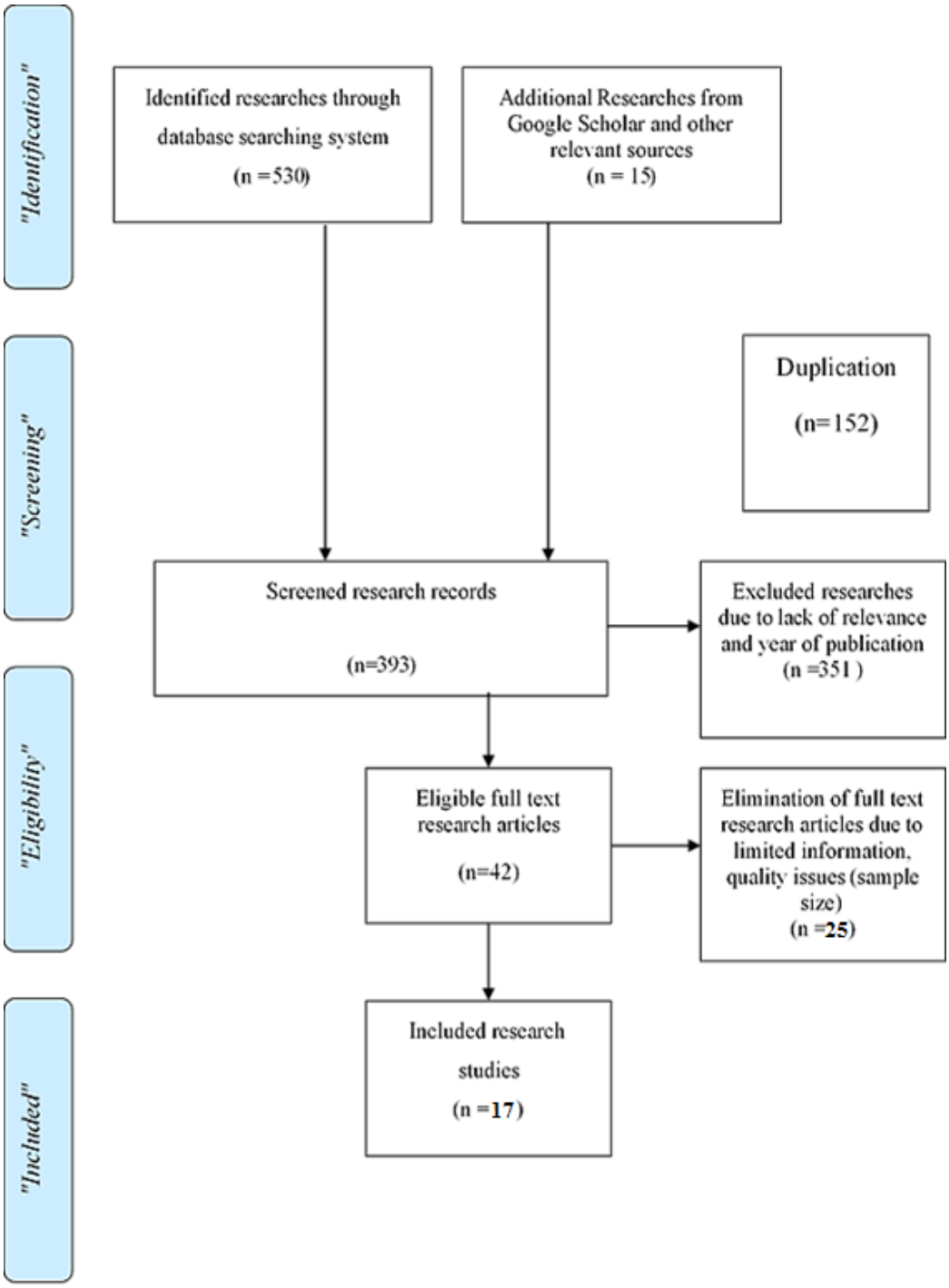

Figure 1. PRISMA flow chart.

as well as healthcare point of view (Ohl and Dodds, 2011).

AMR crisis is gaining much attention from healthcare professionals, researchers and government due to the economic burden it poses on a country nationwide, as well as to the patients infected by it and to their families. For example, according to the studies of Aslam et al. (2018); Naylor et al. (2018) and Allcock et al. (2017), the clustering of patients who are vulnerable to AMR in hospitals was resource-intensive, thereby requiring extensive use of aggressive treatments which require millions of funds for the patients' family to pay. It also increases the burden on hospitals and clinics to efficiently keep their resources stocked up in case of emergencies.

Table 1. Results factors fuelling global amr.

Year	Author	Country	Methodology	Key Findings
2017	Allcock et al.	United Kingdom	Qualitative Review	The study concluded that use, overuse and misuse of antimicrobial are a key contributing element in the spread of AMR. Together with antimicrobial use in animals and humans, its exposure in the environment is thought to be significant selective pressure for AMR worldwide.
2018	Aslam et al.	Pakistan, China and the United Kingdom	Qualitative Review	The findings of the study assert that possible reasons of global AMR are the high use of antibiotics in humans and animals (food, aquatic, pets), poor hygiene/sanitation, increased global travel and non-metabolised antibiotics release or their residues into the environment.
2017	Bengtsson-Palme et al.,	Sweden	Qualitative Review	The factors causing antibiotic resistance include; fixation and emergence of novel resistance genes and transfer and mobilisation of resistance genes to human pathogens. Also, poorly treated sewage, discharge from antibiotic manufacturing and hospital contacts, human-to-human contacts with environmental pathogens through animal husbandry or food fuel AMR.
2013	Huttner et al.	Switzerland	Systematic Review	The key factors behind AMR are the selection pressure caused by antimicrobial use in food-producing animals as well as poultry.
2016	Hwang and Gums	United States	Qualitative Review	The driving factor behind the rise of AMR is associated with the overuse of antimicrobials and reducing arsenal against the fight of resistance.
2013	Laxminarayan et al.	United States	Qualitative Review	It was found that resistance arises as a result of selection pressure from the use of antibiotics and mutations in microbes. Besides, the spread of AMR is facilitated by poor hygiene and sanitation, empirical prescribing, lack of antimicrobial stewardship, poor AMR monitoring; hospital-acquired infections, the transmission of inter-species genes and the growing frequency of global trade, travel and transmission of disease.
2012	Sahoo	India	Mix-method Approach	It was observed that social environmental and behavioural factors such as non-compliance with the use of antibiotics by patients and inappropriate or empirical prescribing of antibiotics in informal healthcare providers were the key contributing factors behind the development of AMR. Furthermore, it was found that physical and natural environmental factors are linked with the rise and prevalence of AMR and infectious diseases.
2015	Ventola	United States	Qualitative Review	It was established that the overuse of antibiotics is the primary factor behind the spread of global AMR. Also, inappropriately prescribed antibiotics add to the spread of resistant bacteria.

These scenarios were further evidenced by healthcare regulatory bodies such as Centres for Disease Control and Prevention. They explained that even in nations where effective drugs are being used, records showed that patients taking such treatments were required to stay longer in hospitals, have to make more routine visits to the doctor and long-term recuperation (Centres for Disease Control and Prevention, 2013). It eventually exhausts the resources for other patients with severe diseases.

Further, it was reported by Wright (2014) and Bartlett et al. (2013) that the total economic burden measured on the economy of the US by AMR ranged from $18,588 to $29,069. The findings of AMR being an economic and clinical burden are further reinforced by the studies of Shrestha et al. (2018) whereby the researcher concluded that oftentimes the economic costs of consuming antibiotic by each AMR patient were considerably exceeding the purchase costs of it; although measuring the economic cost and clinical burden is a challenge identified by Maragakis et al. (2008). The study also strengthened the argument that AMR crisis has a significant impact on economic and clinical burden by explaining that the cost of patients exposed to AMR infections is higher compared to those patients who are not infected by AMR pathogen.

Similarly, Hay et al. (2018) asserted that AMR is a global threat to wellbeing and health of people, damaging the ability to prevent and cure infectious diseases; therefore, it is now considered as an economic burden on healthcare facilities. Similarly, Tacconelli and Pezzani (2019) concluded that since 2007, the clinical burden

Table 2. Economic and Clinical Burden of Global AMR.

Year	Author	Country	Methodology	Key findings
2017	Allcock et al.	United Kingdom	Qualitative Review	The study estimated that globally 700,000 deaths are attributed to infections caused by AMR organisms, and this figure may reach 10 million in the next few decades.
2013	Huttner et al.	Switzerland	Systematic Review	The findings of the study established that the economic burden for AMR has risen. In the US alone, yearly societal cost-of-disease for AMR is roughly around $55 billion.
2018	Hay et al.	United Kingdom	Qualitative Commentary	It was established that AMR is a significant global menace to the health of the people, jeopardising the ability to cure and prevent a number of infectious diseases, hence a burden on the healthcare system.
2013	Laxminarayan et al.	United States	Qualitative Review	Healthcare-related infections are increasing in low-middle income countries (LMICs), and it is nine times more prevalent than in the U.S. Both the burden of resistance and the need for antibiotics are increasing in LMICs. Besides, resistance linked with the inability to make interventions, that is, Chemotherapy and transplantation all increase clinical burden and costs.
2008	Maragakis et al.	United States	Qualitative Review	It was observed that AMR increases clinical burden due to increases in length of hospitalisation, morbidity, mortality and healthcare cost. Similarly, in terms of economic burden, patients had an infection due to AMR pathogens, and it cost them around $6000-$30000 as compared to patients with common infection.
2018	Naylor et al.	United Kingdom	Systematic Review	In terms of clinical burden, mortality has a significant impact from AMR resistance, and around $1 billion annually was the excess healthcare system costs due to resistance.
2018	Shrestha et al.	United Kingdom	Quantitative Approach and Co-relative Design	In Thailand, the economic cost of AMR resistance was $ 0.5 billion, whereas, in the US, it was $2.9 billion.
2009	Roberts et al.	United States	Quantitative Methodology	It was found that in the US, the clinical burden from AMR increased due to excess use of invasive procedures, a high number of vulnerable patients, and increased rates of antibiotic use. Further, the duration of hospital stay for AMR infection patient was around 6 to 12 days. Also, the medical cost per patients ranges from $18,588 to $ 29,069.
2019	Tacconelli and Pezzani	Netherlands and UK	Review based	It was found that the clinical burden has increased since 2007, and it is highest among infants. Further, Italy (400 deaths per 100,000 people) and Greece (1600 + attributable deaths) were found to have the highest health burden.

has increased, and it is highest among infants. Major European countries like Greece and Italy recorded the highest health burdens due to AMR. In terms of the U.S, Roberts et al. (2009) assert that the clinical burden from AMR has increased significantly, largely because of invasive procedures and increasing rates of antibiotic use. Hence, it can be concluded based on these studies, AMR is fuelling globally due to factors as mentioned earlier and is exerting serious economic and clinical burden on the healthcare system and the people.

Conclusion

The study aims to assess the factors behind the global spread of AMR and its clinical and economic burden. The study used a systematic review technique to determine the factors and clinical and economic burden of AMR. Several factors behind the global of the spread of AMER were found using data of 14 relevant journal articles such as; delay in proper antimicrobial treatment; hospital-acquired infections, travel and transmission of disease, poorly treated sewage, empirical prescribing, lack of antimicrobial stewardship, poor AMR monitoring etc. From the review of the studies, it was found that there is a significant economic and clinical burden of AMR infections, that is, high costs of medical treatment around $18, 000 to $29,000 per patient and 6 to 12 days of hospital stays. Therefore, we recommend that well organised antimicrobial stewardship be in place, AMR monitoring, limited access to antibiotics over the counter without prescriptions, mandatory sensitivity tests for the

antimicrobial prescription is required to reduce global AMR. It is also further recommended that proper hygiene in hospitals must be ensured to reduce AMR infections, and this can all be done by coordinated actions at a national and global level.

The study has some limitations as it was review-based research and concluded its findings based on already established facts. Therefore, a primary data-based quantitative approach using an experiment or survey strategy would be significant in acquiring fresh data regarding the spread of AMR and its burden on health and economy. For further research, more studies should be carried out. To achieve more comprehensive findings and provide actionable recommendations on the basis of those findings, which can be implemented at the global level.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

REFERENCES

Allcock S, Young EH, Holmes M, Gurdasani D, Dougan G, Sandhu MS, Solomon L, Török ME (2017). Antimicrobial resistance in human populations: challenges and opportunities. Global Health, Epidemiology and Genomics 2.

Aslam B, Wang W, Arshad MI, Khurshid M, Muzammil S, Rasool MH, Nisar MA, Alvi RF, Aslam MA, Qamar MU, Salamat MKF (2018). Antibiotic resistance: a rundown of a global crisis. Infection and Drug Resistance 11:1645.

Bartlett JG, Gilbert DN, Spellberg B (2013). Seven ways to preserve the miracle of antibiotics. Clinical Infectious Diseases 56(10):1445-1450.

Bengtsson-Palme J, Kristiansson E, Larsson DJ (2017). Environmental factors influencing the development and spread of antibiotic resistance. FEMS Microbiology Reviews 42(1):fux053.

Centres for Disease Control and Prevention (2013). Antibiotic / Antimicrobial Resistance (AR/AMR). Retrieved from: https://www.cdc.gov/drugresistance/

ECDC (2015). European food safety authority, the European centre for disease prevention and control: the European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2013. EFSA Journal 13:3991.

Hay SI, Rao PC, Dolecek C, Day NP, Stergachis A, Lopez AD, Murray CJ (2018). Measuring and mapping the global burden of antimicrobial resistance. BMC Medicine 16(1):78.

Huttner A, Harbarth S, Carlet J, Cosgrove S, Goossens H, Holmes A, Jarlier V, Voss A, Pittet D (2013). Antimicrobial resistance: a global view from the 2013 World Healthcare-Associated Infections Forum. Antimicrobial Resistance and Infection Control 2(1):31.

Hwang AY, Gums JG (2016). The emergence and evolution of antimicrobial resistance: Impact on a global scale. Bioorganic and Medicinal Chemistry 24(24):6440-6445.

KPMG L (2014). The Global Economic Impact of Anti-microbial Resistance. KPMG LLP.

Landers TF, Cohen B, Wittum TE, Larson EL (2012). A review of antibiotic use in food animals: perspective, policy, and potential. Public Health Reports 127(1):4-22.

Laxminarayan R, Duse A, Wattal C, Zaidi AK, Wertheim HF, Sumpradit N, Vlieghe E, Hara GL, Gould IM, Goossens H, Greko C (2013). Antibiotic resistance—the need for global solutions. The Lancet Infectious Diseases 13(12):1057-1098.

Maragakis LL, Perencevich EN, Cosgrove SE (2008). Clinical and economic burden of antimicrobial resistance. Expert Review of Anti-Infective Therapy 6(5):751-763.

Naylor NR, Atun R, Zhu N, Kulasabanathan K, Silva S, Chatterjee A, Knight GM, Robotham JV (2018). Estimating the burden of antimicrobial resistance: a systematic literature review. Antimicrobial Resistance and Infection Control 7(1):58.

O'Neill JIM (2014). Antimicrobial resistance: tackling a crisis for the health and wealth of nations. Review on Antimicrobial Resistance 20:1-16.

Ohl CA, Dodds AES (2011). Antimicrobial stewardship programs in community hospitals: the evidence base and case studies. Clinical Infectious Diseases 53(suppl_1):S23-S28.

Parveen S, Lukasik J, Scott TM, Tamplin ML, Portier KM, Sheperd S, Farrah SR (2006). Geographical variation in antibiotic resistance profiles of Escherichia coli isolated from swine, poultry, beef and dairy cattle farm water retention ponds in Florida 1. Journal of Applied Microbiology 100(1): 50-57.

Pruden A, Arabi M, Storteboom HN (2012). Correlation between upstream human activities and riverine antibiotic resistance genes. Environmental Science and Technology 46(21):11541-11549.

Roberts RR, Hota B, Ahmad I, Scott RD, Foster SD, Abbasi F, Schabowski S, Kampe LM, Ciavarella GG, Supino M, Naples J (2009). Hospital and societal costs of antimicrobial-resistant infections in a Chicago teaching hospital: implications for antibiotic stewardship. Clinical Infectious Diseases 49(8):1175-1184.

Rolain JM, Baquero F (2016). The refusal of the society to accept antibiotic toxicity: missing opportunities for therapy of severe infections. Clinical Microbiology and Infection 22(5):423-427.

Sahoo KC (2012). Antibiotic resistance and environmental factors: focusing on the situation in Odisha, India. Inst för folkhälsovetenskap/Dept of Public Health Sciences.

Schwartz KL, Achonu C, Brown KA, Langford B, Daneman N, Johnstone J, Garber G (2018). Regional variability in outpatient antibiotic use in Ontario, Canada: a retrospective cross-sectional study. CMAJ Open 6(4):E445.

Shrestha P, Cooper BS, Coast J, Oppong R, Thuy NDT, Phodha T, Celhay O, Guerin PJ, Wertheim H, Lubell Y (2018). Enumerating the economic cost of antimicrobial resistance per antibiotic consumed to inform the evaluation of interventions affecting their use. Antimicrobial Resistance and Infection Control 7(1):98.

Smith RD, Coast J (2012). The economic burden of antimicrobial resistance: why it is more serious than current studies suggest. Technical Report. London School of Hygiene and Tropical Medicine, London. DOI: https://doi.org/10.17037/PUBS.00639028

Tacconelli E, Pezzani MD (2019). Public health burden of antimicrobial resistance in Europe. The Lancet Infectious Diseases 19(1):4-6.

Tam CC, O'Brien SJ (2016). The economic cost of campylobacter, norovirus and rotavirus disease in the United Kingdom. PloS One 11(2):e0138526.

Taylor J, Hafner M, Yerushalmi E, Smith R, Bellasio J, Vardavas R, Bienkowska-Gibbs T, Rubin J (2014). Estimating the economic costs of antimicrobial resistance: model and results. Santa Monica, CA: RAND Corporation.

Ventola CL (2015). The antibiotic resistance crisis: part 1: causes and threats. Pharmacy and Therapeutics 40(4):277.

World Health Organization (WHO) (2017). What is antimicrobial resistance? Retrieved from https://www.who.int/features/qa/75/en/

Wright GD (2014). Something old, something new: revisiting natural products in antibiotic drug discovery. Canadian Journal of Microbiology 60(3):147-154.

Vol. 15(3), pp. 132-145, March, 2021
DOI: 10.5897/AJMR2020.9401
Article Number: 2BA544F66314
ISSN: 1996-0808

http://www.academicjournals.org/AJMR

African Journal of Microbiology Research

Full Length Research Paper

Antimicrobial activities of microbially-synthesized silver nanoparticles against selected clinical pathogens in Akure, Nigeria

Ekundayo E. A.[1], Adegbenro A.[2], Ekundayo F. O.[2], Onipede H.[2], Bello O. O.[1*] and Anuoluwa I. A.[1]

[1]Department of Biological Sciences, University of Medical Sciences, Ondo, Nigeria.
[2]Department of Microbiology, The Federal University of Technology, Akure, Ondo State, Nigeria.

Received 14 August, 2020; Accepted 27 January, 2021

Silver nanoparticles (AgNPs) have stimulated interest of scientists due to their wide range of applications, including their potential antimicrobial activity. This study investigated the antimicrobial activities of AgNPs synthesized by rhizospheric soil and fish pond sediment microorganisms against selected clinical pathogens. The samples were cultured and organisms identified in accordance with standard procedures. The synthesis of AgNPs colloidal solution was monitored by UV-vis analysis. Presence of bands was determined by the Fourier Transform Infrared spectroscopy (FTIR). The antimicrobial activity of the synthesized AgNPs against selected clinical isolates was determined using agar well diffusion method. Ten species each of bacteria and fungi were isolated from the samples. Formation of AgNPs was indicated by colour transformation from yellow to brown. All synthesized AgNPs showed intense peak with wavelengths ranging of 410-440 nm in UV-vis. The FTIR revealed band at 3395 cm^{-1} and a strong peak at 3300-3500 cm^{-1}. The AgNPs synthesized by some of the isolates exerted remarkable and varying degrees of antimicrobial activities against the susceptible test organisms. This study revealed that the microbially-synthesized AgNPs obtained from this study possess a high antimicrobial potency against most potential pathogens investigated, and, thus, can be exploited in the development of novel antimicrobial agents.

Key words: Antimicrobial agents, microbial resistance, microorganisms, pathogens, silver nanoparticles, zones of inhibition.

INTRODUCTION

Multidrug resistance is a major concern in the treatment of infectious diseases. The wide and indiscriminate use of broad-spectrum antibiotics has led to resistance to traditional antimicrobial agents for many bacterial human pathogens, and this is a major threat to the global health care (Amr-Saeb et al., 2014). Hence, a new research for

*Corresponding author. E-mail: juwonbello@yahoo.com; obello@unimed.edu.ng.

potent antimicrobial agent is necessary. Nanotechnology is a relatively new field applied in research, especially in biotechnology. New applications of nanoparticles and nanomaterials are also increasing rapidly. Biological method of synthesis provides a wide range of environmentally acceptable methodology with low cost of production and minimum time required (Kannan et al., 2010; Al-Khuzai et al., 2019) since the reducing agent and the stabilizer used during chemical synthesis are replaced by molecules obtained from living organisms such as bacteria, fungi, yeasts, algae, or plants (Narayanan and Sakthivel, 2010). The unique properties of AgNPs such as the size, shape, electrical, and magnetic properties, can be incorporated into antimicrobial applications, biosensor materials, composite fibers, cryogenic superconducting materials, cosmetic products, and electronic components, and these have stimulated researchers' interests in their applications in these fields (Vishwanatha et al., 2018; El-Saadony et al., 2019). Several methods have been used for synthesizing and stabilizing AgNPs such as laser ablation, chemical reduction, gamma irradiation, electron irradiation, microwave processing, photochemical and biological synthetic methods, (Ghareib et al., 2016; Vishwanatha et al., 2018; El-Saadony et al., 2019).

However, the synthesis of AgNPs from bacteria cells has gained much attention because bacterial cells possess a special mechanism of resistance to silver ions in the environment. This innate feature is responsible for growth and survival in the environments with metal ion concentrations and their ability to synthesize nanoparticles (Saklani et al., 2012). Efflux systems, alteration of solubility and toxicity which occur via extracellular complex formation or precipitation of metals, biosorption, bioaccumulation, reduction or oxidation, and lack of specific metal transport systems are the mechanisms involved in resistance (Husseiny et al., 2006).

Silver, when compared with other metals, shows stronger toxicity to microorganisms and the advancement in the field of nanotechnology has tremendously assisted researchers to explore novel means of developing more potent antimicrobial drugs. As a result of the fact that silver and its compounds possess high antimicrobial activity, silver nanoparticles (AgNPs) have stimulated interest of scientists owing to their wide range of applications (Qais et al., 2019). The prevalence of infectious diseases is a worldwide challenge and the problem of development antimicrobial resistance continues to become a global health risk. This necessitates the investigation on the biosynthesis of silver nanoparticles from microorganisms from environmental samples with the determination of their antibacterial and antifungal potentials (Keshavamurthy et al., 2017).

It is widely accepted that the presence of nitrate reductase is solely responsible for biosynthesis of AgNPs. Nitrate reductase is responsible for the conversion of nitrate to nitrite (Keshavamurthy et al., 2017). The *in vitro* demonstration of this mechanism was reported by Kalimuthu et al. (2008) using *Bacillus licheniformis*. The bacterium secreted cofactor NADH and NADH-dependent enzymes, especially nitrate reductase which were speculated to be responsible for the bioreduction of silver ion (Ag^{+}) to Ag^{0} and the subsequent formation of AgNPs. Anil-Kumar et al., (2007) confirmed this speculation with the first direct evidence for the involvement of nitrate reductase in the synthesis of AgNPs.

Fungi have the ability of reducing the metals ions into their corresponding nanometals either intracellularly or extracellularly due to their high binding capacity with metal (Al-Khuzai et al., 2019). They were found to produce larger amounts of nanoparticles compared to bacteria because they can secrete larger amounts of proteins which directly translate to higher productivity of nanoparticles (Mohanpuria et al., 2008). Fungi are unique and can be considered as the best producers of nanoparticlees in relation to bacteria because they are easy to culture on solid substrate fermentation and they can grow on the surface of inorganic substrate during culture leading to efficient distribution of metals as catalyst (Ahmad et al., 2003). Extracellular production of nanoparticles from fungi also has an advantage of producing large quantity of enzymes which are in pure state and free from cellular protein, and which are easy to apply for the simple downstream process. Therefore, this study investigated the biosynthesis and antimicrobial potentials of AgNPs obtained from microorganisms associated with rhizospheric soil and fish pond sediments from the Federal University of Technology, Akure, FUTA, Nigeria.

MATERIALS AND METHODS

Collection of samples

Samples of rhizospheric soil of selected plants (guava, banana, mango, and cassava) and fish pond sediment were collected at different areas and varying depth in FUTA. The samples were taken from a depth of 5-10 cm on the farm then kept in plastic bags and transported immediately to the laboratory for analyses.

Collection of test organisms

Five clinical bacterial pathogens -*Pseudomonas aeruginosa*, *Escherichia coli*, *Klebsiella pneumoniae*, *Staphylococcus aureus* and *Enterococcus faecalis*–and five fungal pathogens – *Aspergillus fumigatus*, *Penicillium notatum*, *Rhizopus stolonifer*, *Aspergillus flavus* and *Trichoderma viride* - were collected from Ondo State Specialist Hospital, Akure, Nigeria, and resuscitated on nutrient agar media to obtain young actively growing culture.

Microbiological analysis of samples

Rhizospheric soil and fish pond sediment samples were separately

sieved with 0.5 mm sieve to remove stones and plant debris. Thereafter, 1 g of each sample was serially diluted to obtain a six-fold dilution factor. An aliquot (0.1 ml) of each dilution was aseptically inoculated on appropriate media. The plate count agar, cetrimide agar, mannitol salt agar, eosin methylene blue agar and blood agar media (Oxoid, England) were used for the cultivation of total heterotrophic bacteria, *P. aeruginosa*, *S. aureus*, *E. coli* and *Bacillus* species, respectively using the pour plate technique. This method was also adopted for cultivation of fungi on potato dextrose agar medium. All bacterial culture plates were incubated at 32°C for 24 h while fungal were incubated at 28°C for 72 h. Pure cultures were obtained by repeated subcultures on fresh media using the streak plate technique (Harrigan and McCane, 1976; Bello et al., 2012).

Identification of microorganisms

Primary identification was done by observation of cultural characteristics of pure isolates while characterization procedures were carried out as described by Cowan and Steel (1985) and Holt et al. (2004). Gram's staining reactions and cell morphology from heat-fixed smears were observed. The motility of isolates was determined by the hanging drop technique, and biochemical tests were carried out on the isolates in accordance with standard procedures. Fungal isolates were identified as described by Beneke and Rogers (2005). The Analytical Profile Index (API) 20E and API 20NE were used for the additional identification of the families Enterobacteriaceae and non-Enterobacteriaceae, respectively. API 20C was used for further identification of fungal isolates. These were done in accordance with the manufacturers' protocols (BioMerieux, Marcy l'Etoile, France).

Synthesis and characterization of AgNPs from microbial isolates

The bacteria isolated from the rhizospheric soil were cultured on both nutrient broth as well as Luria Bertani broth to produce biomass for biosynthesis. The pH was adjusted to 7.0. Incubation was done on an orbital shaker at 27°C at 220 rpm. The biomass of each isolate was harvested after 24 h and centrifuged at 12000 rpm for 10 min. The supernatant was collected and used for extracellular synthesis of AgNPs. The supernatant was added separately to the reaction vessel containing 1 mM of silver nitrate ($AgNO_3$) while the control was set up without the silver nitrate for 24 h in the dark. The reduction of the Ag^+ ions in the solution was monitored by observing changes of the colour. The absorbance was measured at a resolution of 1 nm using UV–visible spectrophotometer with samples in quartz cuvette (Kannan et al., 2010; Vanmathi and Sivakumar, 2012).

The chemical compounds of the synthesized AgNPs were studied by using Fourier Transformed Infra-Red Spectrophotometer (FTIR) (Perkin-Elmer LS-55-Luminescence spectrometer). The solutions were dried at 75°C and the dried powders were characterized to identify possible interactions between the Ag salts and protein molecules which could account for the reduction of silver ions and the stabilization of AgNPs (Vanmathi and Sivakumar, 2012).

Transmission electron microscopy (TEM) analysis of AgNPs

In order to obtain quantitative measures of AgNPs, their size distribution and morphology, analysis of the samples was performed using TEM technique. The size and shape distributions of produced AgNPs were characterized by adding a few drops of AgNPs solution onto a TEM grid, and the residue was removed by a filter paper beneath the TEM grid (Mohammadi et al., 2019). The magnification of TEM was determined by the ratio of the distance between the objective lens and the specimen, and the distance between objective lens and its image plane.

Antimicrobial activities of the microbially-synthesized AgNPs against clinical isolates

Antimicrobial activities of AgNPs synthesized by microorganisms from the rhizospheric soil and fish pond sediments were tested against *P. aeruginosa*, *E. coli*, *K. pneumoniae*, *S. aureus*, *E. faecalis*, *A. fumigatus*, *P. notatum*, *R. stolonifer*, *A. flavus* and *T. viride* using the agar well diffusion assay method as described by Ozcelika et al. (2006). A 20 µl portion of the nanoparticles solution was taken and introduced on Mueller Hinton agar plate incubated at 37°C for 24 h. Ciprofloxacin and ketoconazole were used as positive control for bacteria and fungi, respectively. Zones of inhibition exerted by the microbially-nanoparticles were measured in millimeters with the aid of a metre rule.

RESULTS

Identification of microorganisms isolated from rhizospheric soil and fish pond sediment

The ten bacterial species isolated from the rhizospheres and fish pond sediments were characterized as *S. aureus*, *S. epidermidis*, *P. vulgaris*, *E. coli*, *Micrococcus luteus*, *P. aeruginosa*, *Bacillus subtilis*, *B. cereus*, *Serratia marcescens* and *Streptomyces griseus* (Appendix Table 1). Ten fungal species were also identified and these include *Penicillium frequentans*, *Saccharomyces cerevisiae*, *Aspergillus niger*, *A. flavus*, *Fusarium oxysporum*, *R. stolonifer*, *Mucor mucedo*, *Emericella rugulosa*, *T. viride* and *Geotrichum albidum* (Appendix Table 2).

Distributions of microorganisms in the rhizospheric soil and fish pond sediment

S. aureus was isolated from the fish pond sediment sample and rhizospheric soils of guava and banana plants. *B. cereus* and *E. coli* were encountered in the fish pond sediment and rhizospheric soil samples of guava, banana, mango and cassava plants investigated in this study. *P. vulgaris* was present in the rhizospheric soils of guava, banana and mango. *S. marcescens* was encountered only in the rhizospheric soil of mango and the fish pond sediment while *B. subtilis* was found in the fish pond sediment and the rhizospheric soil of banana only. *S. cerevisiae*, *A. niger* and *F. oxysporum* were present in the fish pond sediment. *P. freqentens*, *A. niger* and *A. flavus*were found in the rhizospheric soils of guava and mango plants in addition to *R. stolonifer* encountered in only mango plants. Only *P. freqentens* and *T. viride* were encountered in the soil associated with banana plant while *P. freqentens*, *A. niger*, *M. mucedo*, *E.*

Table 1. Distributions of microorganisms in rhizospheric soil and fish pond sediment in FUTA, Nigeria.

Group	Organisms	Rhizosphere samples				
		Fish pond sediment	Guava	Banana	Mango	Cassava
Bacteria	*Staphylococcus aureus*	+	+	+	-	-
	Proteus vulgaris	-	+	+	+	-
	Serratiamarcescens	+	-	-	+	-
	Bacillus cereus	+	+	+	+	+
	Escherichia coli	+	+	+	+	+
	Micrococcus luteus	+	-	+	+	-
	Pseudomonas aeruginosa	-	+	+	-	+
	Bacillus subtilis	+	-	+	-	-
	Staphylococcus epidermidis	+	+	-	-	+
	Streptomyces griseus	-	-	-	-	+
Fungi	*Penicilliumfrequentans*	-	+	+	+	+
	Saccharomyces cerevisiae	+	-	-	-	-
	Aspergillusniger	+	+	-	+	+
	Aspergillusflavus	-	+	-	+	-
	Fusariumoxysporum	+	-	-	-	-
	Rhizopusstolonifer	-	-	-	+	-
	Mucormucedo	-	-	-	-	+
	Emericellarugulosa	-	-	-	-	+
	Trichodermaviride	-	-	+	-	-
	Geotrichumalbidum	-	-	-	-	+

+ = Present, - = Absent

rugulosa and *G. albidum* were present in rhizospheric soil of cassava plants (Table 1).

Colour change and UV-vis analysis

The appearance of a yellowish-brown colour in the silver nitrate treated flask indicated the formation of AgNPs (Plates 1 and 2). Among the bacterial species isolated in this study, *B. subtilis, E. coli, M. luteus, P. aeruginosa* and *S. aureus* (Figure 1) were found to synthesize AgNPs with intense peak in wavelength ranging from 416-428 nm while the fungi, which included *Aspergillus niger, S. cerevisiae* and *Fusarium oxysporum* (Figure 2), showed broad and strong spectra ranging from 417-437.5 nm.

FTIR analysis of silver nanoparticles

The FTIR analysis was used to identify possible interactions between the Ag salts and protein molecules in the reduction of silver ions and the stabilization of AgNPs. The bacterial and fungal isolates revealed the existence of spectral band at 3395 cm^{-1} in the FTIR spectrograms and a strong peak at 3300-3500 cm^{-1}. The peak at 2185 cm^{-1} was also significant to most of the isolates. Peaks were also observed at 1675 cm^{-1} in all the isolates as shown in Figure 3A to E for bacterial species and Figure 4A to C for fungal species.

Transmission electron microscopy (TEM) analysis

To gain further insight into the features of the AgNPs, analysis of the sample was performed using TEM technique. The shape and size of the obtained AgNPs were elucidated with the aid of TEM images. Nanoparticles observed from the micrographs were majorly spherical with a small percentage of elongated particles ranging in size from 5 to 30 nm with an average size of 20 nm (Figure 5).

Antimicrobial potency of AgNPs synthesized by the microbial species against selected clinical organisms

The *B. subtilis*-synthesized AgNPs exerted the highest potency against the test organisms as compared with other AgNPs synthesized by other Gram-positive isolates in the study. *B. subtilis*-synthesized AgNPs showed antibacterial activities against *S. aureus, E. coli*, and *E.*

Table 2. Antibacterial activities of AgNPs synthesized by bacterial species against test pathogens.

Group	Bacterial AgNPs/ Test bacteria	Zone of inhibition (mm)				
		P. aeruginosa	***E. coli***	***S. aureus***	***E. faecalis***	***K. pneumoniae***
Gram-positive	*S. aureus*	5.67±0.67^{b}	11.00±1.00^{c}	10.33±0.33^{c}	12.17±0.17^{c}	0.00±0.00^{a}
	M. luteus	0.00±0.00^{a}	8.33±0.33^{b}	0.00±0.00^{a}	11.33±0.88^{c}	10.67±0.67^{c}
	B. subtilis	0.00±0.00^{a}	7.00±0.00^{c}	5.33±1.42^{b}	14.33±0.67^{d}	0.00±0.00^{a}
Gram-Negative	*E. coli*	0.00±0.00^{a}	10.67±0.33^{c}	12.67±0.67^{d}	10.33±0.33^{c}	6.33±0.67^{b}
	P. aeruginosa	8.33±0.33^{b}	10.33±0.33^{c}	10.67±0.33^{c}	4.67±0.33^{a}	10.33±0.33^{c}
	Control	5.33±0.33^{a}	6.00±0.58^{b}	11.33±0.67^{d}	10.67±0.33^{c}	5.67±0.67^{a}

Data are presented as Mean±S.E (n=3). Values with the same alphabetic superscript along same column are not significantly different (P<0.05)

Plate 1. Discoloration of supernatants of bacterial cultures due to $AgNO_3$ reduction. A= *Bacillus*-synthesiszed AgNPs; B = *E. coli*-synthesized AgNPs; C = *Micrococcus*-synthesized AgNPs; D = *Staphylococcus aureus*-synthesized AgNPs; E = *Pseudomonas aeruginosa* synthesized-AgNPs and F = Control.

Plate 2. Discolouration of supernatants of fungal cultures due to $AgNO_3$ reduction. A = *Aspergillus niger*-synthesized AgNPs; B = *Saccharomyces cerevisiae*-synthesized AgNPs; C = *Fusarium oxysporum*-synthesized AgNPs and D = Control.

faecalis with zones of inhibition of 5.33, 7.00 and 14.33 mm, respectively. The antimicrobial activity of AgNPs synthesized by *B. subtilis* against *E. faecalis* was even higher than that exerted by ciprofloxacin (the control) with zone of inhibition of 10.67 mm (Table 2).

E. coli-synthesized AgNPs exhibited antibacterial activity against *K. pneumoniae, E. faecalis, E. coli and S. aureus* with zones of inhibition of 6.33, 10.33, 10.67 and 12.67 mm, respectively. There was no significant difference between this antibacterial effect on *E. coli* and *E. faecalis* (P > 0.05). *P. aeruginosa*-synthesized AgNPs also exerted varying degrees of antibacterial activity against all the test pathogens with zones of inhibition ranging from 4.67 to 10.67 mm. The activities on *E. coli*, *K. pneumoniae* and *S. aureus* showed no statistical difference (P > 0.05) (Table 2).

The antifungal activity of AgNPs synthesized by three of the fungal isolates against selected fungal pathogens is shown in Table 3. The *A. niger*-synthesized AgNPs exerted antifungal effect on *R. stolonifer*, *P. notatum*, *A. flavus* and *T. viride* with inhibition zones of 2.33, 5.33, 10.67 and 10.67 mm, respectively. There was no statistical difference between the antifungal activity exerted against *A. flavus* and *T. viride* (P > 0.05) while that of *P. notatum* and *R. stolonifer* showed significant difference (P < 0.05). The AgNPs synthesized by *S.*

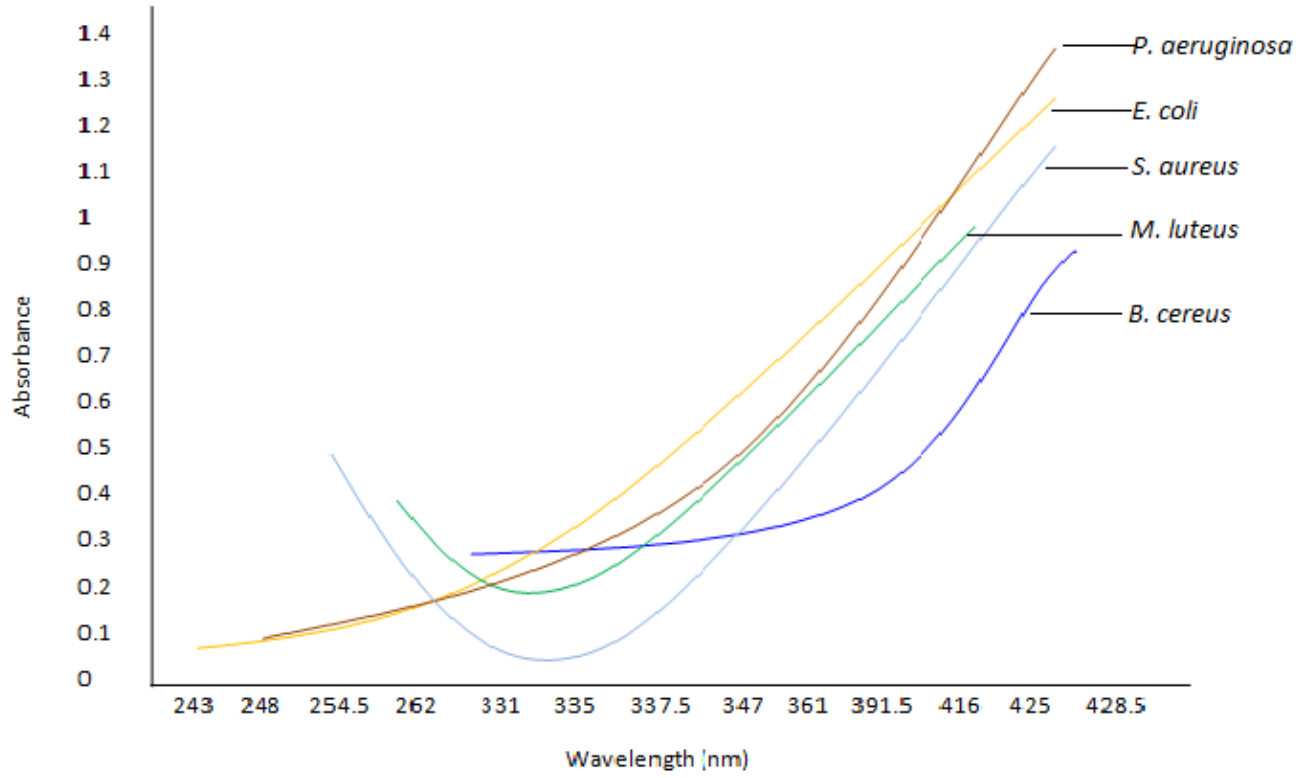

Figure 1. UV–vis spectra of biosynthesized silver nanoparticles by bacterial isolates.

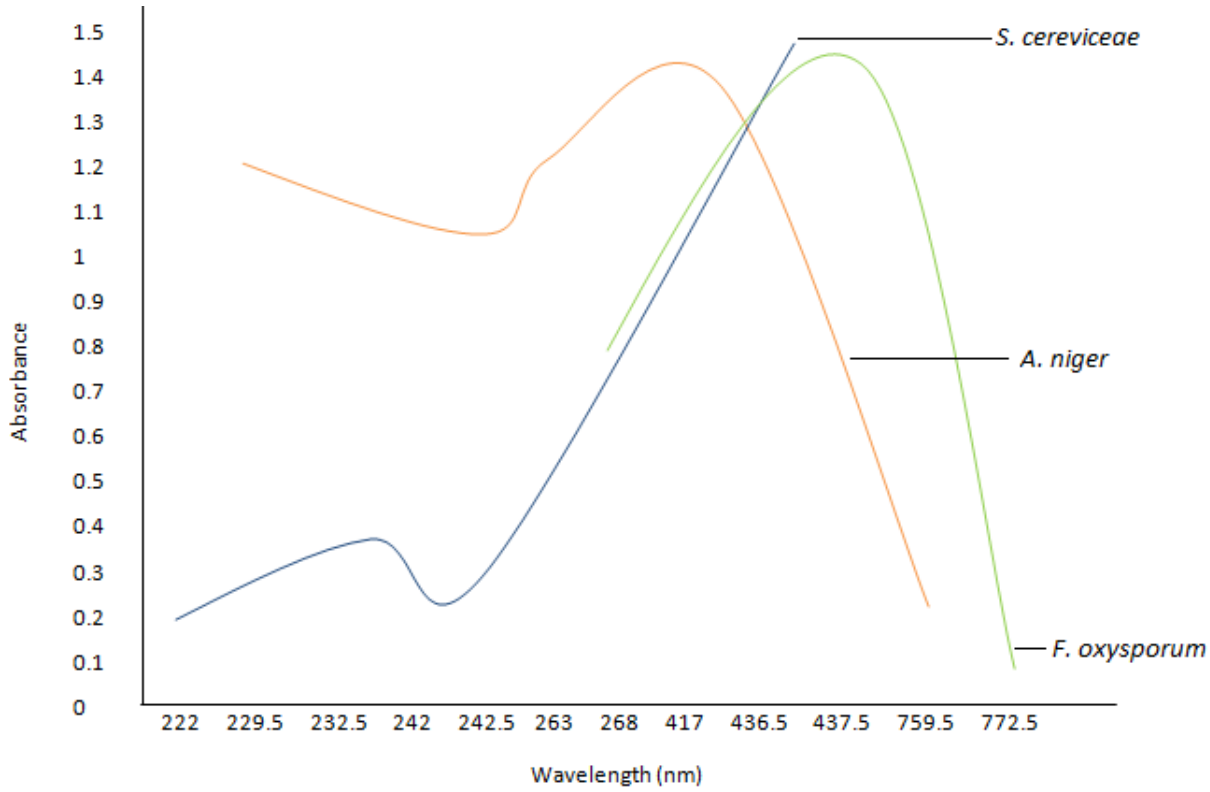

Figure 2. UV–vis spectra of the biosynthesized silver nanoparticles by fungal isolates.

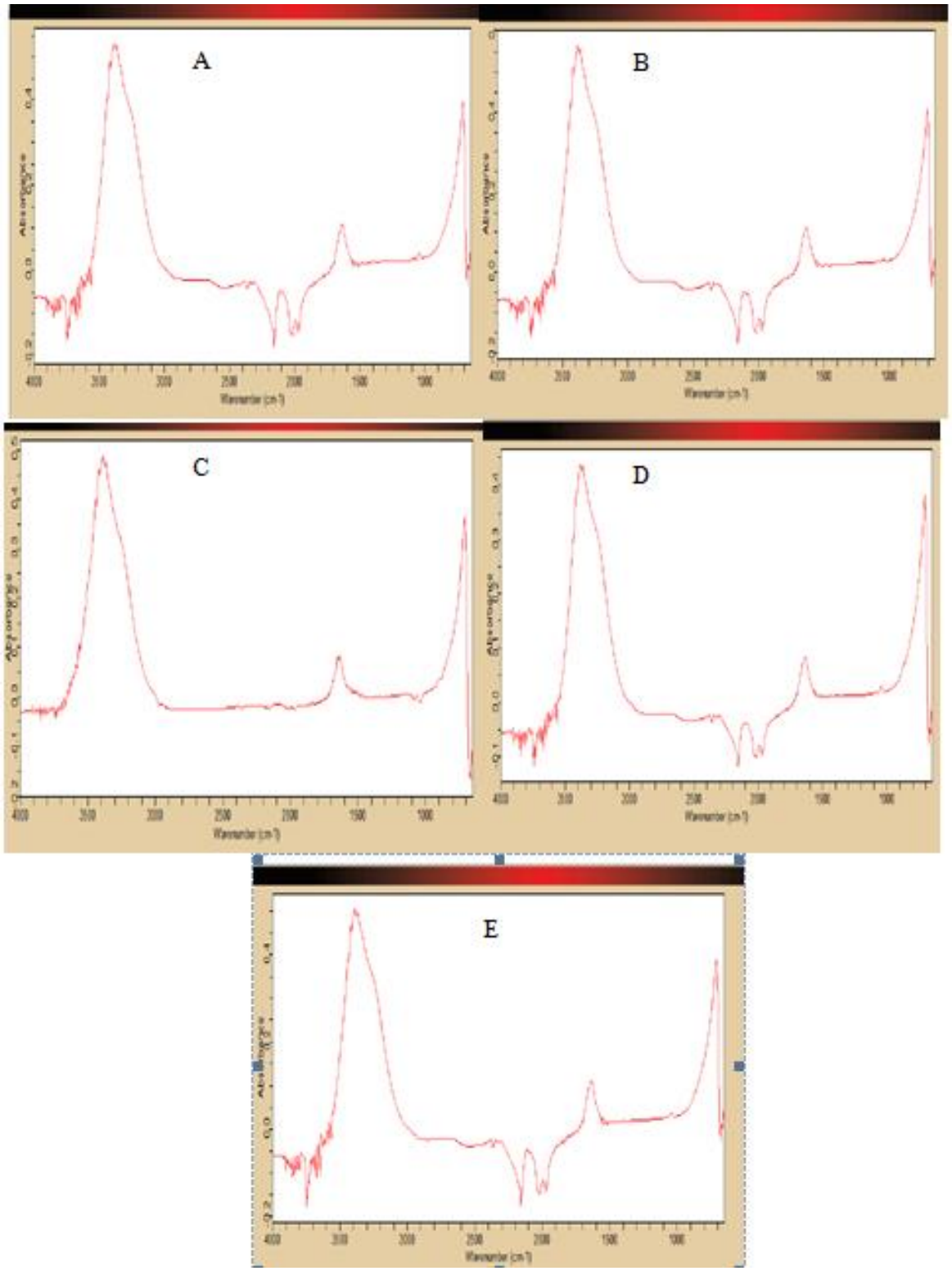

Figure 3(A – E). FTIR spectra of AgNPs of bacterial isolates: A = *B. subtilis*; B = *E. coli*; C = *M. luteus*; D = *P. aeruginosa* and E = *S. aureus.*

cerevisiae exerted the highest antimicrobial potency against *A. fumigatus* and *P. notatum*as compared with other fungal species in that category, and even the control antibiotic (ketoconazole).

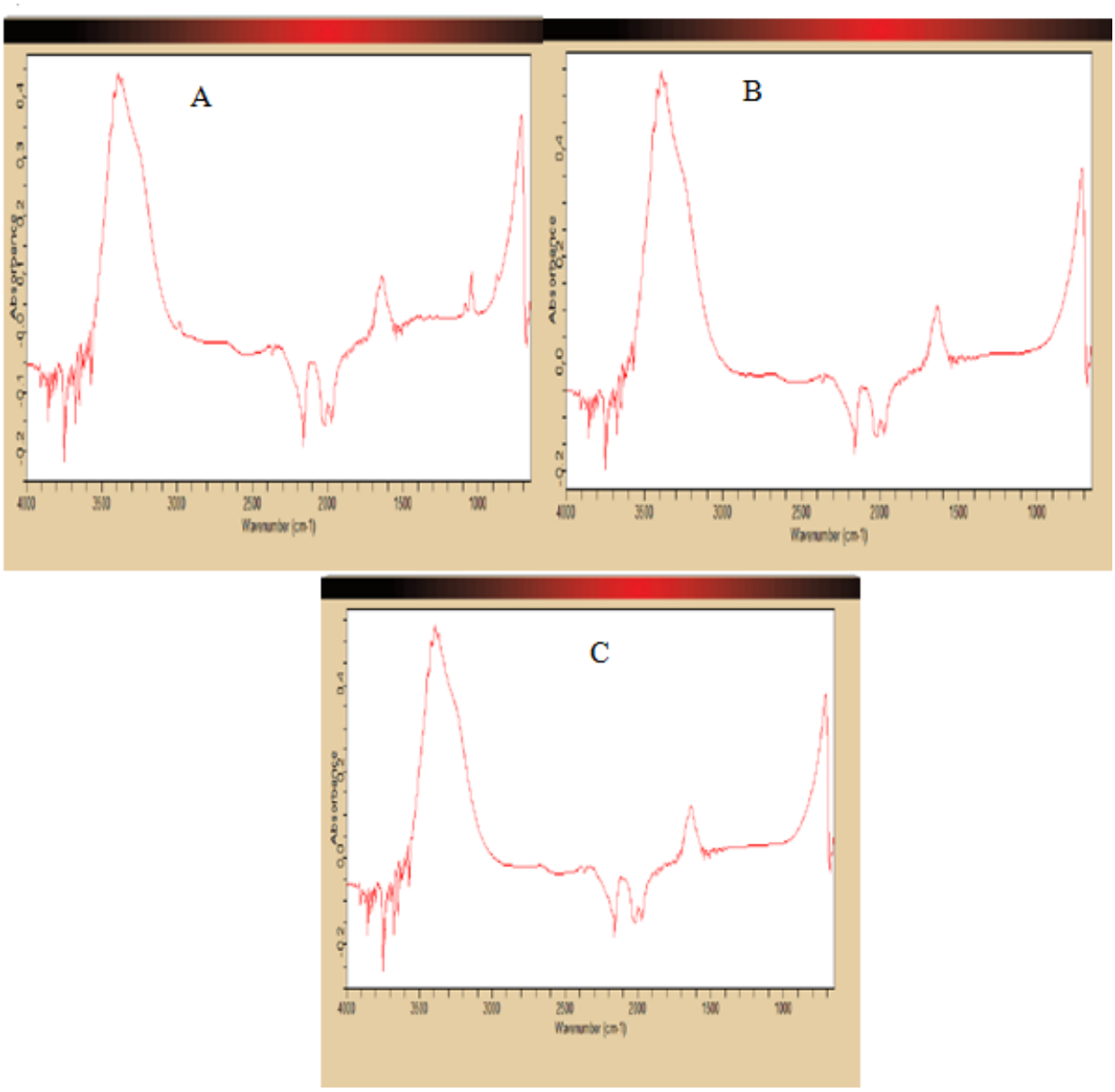

Figure 4(A–C). FTIR spectra of AgNPs by fungal isolates: A = *A. niger*; B = *S. cerevisiae* and C = *F. oxysporum.*

DISCUSSION

The distribution of microorganisms encountered in this study varied and each sample contributed to the microbial diversity investigated. Wolfe and Kilironomas (2005) reported that the quality of root exudates can promote differential recruitment of microorganisms present in the soil. The presence of nitrogen-fixing bacteria in the rhizosphere of plants can also improve plant growth in nitrogen-poor environments, as well as promote increased nitrogen content in the soil, which is often related to the facilitative effect that legume species have on other plant species (Walker, 2003).

The colour transformation from yellow to brownish indicates the formation of AgNPs (Singh et al., 2011). The intensity of the brown colour increased dramatically up to 24 h and this may be as a result of the excitation of surface plasmon resonance (SPR) and the reduction of $AgNO_3$ (Manivasagan et al., 2013; Deljou and Goudarzi, 2016). AgNPs mostly had an absorption peak at 425 nm attributed to their SPR probably due to the stimulation of longitudinal plasmon vibrations (Fig. 1) and which is in line with the report of Kumar and Mamidyala (2012). The SPR property is also responsible for the colour change of the reaction mixture from yellowish to brown (Chaudhari et al., 2012; Yamal et al., 2013). Therefore, the increase

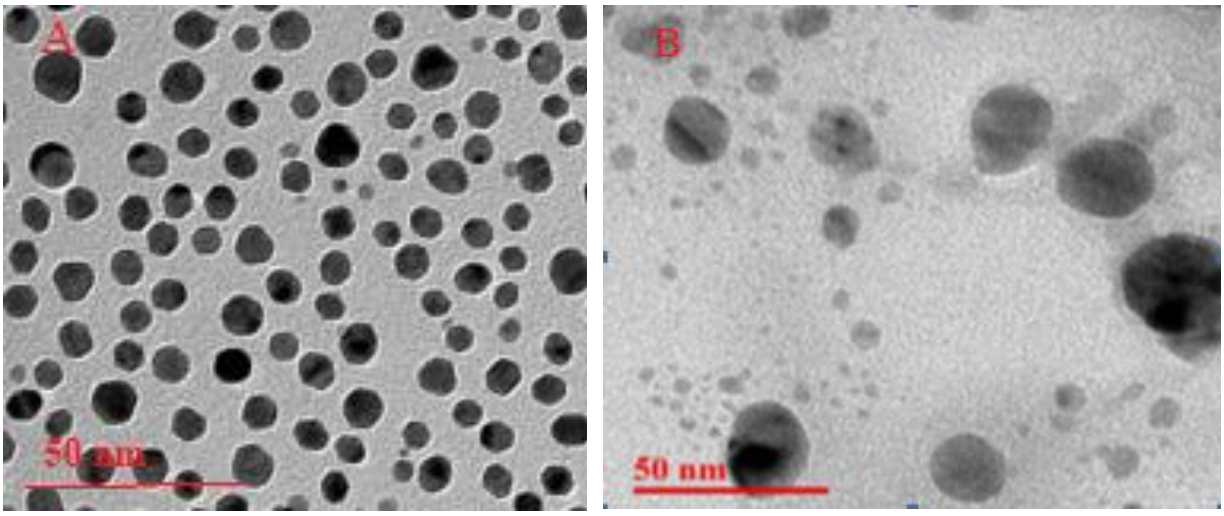

Figure 5. Representative TEM images of produced AgNPs at 50 nm range for A: bacterial species and B: fungal species.

Table 3. Antifungal activities of AgNPs synthesized by fungal isolates.

Fungal AgNPs/ Fungal pathogens	Zone of inhibition (mm)			Control (Ketoconazole)
	A. niger	*F. oxysporum*	*S. cerevisiae*	
A. fumigatus	0.00±0.00^{a}	5.67±0.33^{c}	10.67±0.67^{c}	4.33±0.67^{c}
P. notatum	5.33±0.33^{c}	10.67±0.33^{d}	11.33±0.67^{c}	2.33±0.33^{b}
R. stolonifer	2.33±0.33^{b}	0.00±0.00^{a}	2.67±0.33^{b}	10.33±0.33^{d}
A. flavus	10.67±0.33^{d}	11.00±1.00^{d}	0.00±0.00^{a}	14.33±0.33^{e}
T. viride	10.67±0.67^{d}	2.67±0.33^{b}	0.00±0.00^{a}	0.00±0.00^{a}

Data are presented as Mean±S.E (n=3). Values with the same alphabetic superscript along same column are not significantly different (P<0.05).

of the absorbance at 347 nm is a reliable criterion indicating AgNPs synthesis (Thu et al., 2013).

The FTIR analysis is used for the detection of potential interactions between silver salt that are involved in AgNPs formation (Saha et al., 2010), while it has been suggested that this analysis provides information on the binding of proteins to AgNPs which leads to nanoparticles stabilization (Jain et al., 2011). For these reasons, FTIR has been routinely used by several researchers in nanoparticles characterization (Deljou and Goudarzi, 2016). Similar FTIR spectra were obtained by microbial isolates. They all indicated the existence of proteins in the capping agent of the nanoparticles, and also that the secondary structure of proteins was not affected as a consequence of the reaction with silver ions or the binding to AgNPs. This assertion is supported by the band at 3395 cm^{-1} in the FTIR spectrogram, which is specific to the frequency of extending vibration of primary amines, and the strong band peak at 3300–3500 cm^{-1}, which characterizes the stretching vibrations of N-H, indicating strong hydrogen bonding. This is in accordance with the findings of Saha et al. (2010), who reported 3300-3500 cm^{-1} as strong band peak.

The appearance of a band at about 2185 cm^{-1}, which is assigned to C=O extend vibrations of carboxylic acids, aldehydes and ketones, was remarkable, indicating that the oxidation of the hydroxyl groups of hydrolysates, which is known to originate from the medium peptides, are associated with the reduction of silver ions. The bands observed at 1675 cm^{-1} is a definite indicator of linkages between the amides I and II (Sharma et al., 2012). It is well known that proteins can bind to the AgNPs either through free amine groups or cysteine residues in the proteins (Gole et al., 2001). FTIR spectroscopy thus revealed the possible stabilization of AgNPs with proteins (Venkatesan et al., 2013).

Presence of spectral peaks at 3300 - 3500 cm^{-1} may be attributed to aliphatic C-H stretching vibration of hydrocarbon chains and N-H bending vibration (Cheng et al., 2014). The spectral vibration of aldehydic group (C=O) was shown by wave length 3395 cm^{-1} (Hamouda et al., 2019) while the peak at the range 1675 cm^{-1} could

be attributed to amides (N-H) stretching in addition to peptide bond and C=C stretching involved in stabilizing nanoparticles by proteins as explained by Castro et al. (2013). A spectral band at 3395 cm^{-1} could be designated to the residual amount of $AgNO_3$ (Šeděnková et al., 2009). The presence of absorption bands at 1675 cm^{-1} may also be attributed to vibration of the -C-O group (Hamouda et al., 2019). Spectral peaks at 242.5-417, 242-436.5 and 437.5 cm^{-1} indicated the bending region of the aliphatic chain. Moreover, bands assigned at 3395 cm^{-1} could be attributed to either phosphorus or sulfur functional groups, which possibly attach silver and perform both capping and stabilizing process of nanoparticles (Castro et al., 2013). It had been reported that biologically-synthesized AgNPs are promising therapeutic agents with significant antimicrobial activities (Galdiero et al., 2011; Mohammadi et al., 2019), and a number of these biosynthesized nanoparticles had been described and characterized based on their ability to inhibit microbes (Deljou and Goudarzi, 2016). Vishwanatha et al. (2018) also reported that the biogenic and eco-friendly route for synthesizing AgNPs with antibacterial activity against clinically important pathogens and attributes growing interest on fungi as an emerging source for the synthesis of NPs.

The antibacterial activity of AgNPs synthesized by Gram-positive bacteria revealed that AgNPs from *B. subtilis* showed inhibition on *Enterococcus faecalis*, *Escherichia coli*, and *Staphylococcus aureus* respectively. The result of this research is in accordance with the findings of Deljou and Goudarzi (2015) who reported that AgNPs synthesized by *Bacillus* spp. showed significant inhibition on some selected human pathogens. The AgNPs synthesized from *S. aureus* showed significant inhibition on all the selected isolates except *K. pneumoniae*. The AgNPs synthesized by *M. luteus* showed significant inhibition on *E. faecalis*, and *K. pneumoniae*. This research revealed that only *S. aureus*-synthesized AgNPs showed inhibition on *Pseudomonas aeruginosa*.

Antibacterial activity of Gram-negative-bacteria-synthesized AgNPs revealed that *P. aeruginosa*-synthesized AgNPs showed inhibition on all the selected isolates while *E. coli*-synthesized AgNPs showed inhibition on all the selected isolates except *P. aeruginosa.* The efficacy of AgNPs can be attributed to the fact that their larger surface area gives them a better contact with the microorganisms. This is further supported by the revelation that size dependent interaction of AgNPs with bacteria leads to its antibacterial activity (Pal et al., 2007). The nanoparticles get attached to the cell membrane and also penetrate inside the bacterial cells (Venkatesan et al., 2013). The bacterial membrane is known for its sulphur containing proteins, these might be the preferential sites for the AgNPs to penetrate (Venkatesan et al., 2013). The *A. niger*-synthesized AgNPs exerted antifungal effect on *R. stolonifer*, *P. notatum*, *A. flavus* and *T. viride* while it had activity against *A. fumigatus.* The *F. oxysporum*-synthesized AgNPs exhibited antifungal activities against *T. viride*, A*. fumigatus*, *P. notatum* and *A. flavus* but no activity was exerted against *R. stolonifer.* The *S. cerevisiae*-synthesized AgNPs exhibited activity against *R. stolonifer*, *A. fumigatus* and *P. notatum* while no activity was shown against *A. flavus* and *T. viride.*

The mode of action of AgNPs is triggered by the generation of reactive oxygen species (ROS) inside both bacterial and fungal cells. Feng et al. (2008) reported that nanoparticles are capable of releasing silver ions, while Matsumura et al. (2003) added that these ions can interact with the thiol groups of many vital enzymes and inactivate them. Silver ion is taken in by microbial cells that come in contact with silver which leads to inhibition of several functions in the cell, and thus, damage the cells. The inhibition of a respiratory enzyme by silver ion leads to the generation of reactive oxygen species, which attacks the cell itself (Prabhu and Poulose, 2012).

On the other hand, the toxicity of silver ions, could be by their adhesion to the cell membrane and further penetration inside or by interaction with phosphorus containing compounds like DNA disturbing the replication process or preferably by their attack on the respiratory chain. It has also been suggested that a strong reaction takes place between the silver ions and thiol groups of vital enzymes thus inactivating them (Sunkar and Nachiyar, 2012; Venkatesan et al., 2013). Also, the changes in morphology of bacterial membrane as well as the possible damage caused by the nanoparticles reacting with the DNA will affect the bacteria in cell processes such as the respiratory chain and cell division, finally causing cell death (Sondi and Salopek-Sondi, 2004). Furthermore, nanoparticles might also release silver ions in the bacterial cells, which further enhance their bactericidal activity (Morones et al., 2005).

The shapes and sizes of the obtained AgNPs produced by the bacterial and fungal species were projected by TEM images. The structures of the AgNPs produced by the species of bacteria were identical, which could be attributed to a similarity in the reductive agents present in the species (Jyoti et al., 2016). The AgNPs produced by the bacterial species were well-dispersed without any visible agglomeration or morphological variations. The observations in relation to shapes, sizes and dispersion were same for the AgNPs produced by fungal species. However, the fungi-mediated biosynthesized AgNPs were better dispersed than those of the bacterial species. These reports are in agreement with Singh et al., (2017) and Abdel-Raouf et al. (2018).

Conclusion

This study revealed that microbially-synthesized AgNPs possess a high antimicrobial potency against most

pathogens, and, thus, can be exploited in the development of novel antimicrobial agent.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

REFERENCES

Abdel-Raouf N, Al-Enazi NM, Ibraheem IBM, Alharbi RM, Alkhulaif MM (2018). Biosynthesis of silver nanoparticles by using of the marine brown alga *Padina pavonia* and their characterization. Saudi Journal of Biological Sciences 26(6):1207-1215.

Ahmad A, Mukherjee P, Senapati S, Mandal D, Khan MI, Kumar R, Sastry M (2003). Extracellular biosynthesis of silver nanoparticles using the fungus *Fusarium oxysporum*. Colloids and Surfaces B: Biointerfaces 28:313-318.

Al-Khuzai RAH, Aboud MK, Alwan SK (2019). Biological Synthesis of Silver Nanoparticles from *Saprolegnia parasitica.* Journal of Physics: Conference 1294(6):1-15.

Amr-Saeb TM, Alshammari AS, Al-Brahim H, Al-Rubeaan KA (2014). Production of Silver Nanoparticles with Strong and Stable Antimicrobial Activity against Highly Pathogenic and Multidrug Resistant Bacteria. The Scientific World Journal https://doi.org/10.1155/2014/704708.

Anil-Kumar S, Abyaneh MK, GosaviSulabha SW, Ahmad A, Khan MI (2007). Nitrate reductase mediated synthesis of silver nanoparticles from $AgNO_3$. Biotechnology Letters 29:439-445.

Bello OO, Mabekoje OO, Efuntoye MO, Bello TK (2012). Prevalence of vaginal pathogens associated with genital tract infections in Ogun State, Nigeria. British Microbiology Research Journal 2(4):277-289.

Beneke ES, Rogers AL (2005). Medical mycology manual. 3rd ed. Minneapolis: Burgers Publishing P 226.

Castro L, Blázquez ML, Muñoz JA, González F, Ballester A (2013). Biological synthesis of metallic nanoparticles using algae. IET Nanobiotechnology 7(3):109-116.

Chaudhari PR, Shalaka AM, Vrishali BS, Suresh PK (2012). Antimicrobial activity of extracellular synthesized silver nanoparticles using *Lactobacillus* species obtained from VIZYLAC capsule. Journal of Applied Pharmaceutical Science 2(3):25-29.

Cheng KM, Hung YW, Chen CC, Liu CC, Young JJ (2014). Green synthesis of chondroitin sulfate-capped silver nanoparticles: Characterization and surface modifcation. Carbohydrate Polymers 110:195-202.

Cowan ST, Steel KJ (1985). Manual for the identification of bacteria. Cambridge University Press, Verlage, New York, P. 502.

Deljou A, Goudarzi S (2016). Green extracellular synthesis of the silver nanoparticles using thermophilic *Bacillus* sp. AZ1 and its antimicrobial activity against several human pathogenic bacteria. Iranian Journal of Biotechnology 14(2):25-32.

El-Saadony MT, El-Wafai NA, El-Fattah HIA, Mahgoub SA (2019). Biosynthesis, optimization and characterization of silver nanoparticles using a soil isolate of *Bacillus pseudomycoides* MT32 and their antifungal activity against some pathogenic fungi. Advances in Animal and Veterinary Sciences 7(4):238-249.

Feng QL, Wu J, Chen GQ, Cui FZ, Kim TN, Kim JO (2008). A mechanistic of the antibacterial effect of silver ions on *E. coli* and *S. aureus*. Journal of Biomedical Materials Research 52(4):662-668.

Galdiero S, Falanga A, Vitiello M, Cantisani M, Marra V, Galdiero M (2011). Silver nanoparticles as potential antiviral agents. Molecules 16(10):8894-8918.

Ghareib M, Tahon MA, Saif MM, El-Sayed AW (2016). Rapid Extracellular Biosynthesis of Silver Nanoparticles by *Cunning hamellaphaeospora* Culture Supernatant. Iranian Journal of Pharmaceutical Research 15(4):915-924.

Gole A, Dash C, Ramakrishnan V, Sainkar V, Mandale AB, Rao M (2001). Pepsin-Gold Colloid Conjugates: Preparation, Characterization, and Enzymatic Activity. Langmuir 17(5):1674-1679.

Hamouda RA, Hussein MH, Abo-elmagd RA, Bawazir SS (2019). Synthesis and biological characterization of silver nanoparticles derived from the cyanobacterium *Oscillatoria limnetica.* Scientific Report 9:13701.

Harrigan MG, McCane ME (1976). Laboratory methods in food and dairy microbiology. London: Academic Press pp. 33-200.

Holt GH, Krieg NR, Sneath PH, Staley JT, Williams ST (2004). Bergey's manual of determinative bacteriology. 9th ed. Baltimore: Williams and Wilkins, 787 p.

Husseiny M, Aziz MAE, Badr Y, Mahmoud MA (2006). Biosynthesis of goldnanoparticles using *Pseudomonas aeruginosa.* Spectrochimica Acta Part A: Molecular Spectroscopy 67(3-4):1003-1006.

Jain N, Bhargava A, Majumdar S, Tarafdar JC, Panwar J (2011). Extracellular biosynthesis and characterization of silver nanoparticles using *Aspergillus flavus* NJP08: a mechanism perspective. Nanoscale 3(2):635-41.

Jyoti K, Baunthiyal M, Singh A (2016). Characterization of silver nanoparticles synthesized using *Urtica dioica* Linn. Leaves and their synergistic effects with antibiotics. Journal of Radiation Research and Applied Sciences 9(3):217-227.

Kalimuthu K, Babu RS, Venkataraman D, Bilal M, Gurunathan S (2008). Biosynthesis of silver nanocrystals by *Bacillus licheniformis*. Colloids and Surfaces B: Biointerfaces 65(1):150-153.

Kannan R, Berger C, Myneni S, Technau GM, Shashidhara LS (2010). Abdominal-A mediated repression of Cyclin E expression during cell-fate specification in the Drosophila central nervous system. Mechanisms of Development 127(1-2):137-145.

Keshavamurthy M, Srinath BS, Rai VR (2017). Phytochemicals mediated green synthesis of gold nanoparticles using *Pterocarpussantalinus L.* (Red Sanders) bark extract and their antimicrobial properties. Particulate Science and Technology 36(7):785-790.

Kumar CG, Mamidyala SK (2012). Extracellular synthesis of silver nanoparticles using culture supernatant of *Pseudomonas aeruginosa*. Colloids and Surfaces B: Biointerfaces 84(2):462-466.

Manivasagan P, Venkatesan J, Senthilkumar K, Sivakumar K, Se-Kwon, K (2013). Biosynthesis, Antimicrobial and Cytotoxic Effect of SilverNanoparticles Using a Novel *Nocardiopsis* sp. MBRC-1. BioMed Research International 1:1-9. DOI: 10.1155/2013/287638

Matsumura Y, Yoshikata K, Kunisaki S, Tsuchido T (2003). Mode of bactericidal action of silver zeolite and its comparison with that of silver nitrate. Applied and Environmental Microbiology 69(7):4278-4281.

Mohammadi F, Yousefi M, Ghahremanzadeh R (2019). Green Synthesis, Characterization and Antimicrobial Activity of Silver Nanoparticles (AgNPs) Using Leaves and Stems Extract of Some Plants. Advanced Journal of Chemistry 2(4):266-275.

Mohanpuria P, Rana KN, Yadav SK (2008). Biosynthesis of nanoparticles: Technological concepts and future applications. Journal of Nanoparticle Research 10:507-517.

Morones JR, Elechiguerra JL, Camacho A, Holt K, Kouri JB, Ramfrez, JT, Yacaman M (2005). The bactericidal effect of silver nanoparticles. Journal of Nanotechnology 16(10):2346-2353.

Narayanan KB, Sakthivel N (2010). Biological synthesis of metal nanoparticles by microbes. Advances in Colloid and Interface Science 156(1-2):1-13.

Ozcelika B, Orhanb I, Tokerb G (2006). Antiviral and antimicrobial assessment of some selected flavonoids. Biological Activity of Flavonoids 61(9-10):632-638.

Pal S, Tak YK, Song JM (2007). Does the Antibacterial Activity of Silver Nanoparticles Depend on the Shape of the Nanoparticle? A Study of the Gram-Negative Bacterium *Escherichia coli*. Applied and Environmental Microbiology 27(6):1712-1720.

Prabhu S, Poulose E (2012). Silver nanoparticles: mechanism of antimicrobial action, synthesis, medical applications, and toxicity effects. International Nano Letters 2(1):1-10. https://link.springer.com/article/10.1186/2228-5326-2-32

Saha S, Sarkar J, Chattopadhayay D, Patra S, Chakraborty A, Acharya K (2010). Production of silver nanoparticles by a phytopathogenic fungus *Bipolaris nodulosa* and its antimicrobial activity. Digest Journal of Nanomaterials and Biostructures 5(4):887-895.

Saklani V, Suman JV, Jain K (2012). Microbial synthesis of silver

nanoparticles: A Review. Journal of Biotechnology and Biomaterials S13:007.
Šeděnková I, Trchová M, Stejskal J, Prokeš J (2009). Solid-state reduction of silver nitrate with polyaniline base leading to conducting materials. ACS Applied Materials and Interfaces 1(9):1906-1912.
Sharma N, Pinnaka AK, Raje M, Ashish FN, Bhattacharyya MS, Choudhury AR (2012). Exploitation of marine bacteria for production of gold nanoparticles. Microbial Cell Factories 11:86 doi: 10.1186/1475-2859-11-86.
Singh AK, Tiwari R, Kumar V, Singh P, Khadim SKR, Tiwari A, Srivastava V, Hasan SH, Asthana RK (2017). Photo-induced biosynthesis of silver nanoparticles from aqueous extract of Dunaliella salina and their anticancer potential. Journal of Photochemistry and Photobiology B: Biology 166:202-211.
Singh C, Sharma V, Naik PK, Khandelwal V, Singh H (2011). Green biogenic approach for synthesis of gold and silver nanoparticles using *Zingiber officinale*. Digest Journal of Nanomaterials and Biostructures 6(2):535-542.
Sondi I, Salopek-Sondi B (2004). Silver nanoparticles as antimicrobial agent: a case study on E. coli as a model for Gram-negative bacteria. Journal of Colloid and Interface Science 275(1):17-182.
Sunkar S, Nachiyar CV (2012). Microbial Synthesis and Characterization of Silver Nanoparticles Using the Endophytic Bacterium *Bacillus cereus*: A Novel Source in the Benign Synthesis. Global Journal of Medical Research 12(2):1-9.
Thu TT, Thi THV, Thi HN (2013). Biosynthesis of silver nanoparticles using *Tithonia diversifolia* leaf extracts and their antimicrobial activity. Materials Letters 105:220-223.
Vanmathi SK, Sivakumar T (2012). Isolation and characterization of silver nanoparticles from *Fusarium oxysporum.* International Journal of Current Microbiology and Applied Sciences 1(1):56-62.
Venkatesan KR, Vajrai R, Nithyadevi M, Arun KP, Uma MK, Brindham P (2013). Characterization and Antimicrobial effect of silver Nanoparticles Synthesized from *Bacillus subtilis* (MTCC 441). International Journal of Drug Development and Research 5(4):187-193.
Vishwanatha T, Keshavamurthy M, Mallappa M, Murugendrappa MV, Nadaf YF, Siddalingeshwara KG, Dhulappa A (2018). Biosynthesis, characterization, and antibacterial activity of silver nanoparticles from *Aspergillus awamori*. Journal of Biology and Biotechnology 6(5):12-16.
Walker LR (2003). Colonization dynamics and facilitative impacts of a nitrogen-fixing shrub in primary succession. Journal of Vegetation Science 14(2):277-290.
Wolfe BE, Klironomos JN (2005). Breaking new ground: Soil communities and exotic plant invasion. BioScience 55(6):477-487.
Yamal G, Sharmila P, Rao KS, Pardha S (2013). In built potential of YEM medium and its constituents to generate Ag/Ag2O nanoparticles. PLoS One 8:e61750. https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0061750

Appendix Table 1. Morphological and biochemical characteristics of bacteria isolated from rhizospheric soil and fish pond sediments.

Gram reaction	Cellular morphology	Catalase	Oxidase	Coagulase	Indole	Motility	Methyl-Red VogesProskauer	Urease activity	Citrate Utilization	Starch Hydrolysis	Gelatin Hydrolysis	Casein Hydrolysis	Spore test	NO_3 Reduction	Glucose	Sucrose	Arabinose	Maltose	Mannitol	Xylose	Galactose	Sorbitol	Invositol	Frauction	Most Probable Identity
-ve	R	+	+	-	-	+	-	+	-	+	-	+	-	-	+	+	+	+	+	+	+	+	-	-	+	*P. aeruginosa*
+ve	C	+	-	+	-	-	-	+	+	-	-	+	+	-	+	+	+	-	+	+	-	+	ND	ND	+	*S. aureus*
+ve	C	+	-	-	-	-	-	+	+	-	-	+	-	-	-	+	+	-	-	-	-	-	ND	ND	+	*S. epidermidis*
-ve	R	-	+	-	-	+	+	+	-	+	-	-	+	+	+	+	+	+	-	+	+	-	-	-	+	*E. coli*
+ve	R	+	+	-	-	+	-	+	-	+	-	+	-	+	-	+	+	-	-	+	-	-	-	-	+	*B. cereus*
+ve	C	+	-	-	-	-	-	-	+	-	-	+	-	-	-	+	+	-	+	+	-	-	-	-	-	*M. luteus*
+ve	R	+	+	-	-	+	-	+	+	+	-	+	-	+	-	+	+	-	-	+	-	-	-	-	+	*B. subtilis*
-ve	R	+	+	-	+	-	+	-	+	+	+	-	-	-	+	+	+	+	+	-	-	-	-	+	+	*S. marcescens*
-ve	R	+	-	-	+	+	-	+	+	+	-	-	-	+	+	-	-	-	-	+	-	-	-	-	-	*P. vulgaris*
+ve	R/C	+	+	+	+/-	-	-	-	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+		*S. griseus*

R = Rods; + = Positive reaction; - = Negative reaction; ND = Not determined; A = Zone A; B = Zone B; C =Zone C; D = Zone D and CT = Control.

Appendix Table 2. Cultural and microscopic characteristics of fungi isolated from rhizospheric soil and fish pond sediments.

Fungus	Cultural characteristics	Microscopic characteristics	Carbohydrate Assimilation	Spore Formation	Amino Acid Assimilation	Motility	Hydrolysis	Lipase Activity
Aspergillusflavus	Yellow green texture: fluffy colonies	Conidial heads radiate, conidiophores coarsely roughened. Conidia borne in 360 arrangements covering the upper 2/3 of the conidiophores.	+	-	+	-	-	+
A. niger	Growth begins as yellow colonies that soon develop a black, dotted surface. Conidia are produce within 2-6 days the colony becomes jet black and powdery and the reverse remains cream colour	Exhibits septate hyphae long conidiophores that support spherical vesicle that give rise to mutulae and phalides from which conidia are produce.	+	-	+	-	-	+
Rhizopusstolonifer	Large fluffy white milky colonies which later turns black as culture ages.	Non-septatehyphal with upright sporagioshere connected by stolon and rhizoids, dark pear-shaped sporangium on hemispherical columella.	+	+	-	-	-	-
Trichodermaviride	Fast growth on agar medim with globose conidia. Formation of light green conidia with granules	Arrangement of phialides was in divergent groups of 2-4. Phialides were flask shape	+	-	+	-	-	+
Mucormucedo	Colonies characteristically produce a fluffy white growth that diffusely covers the surface of the agar within 24-48 hours	The hyphae appear to be coarse and fill the entire culture dish rapidly with hyphae dotted with brown or black porangia. Sporangiosphores are branch and have at their tip a sporangium filled with sporangiophores, no rhizoids or stolons.	+	+	-	-	-	-
Emericella rugulosa	Colonies appeared dark grass green with abundant colodial heads. Violet soluble pigment produced and the reverse of plates appeared in shades of pink	Conidiophores appeared with short brown stipes, bearing both metulae and phialides.	+	-	+	-	-	+
Fusarium oxysporium	Rapidly growing wooly to cottonly lemon and yellow	Multicellular distinctive sickle shaped macro coniclia	+	+	+	-	-	+

Contd. Appendix Table 2. Cultural and microscopic characteristics of fungi isolated from rhizospheric soil and fish pond sediments.

Geotrichumalbidum	Rapid growth with white, dry, powdery colonies appearing like ground glass.	Presence of coarse true hyphae and athroconidia. Unicellular arthroconidia appearing in chains. Blastoconidia, conidiophores and pseudohyphae are absent. Round end resembling barrel shape.	+	+	+	-	-	+
Penicillium frequentans	Fast growing colonies in shades of green. Surface texture velutinous, floccose in center. Absence of exudates and reverse pale yellow.	Presence of conidiophores with branching patterns. Wall ornamentation of stipes and conidia. Biverticillate conidiophores have a whorl of three or more metulae between the phialades and the end of the stipe.Phialidesareflask-shaped with cylindrical basal part and distinct neck.	+	-	+	-	-	+
Saccharomyces cerevisiae	Rapid growth with maturity in three days. Flat, smooth, moist, glistening and cream in colour.	Presence of blastoconidia. Unicellular, globose and ellipsoid in shape. Absence of hyphae.	+	+	+	-	-	+

Keys: + = Positive reaction; - = Negative reaction.

Vol. 15(3), pp. 146-151, March, 2021
DOI: 10.5897/AJMR2020.9440
Article Number: 1CBEE3366322
ISSN: 1996-0808

http://www.academicjournals.org/AJMR

ACADEMIC JOURNALS
expand your knowledge

African Journal of Microbiology Research

Full Length Research Paper

Anti-quorum sensing and anti-biofilm activities of *Securidaca longepedunculata* Fresen, an endangered species from Burkina Faso

Issa Karama*, Vincent Ouedraogo, Ablassé Rouamba, Moussa Compaoré and Martin Kiendrebeogo

Department of Biochemistry and Microbiology, University Joseph Ki-Zerbo, Ouagadougou, Burkina Faso.

Received 19 October, 2020; Accepted 19 February, 2021

The emergence of bacterial resistance to antibiotics is a serious challenge to the global health system. The QS inhibition is one of the approaches to fight against antibiotic resistance in bacteria. *Securidaca longepedunculata* is a medicinal plant that roots are the only organ used against microbial diseases. This species is threatened with extinction due to the massive use of its roots in phytotherapy. In this study, the anti-QS and anti-biofilm activities of *S. longepedunculata* leaves methanolic extract at 100 to 400 µg/mL was assessed against the bacterial strains *Chromobacterium violaceum* CV026 and *Pseudomonas aeruginosa* PAO1. The results showed anti-QS and anti-biofilm activity of *S. longepedunculata* leaves which reduced violacein production in *C. violaceum* CV026 by 12 to 59%. The virulence factor pyocyanin in *P. aeruginosa* PAO1 was inhibited from 13 to 46%. Biofilm formation was significantly inhibited (41%) at 400 µg/mL.

Key words: *Securidaca longepedunculata*, anti-quorum, anti-biofilm.

INTRODUCTION

National Institutes of Health (NIH) estimated that more than 80% of human microbial infections are associated with biofilms which is present in about 65% of chronic infections (Jamal et al., 2018). Biofilms are aggregations of microorganisms which live in an extracellular matrix. This matrix is composed by extracellular polymeric substances (EPS), including polysaccharides, nucleic acids, proteins and lipids, at a liquid interface (Li et al., 2020). In biofilms, the bad penetration of antibiotic, reduced nutrition and growth, adaptive stress responses, and persister cells formation would constitute a multi-layered defense system (Stewart, 2002). Cells growing in the biofilm are resistant to both antibiotic therapy and the host immune defense system. This situation is the cause of recurrent and recalcitrant infections (Li et al., 2020). Bacteria attached to a surface and growing in a biofilm are protected against the action of antibiotics, biocides and other control methods (Abebe, 2020). Persistent cells are a subpopulation of bacteria which can transiently survive under the lethal effect of antibiotic treatment and thus contribute to the strong resistance of biofilms (Dincer et al., 2020).

Bacteria control the expression profile of genes according to the size of the microbial population through

*Corresponding author. E-mail: isdok57@gmail.com.

the mechanism of quorum sensing (QS), which allows to form different forms of biofilm (Abebe, 2020). QS acts on the development of the biofilm and controls the production of virulence factors (enzymes, toxins) necessary for resistance to phagocytosis (Li et al., 2020). One of the strategies proposed for overcoming resistance in biofilm is the use of QS inhibitors (Li et al., 2020).

Pseudomonas aeruginosa is an opportunistic bacterium responsible for chronic infections which occurred in hospitals especially in patients suffering from cystic fibrosis. The biofilm formation and the production of several virulence factors characterize the infection process. At the same time, the sessile growth mode leads to a reduction of bacterial sensitivity to both host defenses and antimicrobial agents. Quorum sensing, which is the inter-bacterial communication system, is correlated with their biofilm production and resistance (Ciofu and Tolker-nielsen, 2019).

Medicinal plants offer a variety of phytochemicals with a new potential control of microbial diseases, due to the spectrum of secondary metabolites present in the extracts, which include phenolic, quinones, flavonoids, alkaloids and terpenoids (Asfour, 2018).

As a result, the commonly used plants in ethnomedicine constitute an alternative to search bioactives compounds against virulence factors or their production.

Securidaca longepedunculata is a highly medicinal plant whose roots and stem bark are the only organs used against bacterial disease and for the treatment of chronic wounds in traditional medicine (Mongalo et al., 2015). This plant is threatened because of the massive use of its roots (Compaoré et al., 2018).

This study aimed to assess anti-QS and anti-biofilm activities of *S. longepedunculata* leave extract.

MATERIALS AND METHODS

Bacterial strains and growth conditions

The Plant Biotechnology Laboratory of Université Libre de Bruxelles (Belgium) has kindly offered *P. aeruginosa* PAO1 and *Chromobacterium violaceum* CV026 strains for the biological activities assessment. Luria-Bertani (LB) broth was used as culture medium (37°C for PAO1 and 30°C for CV026).

Plant material collection and extraction

The leaves of *S. longepedunculata* were harvested in Badara locality (Region of "Haut Bassin"). Voucher specimen (CI : 16713) was identified and deposited in the herbarium of "Université Joseph KI ZERBO", Burkina Faso. The powder from dried samples was extracted with methanol for one day. An evaporator was used to concentrate the extracts under vacuum before drying them.

Assessment of inhibition of violacein production in *C. violaceum* CV026

The method of Choo et al. (2006) was used to assess the ability of *S. longepedunculata* extract to affect negatively the QS system through its effect on violacein production in *C. violaceum* CV026. In the presence of exogenous N-hexanoyl-L-homoserine lactone (C6-HSL; Sigma-Aldrich), *C. violaceum* CV026 which is a mutant strain is capable of producing violacein. Briefly, to the mixture of *S. longepedunculata* extract (in DMSO) and C6-HSL, a diluted culture of *C. violaceum* CV026 (18 h at 30°C) was added. The final concentrations were 100 to 400 µg/mL for the extracts and 10 µM for C6-HSL. After tubes incubation at 30°C (24 h, 175 rpm), bacterial growth was assessed by measuring the bacterial turbidity (OD600 nm). The bacterial culture (1 mL) was first centrifuged (7000 rpm, 10 min) and then the violacein was dissolved in 1 mL of DMSO. Quantification of violacein production was performed by measuring its absorbance at 585 nm.

Inhibition of pyocyanin production in *P. aeruginosa* PAO1

The QS controls the pyocyanin production. The inhibitory capacity of *S. longepedunculata* leaves extract on this production was assessed according to the method of Ouedraogo and Kiendrebeogo (2016). Briefly, the extract was used to make a series (dilution by half) of concentrations (in DMSO) which were each added overnight to a culture of *P. aeruginosa* PAO1. The final concentrations of the extracts were 100 to 400 µg/mL. Bacterial growth was assessed by measuring the bacterial turbidity (OD_{600nm}) after 18 h incubation (37°C, 175 rpm) of tubes. To assess the production of pyocyanin (A_{380}), the supernatant was used.

Biofilm formation and quantification

The ability of *S. longepedunculata* extract to inhibit biofilm formation was assessed according to the method of Vandeputte et al. (2010). A volume of 200 µL of *P. aeruginosa* PAO1 culture was supplemented with leaves extract solution for final concentrations ranging from 100 to 400 µg/mL in round-bottomed wells. The supernatant was removed after 24 h incubation at 37°C and the biofilm was fixed with methanol after washing with distilled water. A crystal violet solution (0.1% in water) was added to the wells followed by 30 min incubation at room temperature. The crystal violet stained was dissolved with 200 µL of acetic acid (33% in water) in order to read solution absorbance at 590 nm.

Statistical analysis

One way analysis of variance (ANOVA) followed by Tukey test of Graph Pad Prism software was used to determined statistical significance; p value ≤ 0.05 was considered significant (n=3).

RESULTS

Anti-QS activity of *S. longepedunculata* leaves

To search anti-QS compounds, the strain of *C. violaceum* CV026 is indicated. The leaves extract at different concentrations (100-400 µg/mL) was used to assess the inhibitory capacity of *S. longepedunculata* on violacein production after 24 h of growth. Figure 1A shows that compared to the control (DMSO at 1%), the production of violacein is reduced by 12 to 59% by *S. longepedunculata* leaves extract in concentration-dependent manner. The growth of *C. violaceum* CV026 is not affected by this

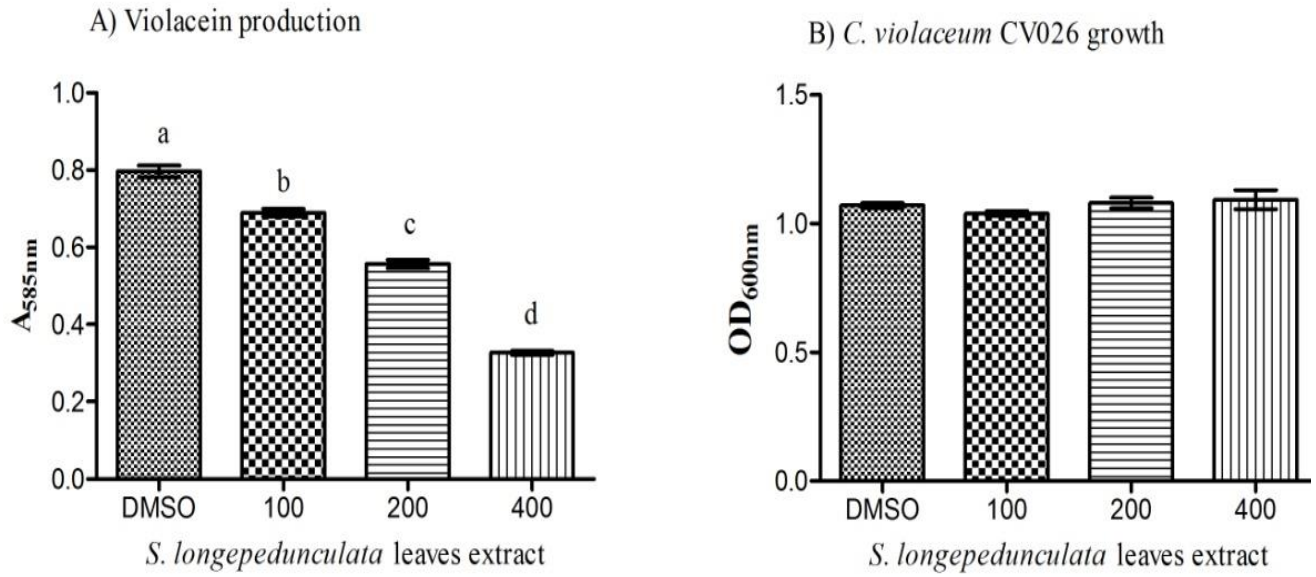

Figure 1. Concentration-dependent manner inhibitory activity of *S. longepedunculata* leaves extract on violacein production in *C. violaceum* CV026. A) Extraction and quantification (A_{585}) of violacein was performed as described in materials and methods section. B) Growth of CV026 assessed at 600 nm. The negative control used was DMSO at 1%. The difference in superscript letters assigned to histograms indicates that values are significantly different ($p < 0.05$).

reduction (Figure 1B). These results confirm that *S. longepedunculata* leaves extract contains anti-QS compounds.

S. longepedunculata leaves extract affects QS-controlled extracellular virulence factor production

The leaves extract reduced significantly the production of violacein, thus showing an inhibitory effect of *S. longepedunculata* on QS system. The QS system in *P. aeruginosa* controls the production of pyocyanin which is the virulence factor. The redox cycle of host cells is altered by a blue-green phenazine pigment which is the pyocyanin produced in culture medium (Liu and Nizet, 2009). The assessment of *S. longepedunculata* ability to inhibit pyocyanin production showed significant reduction effects ranging from 13 to 46% in dose-dependent manner (100 to 400 µg/mL) of leaves extract (Figure 2A). The growth of *P. aeruginosa* PAO1 was not affected by the extracts (Figure 2B).

S. longepedunculata leaves extract affect biofilm formation

The formation of *P. aeruginosa* biofilm PAO1 is related to QS system (Jimenez et al., 2012). Based on anti-QS activity observed, the ability of *S. longepedunculata* leaves extract to inhibit biofilm formation of *P. aerugionosa* PAO1 was evaluated.

Figure 3 shows that biofilm formation is significantly inhibited by *S. longepedunculata* leaves extract at different concentrations (100-400 µg/mL). An inhibition of 41% was recorded at the concentration of 400 µg/mL.

DISCUSSION

Bacterial biofilms are difficult to control and show high resistance to antibiotics (Koo et al., 2017). The destruction of a fully formed biofilm necessarily involves the use of molecules capable of penetrating its structure or destructuring it (Paluch et al., 2020).

This study showed that *S. longepedunculata* leaves methanolic extract have anti-QS activity, inhibit virulence factors and biofilm formation. High concentrations of *S. longepedunculata* extract did not show bactericidal activity against *C. violaceum* and *P. aerugionosa*. This absence of bactericidal activity shows that the leaves of *S. longepedunculata* act only through their inhibitory effects on QS and the formation of the biofilm. An inhibition of biofilm formation would make bacteria accessible and sensitive to the immune system and to antibacterial. This result would suggest a possible combination of *S. longepedunculata* leaves and some antimicrobial compounds in external treatment. Previous work has reported that leaves of *S. longepedunculata* are rich in polyphenol, flavonoids, and alkaloids (Karama et al., 2018). The biological activities of *S. longepedunculata* observed in this work are obviously due to the nature of bioactive compounds contained in its leaves. Vasavi et al.

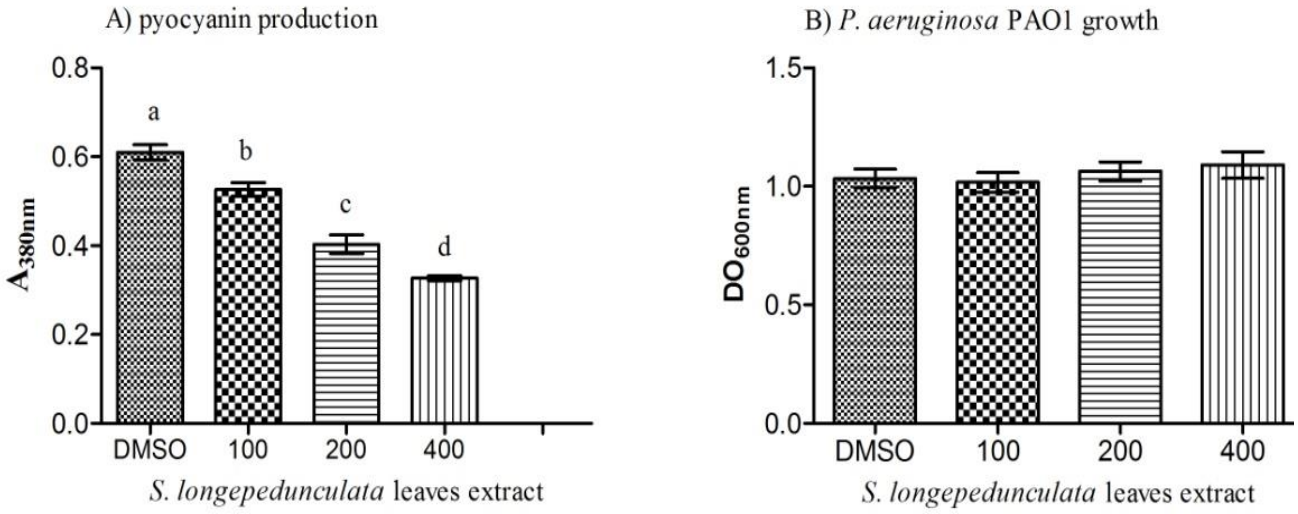

Figure 2. Effect of *S. longepedunculata* extract on *P. aeruginosa* PAO1 pyocyanin production. A) Pyocyanin production; B) Growth. Histogram with different letters is significantly different ($p < 0.05$).

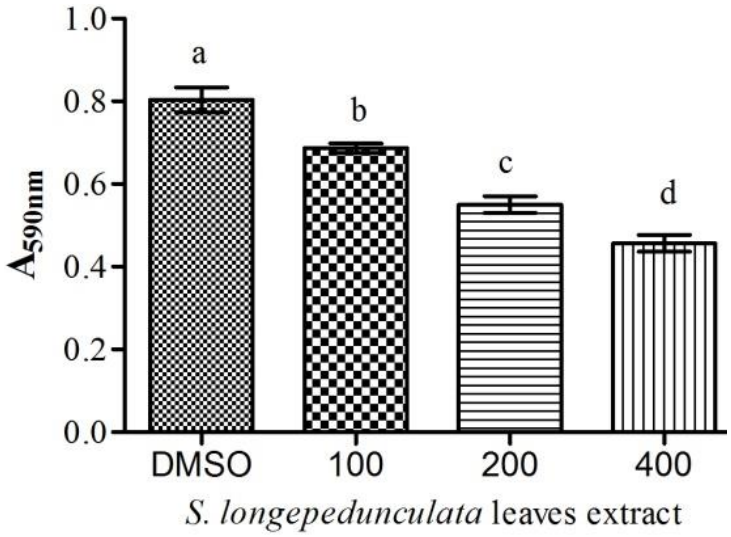

Figure 3. Activity of *S. longepedunculata* extract on *P. aeruginosa* PAO1 biofilm formation. Histograms with different superscript letters are significantly different ($p < 0.05$).

(2016) reported that plant flavonoids have the ability to interfere with inter-microbial communication and have anti-biofilm activity. Karama et al. (2020) reported the presence of compounds such as quercetin, chrysin, rutin, isorhamnetin, luteolin, gallic acid, ellagic acid, ferulic acid and tannic acid in leaves of *S. longepedunculata*. The anti-QS activity against *C. violaceum* of various flavonoids such as quercetin and luteolin have been reported (Bali et al., 2019). Rekha et al. (2016) reported that quercetin showed good inhibition of pyocyanin production.

The anti-biofilm activity of rutin against multidrug-resistant *P. aeruginosa* has been reported (Deepika et al., 2018). Wang et al. (2017) showed that rutin significantly inhibited the biofilm formation of *Streptococcus suis* without impairing its growth *in vitro*.

Likewise, the anti-QS and anti-biofilm effect of luteolin and chrysin have been reported against Gram-positive and Gram-negative bacteria (Cho et al., 2015; Shen et al., 2014).

Another study showed quercetin activity against virulence factors production and biofilm formation of *P. aeruginosa* PAO1 (Ouyang et al., 2016). Significant inhibitions were recorded with quercetin against

pyocyanin, protease and elastase production and biofilm formation (Quecan et al., 2019). Other work had reported that quercetin inhibited the production of violacein in *C. violaceum* 12472, at 50 and 100 µg/mL, respectively (Vasavi et al., 2014).

In addition, Vikram et al. (2010) have shown that quercetin removes *Escherichia coli* O157: H7 and *Vibrio harveyi* biofilm formation. Tannic acid has reduced the QS regulated violacein production up to 47.7% (Sivasankar et al., 2019). According to a past study (Karama et al., 2018), the leaves of *S. longepedunculata* showed an alkaloid content of 245 µg/g of methanolic extract. Studies reported that alkaloids have shown the ability to reverse biofilm resistance (Othman et al., 2019; Su et al., 2020).

The anti-QS and biofilm formation inhibitory activity of *S. longepedunculata* leaves extract would be due to different bioactive compounds it contains. The results observed with S*. longepedunculata* extracts show that this plant could be used against microbial infections of *P. aeruginosa*. However, subsequent studies could accurately identify anti-QS and biofilm inhibitor molecules through bioguided screening. As *S. longepedunculata* is threatened with extinction, the leaves could be used instead of the roots in phytotherapy and thus contribute to a sustainable use of this plant in the management of microbial disease. The biological activity observed with the extract of *S. longepedunculata* would be due to the properties of anti-quorum sensing and anti-biofilm compounds in the phytochemistry of its leaves.

Conclusion

This study showed that methanolic extracts from *S. longepedunculata* leaves have the ability to inhibit significantly the production of QS-controlled factors including violacein and pyocyanin from *C. violaceum* and *P. aeruginosa*, respectively and the formation of biofilm. The extract of *S. longepedunculata* did not affect negatively the growth of bacteria used.

S. longepedunculata leaves would act only by inhibiting QS and biofilm formation. The anti-QS activity of leaves shows that they could be used in the treatment of *P. aeruginosa* infections. This work results show that *S. longepedunculata* leaves would contain anti-QS and anti-biofilm compounds that could be identified by further studies.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

REFERENCES

Abebe GM (2020). The Role of Bacterial Biofilm in Antibiotic Resistance and Food Contamination. International Journal of Microbiology pp. 1-10.

Asfour HZ (2018). Anti-Quorum Sensing Natural Compounds. Journal of Microscopy and Ultrastructure 6(1):1-10.

Bali EB, Türkmen KE, Erdönmez D, Saglam N (2019). Comparative study of inhibitory potential of dietary phytochemicals against quorum sensing activity of and biofilm formation by *Chromobacterium violaceum* 12472, and swimming and swarming behaviour of *Pseudomonas aeruginosa* PAO1. Food Technology and Biotechnology 57(2):212–221.

Cho HS, Lee JH, Cho MH, Lee J (2015). Red wines and flavonoids diminish *Staphylococcus aureus* virulence with anti-biofilm and anti-hemolytic activities. Biofouling 31(1):1-11.

Choo JH, Rukayadi Y, Hwang JK (2006). Inhibition of bacterial quorum sensing by vanilla extract. Letters in Applied Microbiology 42(6):637-641.

Ciofu O, Tolker-nielsen T (2019). Tolerance and resistance of *Pseudomonas aeruginosa* biofilms to antimicrobial agents — How *P . aeruginosa* can escape antibiotics. Frontiers in Microbiology 10:1-15.

Compaoré M, Meda RN, Zerbo P, Karama I, Traoré O, Lamien-meda A, Kiendrebeogo M, Novak J (2018). Availability Evaluation of Twelve Antimalarial Medicinal Plants from Western Regions of Burkina Faso. Journal of Diseases and Medicinal Plants 4(3):80-88.

Dincer S, Uslu FM, Delik A (2020). Antibiotic Resistance in Biofilm.In Bacterial Biofilms, Dincer S, Özdenefe MS, Arkut A, IntechOpen 1-360.

Jamal M, Ahmad W, Andleeb S, Jalil F, Imran M, Asif M, Hussain T, Ali M, Rafiq M, Atif M (2018). Bacterial biofilm and associated infections. Journal of the Chinese Medical Association 81(1):7-11.

Jimenez PN, Koch G, Thompson JA, Xavier KB, Cool RH, Quax WJ (2012). The Multiple Signaling Systems Regulating Virulence in *Pseudomonas aeruginosa*. Microbiology and Molecular Biology Reviews 76(1):46–65.

Karama I, Compaoré M, Djandé A, Traoré O, Lagnika L, Kiendrebeogo M (2020). Antiplasmodial Activities of Flavonoids from Leaves of *Securidaca longepedunculata* Fresen (Polygalaceae). American Journal of BioScience 8(1):1-5.

Karama I, Compaoré M, Traore O, Sanon S, Lagnika L, Kiendrebeogo M (2018). Comparative study of leaves and root bark from *Securidaca longepedunculata* Fresen (Polygalaceae): phytochemistry and antiplasmodial activity. *The Pharma Innovation Journal* 7(10):178-184.

Koo H, Allan RN, Howlin RP, Stoodley P, Hall-Stoodley L (2017). Targeting microbial biofilms: Current and prospective therapeutic strategies. Nature Reviews Microbiology 15(12):740-755.

Li Y, Xiao P, Wang Y, Hao Y (2020). Mechanisms and control measures of mature biofilm resistance to antimicrobial agents in the clinical context. ACS Omega 5(36):22684-22690.

Liu GY, Nizet V (2009). Color me bad : microbial pigments as virulence factors. Trends in Microbiology 17(9):406-13.

Mongalo NI, McGaw LJ, Finnie JF, Staden JV (2015). *Securidaca longepedunculata* Fresen (Polygalaceae): A review of its ethnomedicinal uses, phytochemistry, pharmacological properties and toxicology. Journal of Ethnopharmacology 165:215-226.

Othman L, Sleiman A, Abdel-Massih RM (2019). Antimicrobial activity of polyphenols and alkaloids in Middle Eastern plants. Frontiers in Microbiology 10:1-28

Ouedraogo V, Kiendrebeogo M (2016). Methanol extract from *Anogeissus leiocarpus* (DC) Guill . et Perr . (Combretaceae) stem bark quenches the Quorum Sensing of *Pseudomonas aeruginosa* PAO1. Medicines 3(4):26.

Ouyang J, Sun F, Feng W, Sun Y, Qiu X, Xiong L, Liu Y, Chen Y (2016). Quercetin is an effective inhibitor of quorum sensing, biofilm formation and virulence factors in *Pseudomonas aeruginosa*. Journal of Applied Microbiology 120(4):966-974.

Paluch E, Rewak-Soroczyńska J, Jędrusik I, Mazurkiewicz E, Jermakow K (2020). Prevention of biofilm formation by quorum quenching. Applied Microbiology and Biotechnology 104(5):1871-1881.

Quecan BX, Santos JT, Rivera ML, Hassimotto NM, Almeida FA, Pinto UM (2019). Effect of quercetin rich onion extracts on bacterial quorum sensing. Frontiers in Microbiology 10:867.

Rekha PD, Vasavi HS, Vipin C, Saptami K, Arun AB (2016). A medicinal herb *Cassia alata* attenuates quorum sensing in *Chromobacterium*

violaceum and *Pseudomonas aeruginosa*. Letters in Applied Microbiology 64(3):231-238.
Deepika MS, Thangam R, Sakthidhasan P, Arun S, Sivasubramanian S, Thirumurugan R (2018). Combined effect of a natural flavonoid rutin from *Citrus sinensis* and conventional antibiotic gentamicin on *Pseudomonas aeruginosa* biofilm formation. Food Control 90:282-294.
Shen XF, Ren LB, Teng Y, Zheng S, Yang XL, Guo XJ, Wang XY, Sha KH, Li N, Xu GY, Tian HW, Wang XY, Liu XK, Li J, Huang N (2014). Luteolin decreases the attachment, invasion and cytotoxicity of UPEC in bladder epithelial cells and inhibits UPEC biofilm formation. Food and Chemical Toxicology 72:204-211.
Sivasankar C, Jha NK, Ghosh R, Shetty PH (2019). Anti quorum sensing and anti virulence activity of tannic acid and it 's potential to breach resistance in *Salmonella enterica* Typhi / Paratyphi A clinical isolates. Microbial Pathogenesis 138:103813.
Stewart PS (2002). Mechanisms of antibiotic resistance in bacterial biofilms. International Journal of Medical Microbiology 292(2):107-13.
Su T, Qiu Y, Hua X, Ye B, Luo H, Liu D, Qu P, Qiu Z (2020). Novel Opportunity to Reverse Antibiotic Resistance: To Explore Traditional Chinese Medicine With Potential Activity Against Antibiotics-Resistance Bacteria. Frontiers in Microbiology 11:1-11.
Vandeputte OM, Kiendrebeogo M, Rajaonson S, Diallo B, Mol A, Jaziri M, Baucher M (2010). Identification of catechin as one of the flavonoids from *Combretum albiflorum* bark extract that reduces the production of quorum-sensing-controlled virulence factors in *Pseudomonas aeruginosa* PAO1. Applied and Environmental Microbiology 76:243-253.
Vasavi HS, Arun AB, Rekha PD (2014). Anti-quorum sensing activity of *Psidium guajava* L. flavonoids against *Chromobacterium violaceum* and *Pseudomonas aeruginosa* PAO1. Microbiology and Immunology 58(5):286-293.
Vasavi HS, Arun AB, Rekha PD (2016). Anti-quorum sensing activity of flavonoid-rich fraction from *Centella asiatica* L. against *Pseudomonas aeruginosa* PAO1. Journal of Microbiology Immunology and Infection 49(1):8-15.
Vikram A, Jayaprakasha GK, Jesudhasan PR, Pillai SD, Patil BS (2010). Suppression of bacterial cell-cell signalling, biofilm formation and type III secretion system by citrus flavonoids. Journal of Applied Microbiology 109(2):515-527.
Wang S, Wang C, Gao L, Cai H, Zhou Y, Yang Y, Xu C, Ding W, Chen J, Muhammad I, Chen X, He X, Liu D, Li Y (2017). Rutin inhibits *Streptococcus suis* biofilm formation by affecting CPS biosynthesis. Frontiers in Pharmacology 8:379. doi: 10.3389/fphar.2017.00379.

Vol. 15(3), pp. 152-160, March, 2021
DOI: 10.5897/AJMR2021.9488
Article Number: 20C41E266404
ISSN: 1996-0808

http://www.academicjournals.org/AJMR

ACADEMIC JOURNALS
expand your knowledge

African Journal of Microbiology Research

Full Length Research Paper

Evaluation of yellow fever surveillance in Chad, 2015-2020

Nadlaou Bessimbaye[1,2]*, Djimadoum Mbanga[1,2], Ali Mahamat Moussa[1,2], Djerandouba Yotobumbeti Ferdinand[3], Narbé Djimadengar Maxime[1], Nicolas Barro[4], Abdelsalam Tidjani[2] and Choua Ouchemi[1,2]

[1]Centre Hospitalier Universitaire de Référence Nationale (CHU-RN), de N'Djamena, Chad.
[2]Faculté des Sciences de la Santé Humaine, Université de N'Djamena, Chad.
[3]MENTOR Initiative, Crawley, United Kingdom.
[4]Laboratoire de Biologie Moléculaire, d'Epidémiologie et de Surveillance des Bactéries et Virus Transmissibles par les Aliments (LaBESTA)/Ecole Doctorale Sciences et Technologies, Université Ouaga 1 Joseph KI-ZERBO, 03 BP 7021 Ouagadougou 03, Burkina Faso.

Received 18 January, 2021; Accepted 15 March, 2021

The present study was conducted to determine the seroprevalence of yellow fever virus in patients with clinical signs of febrile jaundice giving suspicion of yellow fever and to evaluate diagnostic techniques for surveillance of yellow fever in the Republic from Chad. From January 2015 to July 2020, an observational study of virological markers was conducted in jaundice patients in Chad. Of the 1730 patients included in this study, a seroprevalence of 0.28% of yellow fever virus was determined. The distribution of pathogens responsible for diseases associated with yellow fever was: 49.47% (*Plasmodium falciparum*), 2% (hepatitis E virus), 4.62% (hepatitis C virus), and 29.00% (hepatitis virus B), respectively. Discrepancies in the results between the regional reference laboratories and the national laboratory of the Republic of Chad were observed. All genders and age groups were affected. Of the 1730 samples taken, 55.49% were female and 44.51% male (p = 0.01, a significant difference in favor of the female sex). The male/female sex ratio was 1.25. This study, the first, made it possible to determine the rate of the yellow fever virus in the absence of an outbreak in forest areas and with heavy rainfall and to evaluate the MAC-ELISA-CDC techniques used between the national laboratory of the Republic of Chad and those regional references. It was recommended that real-time polymerization chain reaction techniques be made available to national laboratories and reagents from the same manufacturing company in order to carry out effective monitoring of yellow fever.

Key words: Evaluation, surveillance, seroprevalence, techniques, yellow fever.

INTRODUCTION

Yellow fever (YF), sometimes called black vomit, American plague, fever or typhus amaril caused by a Flavivirus, is a serious disease for which there is currently no drug treatment (Hunsperger et al., 2016; WHO, 2016b). It is an arbovirus of equatorial forest monkeys and is transmitted from monkey to monkey by various

mosquitoes; the mosquito playing the role of reservoir and vector and the monkey that of biological amplifying host (WHO, 2018). The causative agent of yellow fever is yellow fever virus, and transmission to humans is through a mosquito (*Aedes aegypti*) which bites mostly at dusk and dawn (Paules and Fauci, 2017; WHO, 2016b). It is a hemorrhagic fever with liver damage accompanied by jaundice, or yellowing of the skin and mucous membranes, kidneys with a tendency to bleeding and the nervous system, fatal in 20 to 60% of the cases. The number of yellow fever cases is estimated each year between 18 and 50/100,000 people living in areas at risk of transmission (WHO, 2016b). The disease exists in many countries in Africa, South America and Europe (Abeera et al., 2019; Amraoui et al., 2016).

An observational study was performed in the absence of an outbreak in patients with signs of febrile jaundice suspected of yellow fever.

The current outbreaks of yellow fever worldwide have demonstrated that rapid laboratory confirmation of suspected yellow fever cases is a key part of an effective response (WHO, 2016a). The best way to detect these outbreaks early enough is surveillance. The surveillance system that is currently in place in almost all African countries focuses on the human component only and is based on clinical examination for jaundice, blood sampling and research immunoglobulins M (IgM) by national laboratories with confirmation by a regional laboratory (Santiago et al., 2018; www.signalement-moustique.fr, 2017). This way of proceeding, although strongly recommended, remains limited because it often only gives alert when disease transmission is already well established in the community. It is probably too late for a disease such as yellow fever, which is characterized by a high proportion of asymptomatic cases (WHO, 2016b; HCSP, 2017).

Routine screening for yellow fever virus on all suspected blood samples has helped delineate areas of transmission and reduced the risk of infection with yellow fever virus. There remains, however, a residual risk of transmission of the yellow fever virus; this risk could be due to two factors: a technical error and a viral variant not recognized by certain reagents.

The aim of this study was to determine the seroprevalence of yellow fever virus in patients with signs of febrile jaundice associated with yellow fever during or in the absence of the epidemic outbreak and to assess the performance of yellow fever virus detection techniques between the national laboratory of the Republic of Chad and the regional reference laboratories of Yaoundé (Cameroon) and Dakar (Senegal).

The results of this work will allow corrective measures to be taken on yellow fever diagnostic techniques to ensure the quality of results between laboratories and set up an effective surveillance system for the fight against yellow fever in the Republic of Chad and in the sub-region.

MATERIALS AND METHODS

This study was carried out in agreement with the integrated epidemiological surveillance service of the Chadian Ministry of Public Health, which through the World Health Organization provided the necessary materials for the analysis of blood samples. During this study, we insisted on the confidentiality and informed consent of the participants.

Framework of the study and progress of the work

The study took place in N'Djamena from January 2015 to July 2020 (Chad), Dakar (Senegal), Amsterdam (Holland) and Yaoundé (Cameroon):

(1) Virology Unit of the Laboratory of the National Reference University Hospital (CHU-RN) of N'Djamena (Chad);
(2) Institute Pasteur in Dakar/Senegal where all stages of molecular, immunochemical and serological diagnosis were carried out;
(3) National Laboratory of the Health District of Amtiman (DSA), Province of Salamat (Chad);
(4) Sanquin Amsterdam Laboratory of Holland (LSAH), The Netherlands where the ELISA test with IgM + / IgG + titers greater than 15 was performed on all samples tested with HEV.
(5) Pasteur Center in Yaoundé, Cameroon where all the stages of molecular, immunochemical and serological diagnosis were carried out.

Choice of samples

The screening strategy for the selection of blood samples was summarized in four markers of viral communicable diseases: yellow fever virus, hepatitis B virus, C and E. The selected blood samples were tested either on the system of immuno-serological test with VIDAS (hepatitis B virus) or immuno-chromatographic tests (hepatitis C and E viruses) for the detection of Ag/Ac and an immunochemical test for the detection of yellow fever virus (ELISA).

Inclusion and exclusion criteria

Included was anyone with clinically visible signs: yellow discoloration of the conjunctiva or yellow discoloration of the skin (suggesting jaundice) with hyperthermia at 40°C. Not included were people who did not have signs suggestive of jaundice.

Sample collection

Blood samples were taken systematically from people with signs suggestive of jaundice in towns and villages in the various provinces of the country (Figure 1) after completing the identification forms.

The study population was at least 6 months old to 75 years old, from all occupations and social categories combined. Whole blood

*Corresponding author. E-mail: bnadlaou@gmail.com. Tel: (00235)66373647/90209073.

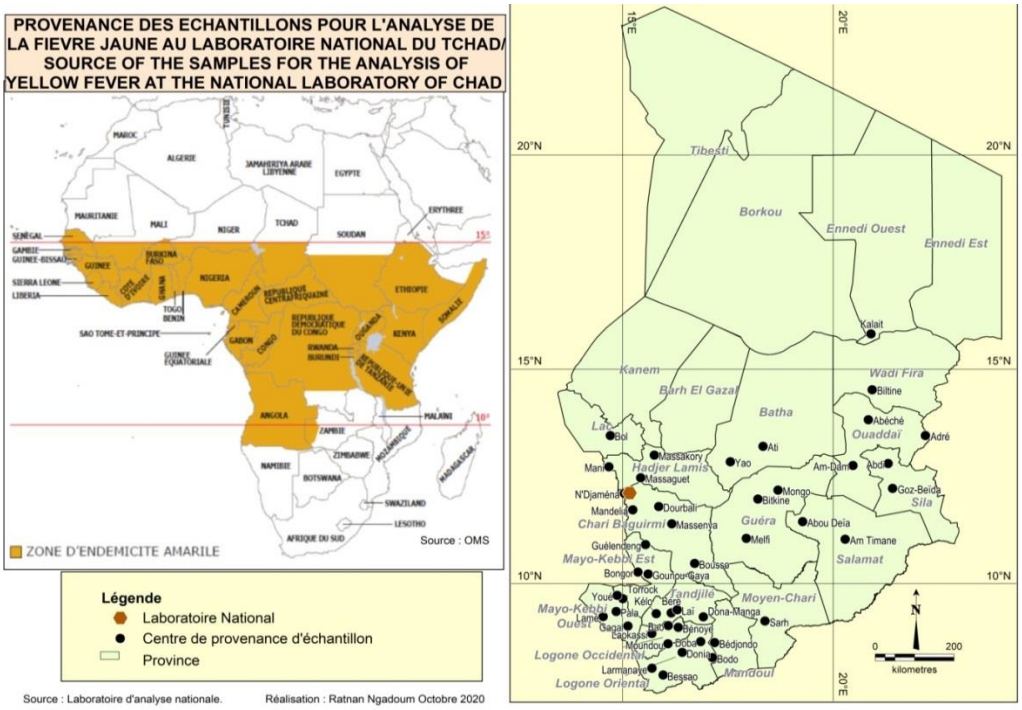

Figure 1. Ecological zones favorable to the development of yellow fever vectors delimited according to rainfall and vegetation.

was collected in 4 mL EDTA tubes from January 2015 to July 2020 in towns and villages in the various provinces of Chad. Whole blood was collected in 4 mL EDTA tubes from January 2015 to July 2020 in towns and villages in the various provinces of Chad. 5 µL of whole blood were taken from each sample for the systematic performance of the rapid diagnostic test (RDT) for *Plasmodium falciparum* malaria.

The blood serum obtained after centrifugation at 3000 revolutions for 5 min was used for the detection of the HBs antigen of the hepatitis B virus with the VIDAS machine. The serum was also packaged in 1.8 mL cryotubes and stored at -80°C for shipment to regional reference laboratories for quality control. The following variables were considered in the people to be sampled such as: sex, age, fever, jaundice, hemorrhage, vaccination status (has the patient received at least one dose of yellow fever vaccine?), marital status, profession, risky behaviors (previous transfusion, surgical procedures, multiple sexual partners, etc.), the system used for serological screening, date of collection, dispatch and receipt of blood samples at the laboratory, areas at risk of yellow fever transmission (presence of mosquitoes). Analysis of these parameters provided a better understanding of the risks of transmission of YF and viral hepatitis.

Data processing

Fisher and Yates Chi-square (χ^2) test was used to compare qualitative variables with a significance level set at 5%.

Microbiological analysis

Presentation of the tests

One Step SD Bioline: The One Step SD Bioline rapid tests were used for the detection of hepatitis C virus (HCV) and *P. falciparum*.

One step anti-HCV SD bioline VHC (standard diagnostics, INC): The MP Diagnostics (MPD) Assure Device (in cassette form) is a rapid immunochromatographic test for the detection of IgM antibodies to hepatitis E virus (HEV). The test was performed according to the manufacturer's instruction. The test is specific for HCV in human serum, plasma and whole blood. The colloidal conjugate and the serum migrate by chromatography along the membrane to the test region (T) and form an antigen-antibody-antigen complex of high sensitivity and specificity which manifests itself as a visible line. This test consists of two marks T (test line) and C (control line), neither of these lines is visible before the test. The control line is used for procedural control and should appear each time if the test is performed correctly.

HEV detection with MP Assure Device HEV IgM rapid diagnostic test: The MP Diagnostics (MPD) Assure Device (in cassette form) is a rapid immunochromatographic test for the detection of IgM antibodies to hepatitis E virus (HEV). The test was performed according to the manufacturer's instruction. The test is specific for HEV in human serum, plasma and whole blood. The colloidal conjugate and the serum migrate by chromatography along the membrane to the test region (T) and form an antigen-antibody-antigen complex of high sensitivity and specificity which manifests itself as a visible line. This test consists of two marks T (test line) and C (control line), neither of these lines is visible before the test. The control line is used for procedural control and should appear each time if the test is performed correctly.

General principle of SD Bioline *Plasmodium* rapid diagnostic test: The SD Bioline Rapid Diagnostic Tests (RDTs) for malaria detect specific antigens produced by plasmodial species. These antigens are: Histidine Rich Protein 2 (HRP2), specific for *P. falciparum*; Lactate dehydrogenase (pLDH) and aldolase produced by all species of *Plasmodiun*.

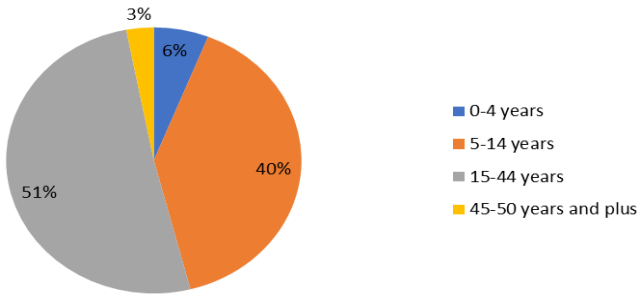

Figure 2. Distribution of samples according to the age group.

Qualitative detection of *P. falciparum* antigen by the SD Bioline *P. falciparum* rapid diagnostic test: The choice for detection of *P. falciparum* is the fact that this species represents 98% of all *Plasmodium* species in Chad (ENIPT, 2017). The *P. falciparum* (whole blood) rapid malaria test device is a qualitative membrane test for the detection of *P. falciparum* in whole blood. The membrane is pre-coated with *P. falciparum*. During the test, the whole blood sample reacts with the dye conjugate, which has been precoated in the test strip. The mixture then migrates over the membrane by capillary action and reacts with the *P. falciparum* on the membrane, at the level of the test line. If the sample contains the *P. falciparum* antigen, a pink line appears in the test area. The absence of a pink line in the test area indicates that the sample does not contain *P. falciparum*. A pink line always appears in the control area; it indicates that an adequate volume of sample has been added and the membrane has been impregnated.

Qualitative determination of HBsAg by the VIDAS machine (BioMérieux)

The VIDAS machine was used for the detection of HBs antigen of hepatitis B virus. The composition and concentration of reagents for HBsAg are: S1: 1 vial containing 1 mL of HBsAg (red cap): standard calibration; C1: 1 vial containing 1.9 mL of HBsAg (white cap): control; C2: 1 vial containing 1.9 mL of HBsAg (white cap): control; Cartridge or strip of HBsAg (single use) containing 10 wells, one of which is for the sample and 9 for the conjugates; HBsAg cone (single use): AC-Ag binding.

The BioMérieux VIDAS machine was used for the detection of Antigen-HBs. A positive and negative quality control system for each run is available to validate a system test kit and internal control for each sample. Single-use, barcode, ready-to-use (reconstitution for some) reagents were used.

The software supplied with the VIDAS system includes programs for analysis and data management. A two-way computer interface automatically transfers results to the user's Laboratory Information System (LIS) and to various product and patient reports. This avoids human errors in reading the results. A quality control system is available to validate a VIDAS system test kit.

As part of the work, VIDAS HBsAg cartridges (BioMérieux) were used for the detection of HBs antigen. The VIDAS has 5 compartments. After collection of whole blood in a dry tube or lithium tube and centrifugation, 150 µl of serum was taken and transferred to the first well of the VIDAS HBsAg cartridge. The cartridge is placed in the corresponding compartment of the VIDAS. The automaton is started by clicking on the appropriate compartment and the test result is expected in 1 h and 21 min.

Qualitative determination of yellow fever virus by ELISA chain

The main test necessary for the confirmation at the national laboratory of the Republic of Chad of yellow fever in an outbreak situation or in the absence of an epidemic outbreak was the enzyme-linked immunosorbent assay (MAC-ELISA-CDC) for the detection of IgM virus, yellow fever. The test was performed following the protocol developed by the American Center for Disease Control and Prevention (CDC) enacted by the World Health Organization.

RESULTS

Origin of samples and mapping of areas at risk for yellow fever

Figure 1 illustrates the geographical origin of blood samples for the detection of yellow fever virus at the national laboratory in Chad. The areas at risk for yellow fever transmission are located between the 10 and 15th parallels. These are forested areas with heavy rainfall containing rivers, lakes, streams favorable to the development of mosquitoes, vectors of yellow fever and associated diseases. In these areas, the virus can circulate continuously and silently between mosquito and monkey populations. It can circulate throughout the year because climatic conditions remain favorable for mosquito activity. In these areas eco-geographic (Figure 1), the mosquito acts as a reservoir and which maintains the presence of the virus by vertical transmission.

Distribution of samples in terms of the category of persons

All sexes are affected. Of the 1730 cases seen and collected in the various health structures of the country, 55.49% (960/1730) were female and 44.51% (770/1730) male (x2 = 6.904> x20> 3, 84, p = 0.01, dof = 1), there is a significant difference in favor of the female sex. The male/female sex ratio in the present study is 1.25 (960/770). All ages were also affected with extremes of 6 days and 75 years. The most affected age group was 15 to 45 years old with 887 cases (51%). It is the working population, the most mobile and certainly the most exposed. Under 15 years (Pediatrics) represent 46% (Figure 2). Note that out of all cases, 29 pregnant women were recorded.

Table 1. Distribution of the proportions of the main diseases associated with jaundice.

Year	NEST	Number of cases of signs (%)				
		Fever	Headache	Vomiting	Yellow conjunctiva	Yellow skin
2015	240	92 (38.33)	111 (46.25)	28 (12)	6 (2.50)	3 (1.25)
2016	340	123 (36.17)	157 (46.17)	54 (16.00)	5 (1.47)	1 (0.30)
2017	320	121 (38.00)	139 (43.43)	57 (18.00)	3 (0.90)	0 (0.00)
2018	250	89 (35.60)	135 (42.18)	25 (1.00)	1 (0.40)	0 (0.00)
2019	340	151 (44.41)	163 (48.00)	24 (7.05)	2 (0.58)	0 (0.00)
2020	240	87 (36.25)	123 (51.25)	30 (12.5)	0 (0.00)	0 (0.00)
Total (%)	1730	663 (38.32)	828 (48.00)	218 (13.00)	17 (1.00)	4 (0.23)

NEST = Number of samples systematically tested at each marker each year; % = percentage.

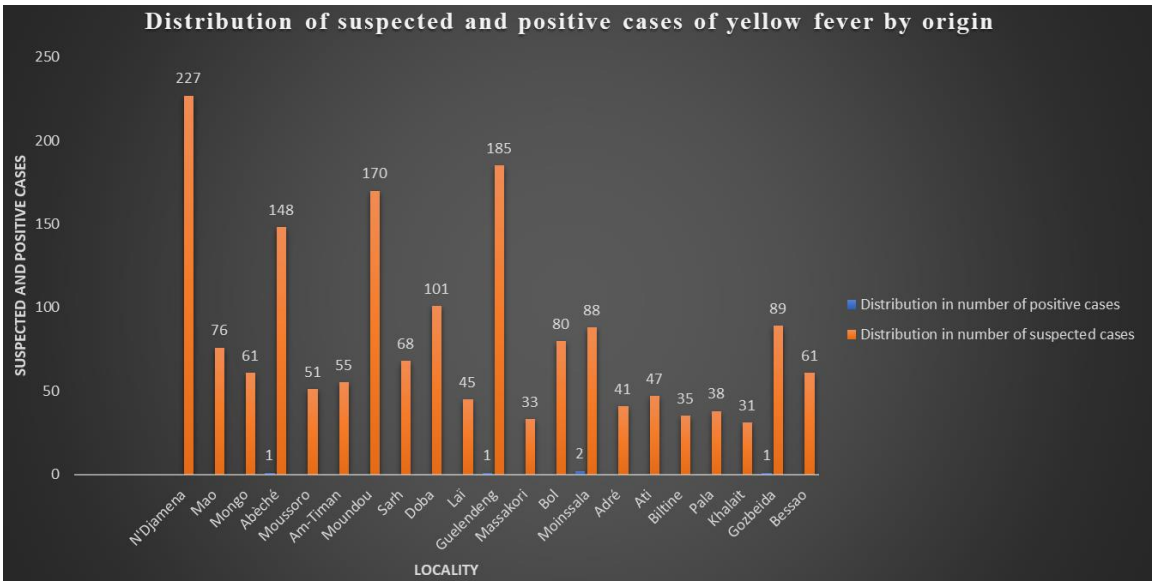

Figure 3. Suspected and confirmed distribution of yellow fever in different localities.

Distribution of proportions of the main symptoms of suspected yellow fever cases

Headache comes first, followed by fever and vomiting. This breakdown showed that the signs suggestive of jaundice are not always linked to yellow fever but associated with other infectious viral and parasitic agents (Table 1).

Distribution of suspected and positive cases of yellow fever by origin

Figure 3 illustrates the frequencies of receipt of suspected cases and confirmed positive cases by the National Laboratory of the Republic of Chad during the study period. The localities of Guelendeng, Laï, Moundou and N'Djamena located in the forest and marshy areas of the Republic of Chad (Figure 1), reported large numbers of suspicious samples: 185, 165, 150 and 127, respectively. The locality of Moinssalla notified two presumptive positive cases followed by Guelendeng, Laï and Bol with one positive case each (Figure 3).

Distribution of the proportions of diseases associated with yellow fever without proof of competence by year

A total, 1730 blood samples were collected for the detection of yellow fever. It emerges from this study that 5/1730 (0.28%) were detected presumptive positive for ELISA-IgMYF by the National Laboratory of Chad, 856/1730 (49.47%) positive for *P. falciparum*, 29/1730 (2%) positive for HEV, 80/1730 (4.62%) positive for HCV and 500/1730 (29%) for HBV, respectively (Table 2). Of the two cases of yellow fever confirmed positive (2/1730 (0.11%)) with the seroneutralization test technique by reduction of lysis plaque (PRNT), one was confirmed

Table 2. Results of the markers tested by years.

Year	NEST	Positive status for markers tested (%)				
		SDBioline Pf	MPDAssure Device IgMVHE	SDBioline Ac VHC	VIDAS-AgHBs	ELISA-IgMYF
2015	240	141 (59.00)	0 (0.00)	6 (2.50)	37 (15.42)	0 (0.00)
2016	340	187 (55)	29 (8.53)	13 (4.12)	42 (12.35)	1 (0.29)
2017	320	191 (60)	0 (0.0)	21 (6.50)	108 (56.25)	0 (0.00)
2018	250	118 (47.2)	0 (0)	13 (5,2)	35 (14.00)	0 (0.00)
2019	340	121 (35.50)	0 (0.00)	16 (4.70)	143 (42.06)	2 **(**0.58)
2020	240	98 (41.00)	0 (0.00)	11 (4.60)	132 (55.00)	2 (0.11)
Total (%)	1730	856 (49.47)	29 (2.00)	80 (4.62)	500 (29.00)	5 (0.28)

NEST = Number of samples systematically tested at each marker each year; % = percentage; Id = undetermined; Pf = *Plasmodium falciparum*, IgMFY = Immunoglobulin yellow fever.

positive at the regional reference laboratory, Center Pasteur of Cameroon (LRRCPC) and the other by the regional reference laboratory, Institute Pasteur de Dakar, Senegal (LRRIPDS). Of the five presumptive positive yellow fever cases, four were unvaccinated and one was of unknown vaccination status. A death was recorded in two cases in the locality of Moinssala as a result of complication of the patient's disease, a case imported from Cameroon since the patient claimed to be back after spending ten years in southern Cameroon and that he was ill only two weeks after arriving in the locality of Nguelendeng in Chad (Figure 3). This proportion showed that malaria was the main cause of consultation in the centers of health facilities in Chad. In 2016, out of a total of 340 samples analyzed, 1 (0.29%) IgMYF was detected by the ELISA chain, 55.00% (malaria), 8.53% (hepatitis E), 4.12% (hepatitis C and 12.35% (hepatitis B) were associated with suspected yellow fever cases, respectively, and infectious markers of hepatitis B, C, E viruses were also noted with yellow fever virus (Table 2).

Distribution of the results of all laboratories

The consistency analysis carried out on the results obtained with the reagents having been used by five laboratories (four techniques concerned), made it possible to identify a certain number of discordant values.

A total of 1,730 whole blood samples were collected and systematically analyzed at the National Reference Laboratory (LNR) of the National Reference University Hospital (CHU-RN) of N'Djamena for the detection of yellow fever, malaria and the virus hepatitis B, C, E. Of the 1,730 samples, nine (9) samples, including three (3) positive, were sent for confirmation to the Regional Reference Laboratory in Dakar, Senegal, but the results obtained from these two laboratories showed no match. Two (2) samples, one of which was detected presumptively positive and the other undetermined by the national laboratory of Chad were confirmed positive and undetermined by the Regional Reference Laboratory of the Pasteur Center of Cameroon and yet the three laboratories (LNT, LRRIPDS and LRRCPC) used the same ELISA technique (Table 3). Twenty-five (25) samples which tested positive in the rapid diagnostic test (MPD Assure Device IgMVHE) were sent to the Sanquin Laboratory in Amsterdam for the confirmation of the hepatitis E virus by ELISA, of which 13 were confirmed positive (IgM), 7 (IgG) and 13 both IgM/IgG, respectively. Sample 007/2016 was detected IgMYF positive on ELISA by the National Reference Laboratory (LNR), undetermined (ELISA), negative (PCR) but positive on the seroneutralization test by reduction of lysis ranges by the Regional Laboratory of Reference (LRR) of Dakar. On the other hand, the sample 389/2015 was declared negative on the ELISA-IgMYF by the National Laboratory of Chad and it was detected positive by ELISA-IgMYF of the Regional Reference Laboratory of Dakar, and seven samples were declared negative by the ELISA-IgMYF by the LNR and were confirmed negative by the Dakar LRR (Table 3).

On October 11, 2016 (Week 38), the Amtiman Health District, a locality in the Salamat province of Chad experienced an outbreak of febrile jaundice, the first case of which was reported on August 1, 2016 (week 31). The Amtiman Health District supported by Médecin Sans Frontière Holland (MSF-H) decided to conduct active research on cases of febrile jaundice in the community and health structures with rapid diagnostic tests (TDR Assure Device IgM) for the detection of the Hepatitis E virus by the laboratory of the Amtiman hospital. This outbreak of febrile jaundice caused an overall case fatality of 4.16% (5/120). From week 37, out of a total of 120 cases of febrile jaundice, 20 RDTs were positive. Samples from the 20 RDT positive cases were sent to N'Djamena to the national surveillance laboratory for confirmation. Faced with information about the lack of a confirmatory test in Chad, and the need to send samples for confirmation outside the country, MSF-H had sent the 20 samples and 5 others to the Sanquin Laboratory in Amsterdam. Of the total of 25 samples, 13 were confirmed IgG and IgM positive by ELISA for Hepatitis E virus (Table 3), 5 samples were negative (IgG and IgM) and 7 samples were negative for IgM but IgG positive indicative of HEV infection.

Table 3. Distribution of the results of all laboratories.

Reference and number of specimen			Positive result of laboratory tests										
			LNTCHUHGRN	LRRIPDS			LRRCPC			LDSST	LSAHPB		
No. LRRIPDS	No. LNT CHUHGRN	Nb	IgMYF (ELISA)	IgMYF (ELISA)	PCR	PRNT	IgMYF (ELISA)	PCR	PRNT	IgMVHE (TDR)	IgMVHE (ELISA)	IgGVHE (ELISA)	IgM/IgGVHE (ELISA)
282199	001/2016		-	-	NF	NF							
282200	003/2016		-	-	NF	NF							
282201	007/2016		+	Ind	-	+1/10							
282202	012/2016		-	-	NF	NF							
282203	013/2016		-	-	NF	NF							
282204	014/2016		-	-	NF	NF							
282205	020/2016		-	-	NF	NF							
282207	380/2015		-	-	NF	NF							
282208	389/2015		-	+	-	-							
LSAHPB													
2016LSHPB	2016LNT	25								20	13	7	13
No. LRRCPC													
20V-13836	144/2020		+				+	NF	+≥320				
20V-13837	187/2020		Ind				Ind	NF	+≥320				

(+) = Positive, (-) = negative, ind = undetermined, NF = not done, Nb = number. PRNT: Seroneutralization test by reduction of lysis plaques; LRRIPDS = regional reference laboratory, Institut Pasteur in Dakar, Senegal; LRRCPP = regional reference laboratory, Center Pasteur in Cameroon; LDSST = Laboratory of the Salamat Chad Health District; LSAHPB = Sanquin Amsterdam Laboratory in Holland/The Netherlands; PCR = Polymerization Chain Reaction; IgMYF = Immunoglobilin M Yellow Fever; ELISA = Enzyme Linked Immunosorbent Assay (Enzyme Linked to Substrate).

DISCUSSION

At the end of this study, which focused on the surveillance assessment of the seroprevalence of yellow fever in Chad, it appears that 5/1730 (0.28%) were detected presumptive positive. This rate is lower than those obtained during yellow fever epidemics by other authors (Kean, 2017; Sylva et al., 2020). For the sample (007/2016), two (2) isolated values were observed for the same technique (MAC-ELISA-CDC) with a titer (1/10) of seroneutralization by reduction of the lysis plaques. Furthermore, there are discrepancies in the results between the laboratories (Poisson et al., 2011). This discrepancy could also be due to pipetting errors by the technicians. On the other hand, for the sample (187/2020), there was no discrepancy for the technique (MAC-ELISA-CDC) with seroneutralization by reduction of the lysis plaques to a high titer (≥320). This result corroborates with laboratory performance evaluation work for the detection of Hepatitis B Virus DNA (HBV-DNA) in terms of inter-laboratory variability (WHO, 2016a; Monath et al., 2015).

This study showed a strong participation of female donors. Female donors were 55.49% and 525 (44.51%) donors were male. The massive participation of women in the study could be explained by the high level of compassion of women towards a sick relative and also by the proportion of women of 52% in Chad (RGPH3, 2014).

The results (Table 2) have sufficiently shown that there are mosquitoes involved in diseases associated with yellow fever in Chad, but without any proof of their competence as vectors. The malaria rate (49.47%) obtained from this study corroborates with the prevalence of malaria in the general population (40.9%) in Chad (ENIPT, 2017). The proportions of seropositivity for viral hepatitis B (29.00%) and C (4.62%) obtained in suspects with jaundice signs could testify to the association of yellow fever with other diseases in terms of markers tested and symptoms (Tables 1 and 2). These results corroborate with the results of previous work carried out by Bessimbaye et al. (2014) on viral hepatitis B and C which reported

similar proportions. In addition, several authors have indexed the involvement of mosquitoes other than *Aedes aegypti* in the urban-rural transmission cycle of yellow fever with association of other diseases (Kean, 2017). The discrepancy in the laboratory results (LNR and LRR) would be linked to these factors (vaccination coverage, cross reactions with other Flaviviruses) which could influence the analysis of the samples since the two laboratories use the same MAC-ELISA technique CDC (Table 3). On the other hand, the tests used did not make it possible to distinguish the antibodies generated against the wild virus (natural immunity) from those aroused by the vaccine virus (acquired immunity), including the seroneutralization test by reduction of the lysis plaques, however more specific. The low frequency of the detection rate for the circulation of the yellow fever virus in Chad could be linked either to the delay in sending samples from most of the different localities (towns and villages) to the National Laboratory, or to the vaccination coverage of certain study areas.

As a result, the usefulness of serological surveys is less in places where vaccination coverage is high. Therefore, one should either avoid sampling where immunization coverage is high or determine by calculation what size of the sample would avoid this problem.

Antibodies from cross-reactions are more likely to be present in older people because older people have probably had more opportunity to come into contact with Flaviviruses in their lifetime than in older people, children, who are less likely to be exposed. Younger children may have antibodies to maternal immunoglobulin G (IgG).

The low frequency of the detection rate of the circulation of the yellow fever virus in Chad could be linked either to the delay in sending samples from the different localities (towns and villages) to the National Laboratory, or to the vaccination coverage of certain areas of the study (Figures 1 and 3). In addition, transport time, vaccination coverage and sample handling have also reported the impact of these factors on the quality of diagnostic results (WHO, 2014; Ruta et al., 2020). As a result, the usefulness of serological surveys is less in places where vaccination coverage is high. Therefore, one should either avoid sampling where immunization coverage is high or determine by calculation how large the sample is to avoid this problem. For localities where the survey could not detect the presence of yellow fever virus, it would be wise to test for the presence of yellow fever virus in mosquitoes (detected by real-time PCR) to isolate the virus.

Conclusion

This is the first National Quality Control operation dedicated to the search for RNA of the yellow fever virus. It involved 4 laboratories which all returned a reply slip.

Overall, there is a number of outlier or isolated inter-laboratory values, showing heterogeneity in the results obtained for the same technique.

Detection of the isolated yellow fever virus remains a major challenge for developing countries and in Chad. The evaluation of the circulation of the yellow fever virus requires, on one hand, a better selection and a better loyalty of the places where the vaccination coverage is less or absent in order to carry out a serological survey and on the other hand, by the method which would make it possible to distinguish the antibodies generated against the wild virus (natural immunity) from those aroused by the vaccine virus (acquired immunity). It would be desirable to avoid sampling where the vaccination coverage is high, or to determine by calculation what is the size of the sample which makes it possible to avoid this problem and also to promote vaccination against the virus, in particular from ages 9 and above, and depending on gender.

To the WHO, CDC Atlanta and CDC Africa which are involved in the research of the yellow fever virus, it is recommended that they should make available the standard MAC-ELISA-CDC kits to the national laboratories to carry out adequate serological analyses for the detection of yellow fever virus IgM and/or RT-PCR tests containing the same reagents with the same concentrations and from the same manufacturing company to perform differential tests (such as dengue viruses, West Nile virus and Zika virus) in order to minimize discrepancies results between national and regional reference laboratories for fever.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

ACKNOWLEDGEMENTS

The authors thank all the people who allowed and facilitated the realization of this investigation in the Health Districts of the different provinces: Yellow fever surveillance focal points for the collection and delivery of samples to the national laboratory; WHO-Chad and MSF-Holland, for sending samples to regional laboratories for quality control and confirmation of results; Mr. RATNAN for drawing up the map of yellow fever for the study areas.

REFERENCES

Abeera A, Fahim A, Yasir R, Nasir A R, Shugufta Y, Irfan N S (2019). Epidemiology of dengue fever and utility of dengue ns1 antigen rapid diagnostic point of care test at combined military hospital malir. Pak Armed Forces Medicine Journal 70 (4):1019-1023.

Amraoui F, Vazeille M, Failloux AB (2016). French *Aedes albopictus* are able to transmit yellow fever virus. Euro Surveillance 21(39):30361.

Bessimbaye N, Moussa AM, Mbanga D, Tidjani A, Mahamat SO, Ngawara MN, Ngarnayal G, Fissou HY, Sangare L, Ndoutamia G, Barro N (2014). HBsAg and Anti-HCV antibody seroprevalence in people infected with HIV1 in N'Djamena, Chad. Bulletin Société Pathologie Exotique 107:327-331.

ENIPT (2017). National Survey on Malaria Indicators in Chad 2017. 165p.

HCSP (2017). Notice on what to do with an imported or indigenous case of yellow fever. Bulletin épidémiologique hebdomadaire (www.hcsp.fr), pp. 1-26.

Hunsperger EA, Muñoz-Jordán J, Beltran M, Colón CJ, Carrión J, Vazquez J, Acosta LN, Medina-Izquierdo JF, Horiuchi K, Biggerstaff BJ, Margolis HS (2016). Performance of Dengue Diagnostic Tests in a Single-Specimen Diagnostic Algorithm. Journal Infectious Disease 214(6):836-844.

Kean S (2017). On the trail of yellow fever. Science 357(6352):637-641.

Monath TP, Pedro FC, Vasconcelos PFC (2015). Yellow fever. Journal of Clinical Virology 64:160-173.

Paules CI, Fauci AS (2017). Yellow Fever - Once Again on the Radar Screen in the Americas. England Journal Medicine 376:1397-1399.

Poisson F, Cordobes DM, Laperche S (2011). Evaluation of laboratory performance for the detection of Hepatitis B Virus DNA (HBV-DNA) in terms of inter-laboratory variability. Ed AFSSAP (www.afssap.fr), pp. 1-13.

RGPH 3 (2014) Second General Population and Housing Census. N'Djamena. INSEED, pp. 1-164.

Ruta K, Meera Modak , Mrunal Gosavi , Dileep Wani , Akhilesh C Mishra , Vidya A Arankalle (2020). Comparative assessment of commercial enzyme-linked immunosorbent assay and rapid diagnostic tests used for dengue diagnosis in India. Indian Journal Medicine 151(1):71-78.

Santiago GA, Vazquez J, Courtney S, Matias KY, Andersen LE, Butler AE, Roulo R, Bowzard J, Villanueva JM, Munoz-Jordan JL (2018). Performance of the Trioplex real-time RT-PCR assay for detection of Zika, dengue, and chikungunya viruses. Nature Communication 9(1):1391-1401.

Sylva NIO, Sacchetto L, de Rezende IM, Trindade GS, LaBeaud AD, de Thoisy B, Drumond BP (2020). Recent sylvatic yellow fever virus transmission in Brazil: the news from an old disease. Virology Journal 17(1):1277-1289.

WHO (2014). Assessment of the risk of circulation of the yellow fever virus in endemic countries 2nd Report: 1-54.

WHO (2016a). "Yellow fever: epidemiology 2015". Report, pp. 1-38. www.who.int.

WHO (2016b). Laboratory diagnosis of yellow fever in Africa 1:1-7.

WHO (2018). A global strategy to Eliminate Yellow fever Epidemics (EYE) 2017 – 2026. Geneva: World Health Organization: 1-56. www.signalement-moustique.fr (2017). Tiger Mosquito Reporting Portal (*Aedes albopictus*).

Vol. 15(3), pp. 161-170, March, 2021
DOI: 10.5897/AJMR2021.9498
Article Number: 596529A66411
ISSN: 1996-0808

http://www.academicjournals.org/AJMR

ACADEMIC JOURNALS
expand your knowledge

African Journal of Microbiology Research

Full Length Research Paper

Degradation of lignocellulosic content of rice straw using aerobic cellulolytic bacteria isolated from forest soil of Bangladesh

Md. Rashidul Islam[1]*, Md. Morshedur Rahman[2], Shilpi Islam[1], Shabbir Ahmed[2], Mueena Jahan[3], Sulav Indra Paul[4], and Abu Sadeque Md. Selim[1]

[1]Department of Animal Science and Nutrition, Faculty of Veterinary Medicine and Animal Science, Bangabandhu Sheikh Mujibur Rahman Agriculture University (BSMRAU), Gazipur 1706, Bangladesh.
[2]Department of Dairy and Poultry Science, Faculty of Veterinary Medicine and Animal Science, BSMRAU, Gazipur - 1706, Bangladesh.
[3]Department of Microbiology and Public Health, Faculty of Veterinary Medicine and Animal Science, BSMRAU, Gazipur -1706, Bangladesh.
[4]Institute of Biotechnology & Genetic Engineering (IBGE), Faculty of Agriculture, BSMRAU, Gazipur -1706, Bangladesh.

Received 8 February, 2021; Accepted 23 March, 2021

The present research work was conducted to enumerate and isolate aerobic cellulolytic bacteria from forest soil using carboxymethyl cellulose (CMC) as substrate. The identified isolate was further tested as potent candidate to improve the nutritional value of rice straw. The forest soil was loaded with substantial amount (2.45×10^7) of cellulolytic bacteria. A total of 10 cellulose degrading bacteria (CDB) were isolated, identified and monitored for their cellulolytic activity. The isolate having the highest cellulolytic index (2.5) was identified as *Bacillus subtilis* targeting the 16S rRNA gene which was labeled as *B. subtilis* strain CDB7 and investigated for nutritional improvement of rice straw. Solid state fermentation of each group was carried out at 37°C for a period of 0, 3 and 6 days in 0 (no bacterial inoculum), 1, 5 and 10% inoculum group. There were a tendency of reduction in Acid Detergent Fiber (ADF), Neutral Detergent Fiber (NDF) and lignin at concentrations of 1, 5 and 10% inoculums at every interval of fermentation. Highest reduction of ADF (4.8%), NDF (10.78%) and lignin (37.6%) were observed after 6 days of fermentation at 10, 10 and 5% inoculum group, respectively. The crude protein content was increased (5.3 ± 0.4 to 6.4± 0.3) in 10% inoculum group. Taken together, the identified isolate could be a potent candidate to degrade lignocellulosic content through breaking of lignin-cellulose bondage and to improve the nutritional value of rice straw.

Key words: Rice straw, *Bacillus subtilis* strain CDB7, solid state fermentation, acid detergent fiber (ADF), neutral detergent fiber (NDF), lignin.

INTRODUCTION

The global demand for livestock products is expected to double by 2050 (Conforti, 2011). However, the acute scarcity of feeds and fodder has been considered as one of the biggest problems in the development of livestock

production. Because more than 70% expenditures are incurred on feed while raising livestock, a huge gap in demand and supply of feeds and fodders in many developing countries including Bangladesh has already been reported (Huque and Sarker, 2014). Ruminant nutrition is mostly dependent on naturally grown grasses, various crop residues like straws, stovers, stalks and cereal by-products. Rice straw is considered as the prime crop residue roughage feed which farmers usually store and use in tropical areas especially during the long dry season. However, rice straw is often termed as very poor quality roughage feed due to low protein content (2 to 5%), fiber and lignin contents (Neutral Detergent Fiber (NDF) > 50%) and also having low digestibility (<60%). The microbial treatments of rice straw can improve the accessibility and is considered as a cheap and sustainable approach. Thus improving their digestibility and feeding value have been attracting the extensive interests among researchers (Zhang and Lynd, 2004); although this process has a long history. The reason behind the low degradability, rice straw consists of cellulose, hemicelluloses and lignin that are strongly intermeshed and chemically bonded in its structure. So feeding rice straw only provides inadequate nutrients for optimum production requirements for livestock (Wanapat et al., 2013). However, the use of crop residue as animal feed is limited due to its structure, low nutritive value, and high structural carbohydrate content (Chanjula et al., 2017).

The breakdown of cellulose is performed by the enzyme called cellulase which is generally secreted by anaerobic cellulolytic bacteria found in the rumen. They also produce some lignocellulytic enzymes which make more easily available nutrients to animal. These enzymes also were reported to be produced by fungi, bacteria, and protozoans existing in the environmental samples (Immanuel et al., 2006). This enzyme is produced by several microorganisms, commonly by many cellulolytic bacteria. Cellulolytic enzymes are synthesized by a number of microorganisms. Abdel-Rahman et al. (2016) showed an efficient composting process of rice straw when inoculating with mixed culture of *Bacillus* species that reduced the composting time by 40 to 43%.

For aerobic and anaerobic mesophilic bacteria, certain protozoa are able to utilize lignocellulose in different fibrous feed (Alexander, 1978) and they play an important role in conversion of lignocelluloses in straw into readily available nutrients for the animal.

The major obstruction in biological conversion of lignocelluloses is the physical protection of cellulose by lignin against cellulolytic enzymes. As lignocellulytic bacteria have been isolated and characterized from variety of sources such as soil, organic matters, decayed plant materials, feces of ruminants and composts, therefore, soil might be the good source of aerobic cellulolytic bacteria. Cellulolytic properties of some bacterial genera such as *Cellulomonas*, *Pseudomonas*, *Bacillus* and *Micrococcus* species were reported (Abou-Taleb et al., 2009). Recently, probiotics are randomly used to increase the nutritional value of fiber constituents of roughage. So, cellulolytic bacteria can play an active role to degrade the fibrous part (Acid Detergent Fiber (ADF), NDF and lignin) of rice straw by producing the enzyme. This will convert fiber into readily available nutrients.

The aim of this study was to improve the nutritional value of rice straw to make more available nutrients for ruminants by using cellulase producing aerobic cellulytic bacteria from forest soil.

MATERIALS AND METHODS

Soil sample collection and preparation

Soil samples were collected from the Bhawal National Forest, Gazipur, Bangladesh. For each sample, soil was first dug out with clean shovel up to 10 cm depth using disposable and sterile spatula. Soil was then mixed thoroughly and foreign materials like tree roots, leaves were removed. Approximately 50 g was transferred to a sterile zip lock bag. All the soil samples after collection were properly sealed, labeled and sent to the laboratory where they were kept at 4°C. At first the soil sample was ground to fine particles and 10 g of the sample was added in 90 mL of distilled water and was shaken thoroughly. After the proper mixing, the suspension was filtered to remove the unwanted portion of the soil. The filtrated suspension was used for the isolation of bacteria.

Enumeration and isolation of cellulolytic bacteria

The carboxymethylcellulose (CMC) agar medium containing 1.0% peptone, 1.0% CMC, 0.2% K_2HPO_4, 1% agar, 0.03% $MgSO_4.7H_2O$, 0.25% $(NH_4)_2SO_4$ and 0.2% gelatin adjusted to pH 7.0 was used for enumeration as well as isolation of aerobic cellulolytic bacteria. Serially diluted soil suspension was spread onto CMC agar plate and incubated at 37°C for 48 h. The total cellulolytic bacterial load was expressed as colony forming unit per gram (cfu/g). For isolation, from each highest dilution, plate colonies having different shapes were selected and purified by repeated streaking onto the same agar media. The purified isolates were Gram stained and then preserved at 4°C in agar slant media for further study.

Congo red counter staining

After grown overnight in nutrient broth, each pure isolate was

*Corresponding author E-mail: rashedasn@bsmrau.edu.bd, Tel. +88-01760374783.

Table 1. Total viable cellulose-degrading aerobic bacterial load.

Type of soil	Viable bacterial load (log cfu/g)
Forest soil	7.4[a]
Farming soil	5.1[b]

Means with different superscripts within a column are significantly different ($p < 0.05$).

spread onto CMC agar plates followed by incubation at 37°C for 48 h. The CMC agar plates were then flooded with 1% Congo red and allowed to stand for 15 min at room temperature. The plates were counterstained with 1 M NaCl solution. Clear zones appeared around grown colonies indicating cellulose hydrolysis.

Measurement of hydrolytic capacity

The hydrolytic capacity of each isolate was determined by measuring the diameter of the colony and surrounded clear zone. The hydrolytic capacity was expressed as cellulytic index calculated from the differences of diameter of hydrolytic zone and bacterial colony divided by diameter of bacterial colony (Ferbiyanto et al., 2015). The isolate having the highest hydrolytic capacity was selected for molecular identification and degradation ability of rice straw under solid-state-fermentation.

Molecular identification

The isolate having the highest hydrolytic capacity was used for molecular identification. The isolate was cultured in nutrient broth at 37°C for 48 h. After incubation, the genomic DNA of the isolate was extracted using a commercial DNA Purification Kit (Thermo Fisher Scientific, USA) following the manufacturer's protocol. The PCR reaction mixture was prepared as a volume of 100 µL containing 6 µL of 25 mM $MgCl_2$ (Thermo Fisher Scientific, USA), 10 µL of 10× Dream *Taq* buffer (Thermo Fisher Scientific, USA), 2 µL of 10 mM dNTP mix (Thermo Fisher Scientific, USA), 5 µL each of forward primer (27F: 5'-AGAGTTTGATCATGGCTCAG-3') and reverse primer (1492R: 5'-AGGAGGTGATCCAACCGCA-3') (Thermo Fisher Scientific, USA), 5 µL of template DNA, 1 µL of *Taq*DNA polymerase (Thermo Fisher Scientific, USA) and 66 µL of nuclease-free water were used for the amplification of 16s rRNA universal sequence (Frank et al., 2008). The PCR amplification of 16S rRNA gene was performed in a PCR Thermocycler (Applied Biosystems, Thermo Fisher Scientific, USA). The thermal profile for PCR was set as follows: an initial denaturation step at 94°C for 5 min; 35 cycles of a denaturation step at 94°C for 1 min, an annealing at 57°C for 40 s and an extension at 72°C for 1 min and a final extension step at 72°C for 10 min. The amplified PCR product was purified by using a commercial PCR Purification Kit (Thermo Fisher Scientific, USA) following the manufacturer's protocol. Raw sequence reads were analyzed by using MEGA 7.0. Sequence homologies of the bacterial isolates with closest similar isolates were determined by using web-based BLAST (Basic Local Alignment Search Tool) program of the NCBI website (ncbi.nlm.nih.gov/BLAST). After analysis, the FASTA sequences are submitted to the NCBI database for GenBank accession number. Phylogenetic trees were prepared by neighbor joining method in MEGA7.

Soild-state fermentation of rice straw

The solid-state-fermentation of rice straw was carried out using the isolate having highest hydrolytic capacity. The fermentation was arranged according to a factorial arrangement in a complete randomized design (CRD). A total of four treatments comprising (1) untreated rice straw, (2) rice straw treated with 1% inoculum, (3) rice straw treated with 5% inoculum, and (4) rice straw treated with 10% inoculum were performed. The rice straw collected from the local market was ground into 2 to 3 mm length using a mechanical grinder which was then sterilized by autoclaving at 121°C for 15 min. One hundred fifty grams of sterilized rice straw was taken in a Ziplock bag and mixed with sterilized distilled water at a ratio of 3:1. The isolate having the highest cellulolytic index was grown overnight in the CMC broth at 37°C for 48 h. Then, the CMC broth without bacteria added to the control group and CMC broth having 1.2×10^{10} cfu/ml bacteria was added at 1, 5 and 10% in the treatment group. Then, all the bags were placed in an incubator at 37°C and kept for a period of 6 days.

Estimation of changes in rice straw composition during fermentation

The changes of composition in rice straw were estimated at every 3 days interval of solid-state fermentation. The DM, CP, ash, ADF, NDF and lignin of rice straw in both control and treated groups were measured according to AOAC (2000).

Statistical analysis

Data were recorded, checked for completeness and consistency and subjected to analysis using Excel and R (4.0.2 version) software packages. One-way ANOVA was used for multiple mean comparisons. $P< 0.05$ was considered significant during the analysis.

RESULTS AND DISCUSSION

Viable cellulolytic bacterial load in soil samples

The total aerobic cellulolytic bacterial load in pooled forest soil samples were 7.4 log cfu/gm. The bacterial load was also compared with the farming soil sample (Table 1) which showed a significant difference among the samples suggesting the forest soil as a predominant source of cellulolytic bacteria.

The number of colonies in forest soil was higher than of the load of farming soil. Hatami et al. (2008) found the number of colonies forming unit was 138 in forest soil and in farming soil it was 126 which indicates forest soil contain more load of cellulytic bacteria. The organic matter in soil is utilized as energy and carbon resources by cellulolytic bacteria. Higher number of cellulolytic bacteria in forest soil can be attributed to higher organic carbon content in forest soils. The number of cellulolytic bacteria in forest soil samples is more than farm soils due to the type of organic matter in forest soils. This also may

Table 2. Characteristics of cellulose-degrading aerobic bacteria from the forest soil.

Name of isolate	Colony characteristics		Cellulytic index
	Morphology	Gram staining	
CDB1	Thin, watery, small round colony	Negative	ND
CDB2	Watery, medium round colony	Negative	ND
CDB3	Thick, round, light cream color colony	Negative	0.26
CDB4	Thick, round, light cream color colony	Negative	0.62
CDB5	Very small round shaped, pinpoint colony	Negative	1.04
CDB6	round shaped, pinpoint colony	positive	ND
CDB7	Large, round but outside branching, thick, cream color colony	positive	2.50
CDB8	Thick, round, light cream color colony	Negative	ND
CDB9	Thick, round, light cream color colony	Negative	ND
CDB10	Thin, round, light cream color colony	positive	ND

ND: Not detected.

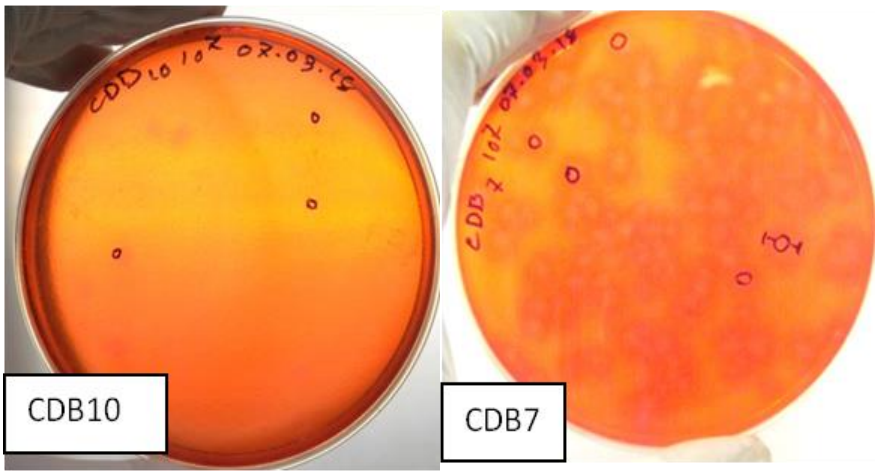

Figure 1. Representative cellulolytic index (z-c)/c (mm) of different CDB isolates.

be due to the fact that micronutrients discharged into the forest soil through plant wastes reduce the porosity of the soil.

Morphological characteristics and hydrolytic capacity of the isolate

After spreading forest soil suspension on CMC agar plates, a total of 10 colonies were isolated randomly. The isolates were labeled as CDB1, 2, 3….CDB10. As shown in Table 2, the isolates varied in shape and color (round, elongated, watery, and cream). Of the 10 isolates, 3 were identified as Gram positive and the remaining 7 were Gram negative. Out of the 10 isolates, only 4 showed hydrolytic capacities and produced a clear hydrolytic zone around the colonies (Figure 1). Conversely, the isolates having less/no hydrolytic capacity showed absence of such zone (Figure 1). The highest cellulolytic index was observed by the isolate CDB 7 indicating the ability to produce cellulase enzyme. Consequently, the isolate was selected to test as a candidate for improving the nutritional value of rice straw and also to identify at molecular level.

In the cellulolytic Index study, the highest value was 2.5 found in CDB7 and the lowest value was 0.2 in CDB3. This finding was similar to Ferbiyanto et al. (2015). Ferbiyanto et al. (2015) measure cellulolytic activity of bacterial isolates based on a clear zone of degraded CMC area around the colony. Cellulolytic activity test showed that the isolate has the largest cellulolytic index of 2.5 and the isolate has the smallest cellulolytic index of 0.75. Cellulytic index is an indicator of producing cellulase enzyme and based on these criteria, CDB7 was

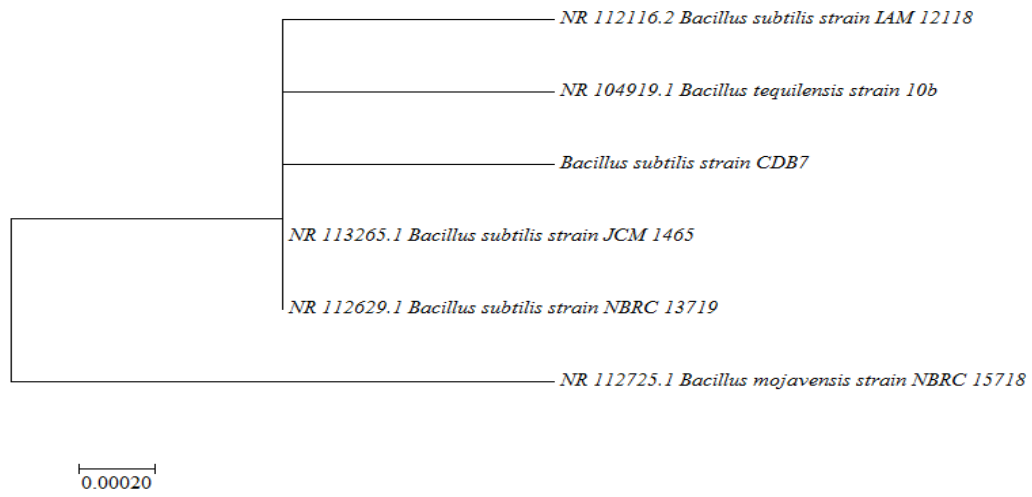

Figure 2. Phylogenetic tree of *Bacillus subtilis* strain CDB7.

selected to be used in the solid state fermentation. CDB7 showed hydrolysis capacity of 2.5 (Table 2) in the Congo red assay having similarities with Khianngam et al. (2009) who isolated cellulolytic bacteria of *Bacillus*, *Lysinibacillus* and *Paenibacillus* genus from the sample of oil palm meal, which showed cellulolytic index in the range from 1.56 to 4.14 in the Congo red assay. Maki et al. (2011) showed that cellulolytic bacteria were isolated from soil samples from natural reserves producing zones of hydrolysis in Congo red method and found *Paenibacillus terrae* ME27 as the highest cellulase producer. The appearance of the clear zone around the colony when the Congo red solution was added (Wood and Bhat, 1988) was strong evidence that the bacteria produced cellulase in order to degrade cellulose.

Hydrolytic capacity of the isolates

Four isolates (CDB3, CDB4, CDB5 and CDB7) out of ten showed hydrolyzing capacity on agar plates containing CMC as core carbon source, after Congo-red staining. The hydrolyzing zone diameter and colony diameter were listed as shown in Table 2.

In Figure 1, it is clear that cellulolytic bacteria produced cellulase enzymes and hydrolyzed the agar media around its colony. The bacterial colony produced a hydrolytic zone and showed differential cellulolytic index. In the case of *Bacillus subtilis* strain CDB7 colony's highest clear zone was found.

Molecular characterization

The 16S rRNA gene sequencing data of the isolate CDB7 exhibited 99.79% homology with *B. subtilis*. In the phylogenetic tree analysis, *B. subtilis* strain CDB7 formed clusters with its corresponding species (Figure 2). The accession number of *B. subtilis* strain CDB7 is MW159692.

Rice straw fermentation

According to the highest hydrolytic capacity and the result of biochemical test CDB7 was selected for the further fermentation study. These isolates were selected for the solid state fermentation in rice straw. At first the pure culture of CDB7 was grown in the CMC broth for 48 h and then applied to rice straw to be fermented.

The physical appearance (color and smell) of rice straw during solid state fermentation with the target isolate (CDB7) for a period of 6 days were observed. The color of straw changed from brownish yellow to yellowish green (Figure 3). The smell was slightly acidic in the treated group compared to untreated. These indicate bacteria might have some action on rice straw. However, no fungal appearance was observed in any group during the entire fermentation period.

Improvement of nutritional value of treated rice straw

During the solid state fermentation for a period of 6 days the proximate composition of both rice straw samples treated with or without the target isolates were measured at every 3-days interval. The parameters of the proximate component included dry matter (DM), ash and crude protein (CP) shown in Table 3.

Among the proximate component, the CP content was

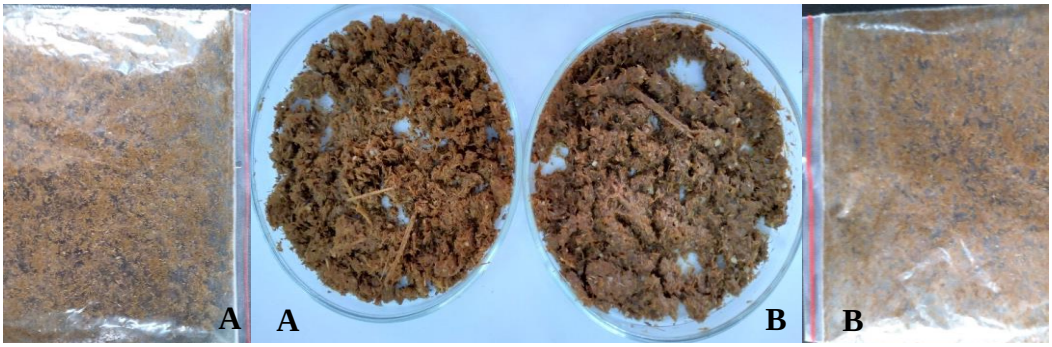

Figure 3. The change of color due to solid state fermentation of rice straw with *Bacillus subtilis* strain CDB7; A: Control, B: Treated.

Table 3. Proximate composition of the samples during solid state fermentation.

Inoculum level (%)	Fermentation period (days)	DM (%)	CP (% of DM)	Ash (% of DM)
0 (control)	0	23.4±0.4^{a}	5.4±0.6^{a}	16.1±1.2^{a}
	3	23.5±0.6^{a}	5.5±0.6^{a}	15.8±0.7^{a}
	6	23.5±1.16^{a}	5.4±0.5^{a}	15.4±1^{a}
1	0	21.51±0.8^{c}	5.5±0.4^{a}	16.8±1.2^{a}
	3	24.3±0.7^{a}	5.5±0.4^{ab}	14.6±0.7^{b}
	6	22.9±0.7^{b}	6.2±0.6^{b}	13.7±0.7^{c}
5	0	23.6±1.1^{a}	5.6±0.5^{a}	15.8±1.1^{a}
	3	22.1±1.2^{a}	6.2±0.8^{b}	14.2±0.7^{b}
	6	22.0±1.3^{a}	6.4±0.7b	13.1±0.5^{c}
10	0	22.9±1.1^{a}	5.3±0.4^{a}	16.5±0.9^{a}
	3	24.2±1.25^{a}	6.3±0.4^{b}	14.8±0.5^{b}
	6	23.7±2.17^{a}	6.4±0.3^{b}	15.5±0.2^{b}

*Values are expressed as mean ± SD (n = 3). Means with different superscripts within a column are significantly different ($p < 0.05$).

increased significantly with the increment of inoculum level as well as fermentation period as compared to control group. The highest CP% was observed after 6 days of fermentation in 10% inoculum groups (5.36±0.4 to 6.4±0.3). At the beginning of fermentation, the CP content was almost similar to all groups (5.3 to 5.6). The CP content was increased sharply after 3 days of fermentation and then the trend of increment was appeared to be static. These findings showed the similarities with the Sembiring et al. (2002), showed that the level of crude protein content of rice straw fermented with probiotic was 5.63 and it was in the range of other reports that was 5.63 to 11.25%.

The highest reduction (18.5%) of ash content was found in the 1% level of fermentation. The results are in agreement with Syamsu et al. (2013). Syamsu et al. (2013) showed that the ash value decreased from 22.34 to 20.19 (% of DM) when rice straw fermented with a starter of microbes. The present findings are also supported by Sariubang et al. (2002) who found the ash value decreased range 22.54-33.50% to 21.89-31.02% after treating with the probiotics. Akter et al. (2013) found that rice straw contained 15.02% ash which changed very little to 15.06, 15.09, 15.12 and 15.15% by the treatment with different concentration of urea and midden soil (3.0% urea + 2.0% midden soil, 3.0% urea + 3.0% midden soil, 3.0% urea + 4.0% midden soil and 3.0% urea + 5.0% midden soil, respectively).

ADF

ADF indicates the least digestible plant components which include cellulose and lignin and insoluble ash

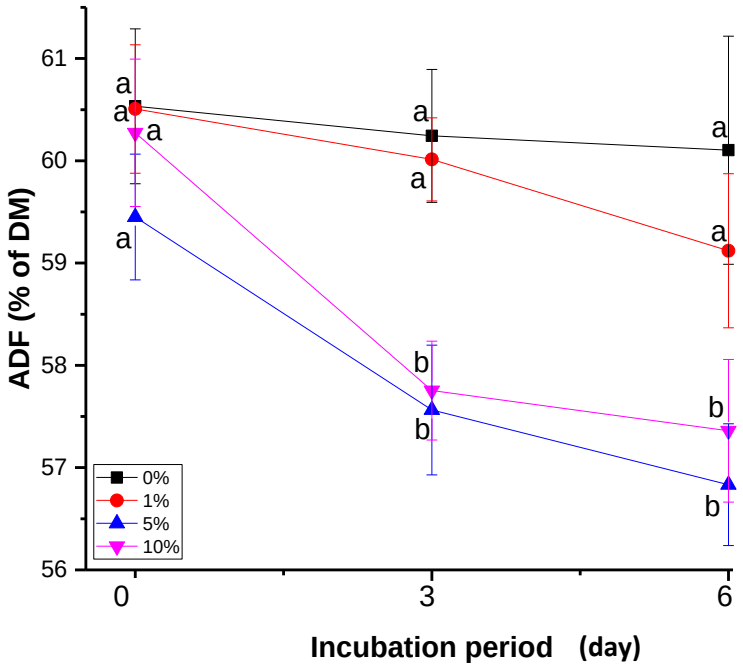

Figure 4. Degradation of ADF fraction of fiber in rice straw by *Bacillus subtilis* strain CDB7 isolates.

(mainly silica). The ADF values are inversely related to digestibility of a specific forage. Therefore, forages with low ADF concentrations usually contain higher amount of energy. The ADF value in the control and treated group of rice straw for 0, 3 and 6 days of fermentation is as shown in Figure 3. The ADF content was decreased from 60.5±0.6 to 59.1±0.8, 59.5±0.6 to 56.8±0.6 and 60.3±1.1 to 57.4±0.7 in 1, 5 and 10% inoculum level group, respectively. In control group, the ADF content was almost unchanged throughout the fermentation period. The highest reduction of ADF (4.8%) was observed in 10% of inoculum level group after 6 days of fermentation.

Figure 4 shows that ADF value was affected by treatment with different concentrations of inoculums at 0, 3 and 6 days showing similarities with Syamsu et al. (2013), who found that the ADF value decreased from 57.76 to 47.64 when treated with microbial starter.

NDF

NDF indicates structural components of the plant specially the cell wall which contains hemicellulose, cellulose, lignin, and insoluble ash. The level of NDF in the animal ration influences the animal's intake of dry matter. It is an important parameter to measure the relative feed value. As shown in Figure 4, the NDF value of rice straw decreased greatly from 90.7±1.1 to 87.7±1.4, 91.1±0.7 to 81.6±1.7 and 90±0.9 to 80.3±1.4 in 1, 5, and 10% of inoculums group, respectively. The highest percentage (10.78%) of NDF reduction was observed after 6 days of fermentation in 10% inoculums group (Figure 5).

These results of NDF are in agreement with the results of Bansi et al. (2012) and Sariubang et al. (2002). Bansi et al. (2012) showed that the decreased crude fiber level of rice straw fermented with commercial probiotic (P1) was decreased 6.07% than control. It was lower than reported by Sairubang et al. (2002) who reported that crude fiber content in rice straw fermented using probiotics decreased by 25.73 and 14.79%. Sariubang et al. (2002) found that the NDF value decreased from 79.78 to 77.0 when treated with probiotics.

The NDF was also decreased about 2% when compared with untreated sugarcane bagasse after treatment with a combination of cellulase, TH14 and molasses (Yoo et al., 2020). Though enzymes were often added with a variety of bacterial inoculations in their experiment, the decreased cellulose and lignin level of rice straw fermented interpret that probiotic microbes are able to penetrate the fibrolytic structure of rice straw and detach the binding of lignified carbohydrate and in some extent, degrade cellulose and hemicellulose. Selim et al. (2017) showed that the NDF content was reduced by probiotics at days 2 to 4. It was also reduced by urea, *Trichoderma* and *Aspergillus* treatments at all-time points.

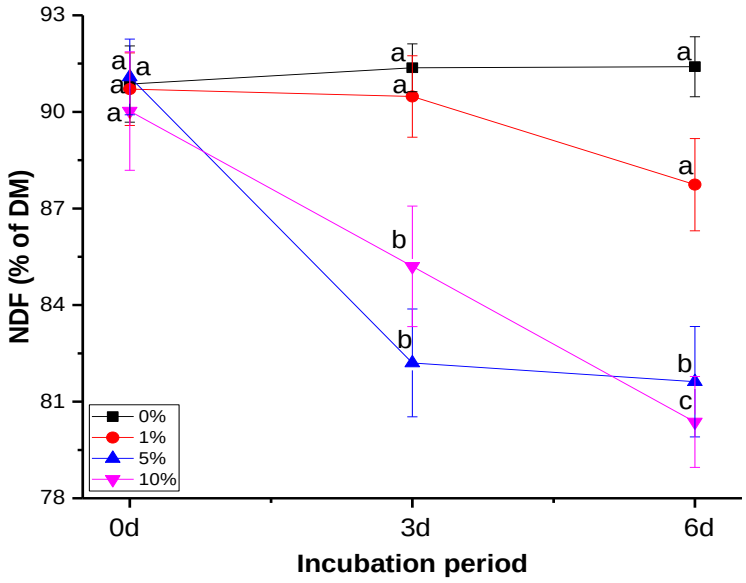

Figure 5. Degradation of NDF fraction of fiber in rice straw by *Bacillus subtilis strain CDB7* isolates.

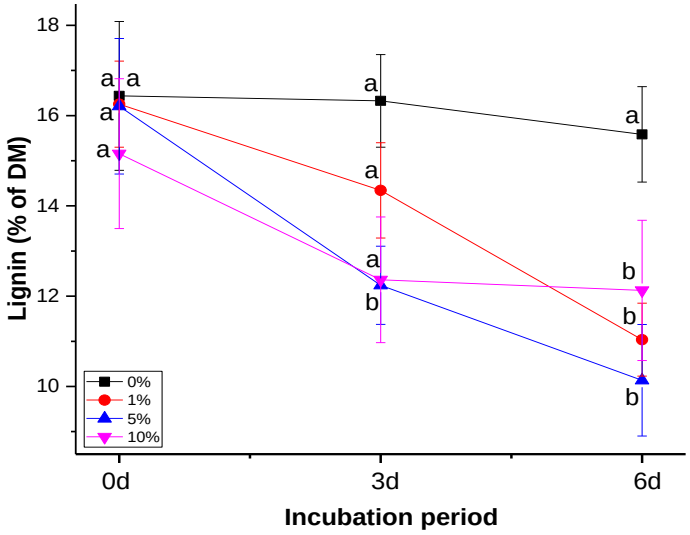

Figure 6. Degradation of lignin fraction in rice straw by *Bacillus subtilis strain* CDB7 isolates.

Lignin

Lignin is a cell wall component in plant and is considered an anti-nutritive component of forages as it cannot be readily fermented by rumen microbes. In terms of energy yield from biomass, the role of lignin depends on the conversion process. It reduces the total digestibility of forage. Depending on the kind of plant, lignin makes up approximately 10 to 25% of lignocellulosic biomass and is the second most abundant natural polymer (Min et al., 2013). The lignin content in the control and treated group of rice straw for 0, 3 and 6 days of incubation is as shown in Figure 6.

The lignin value decreased from 16.3±1.9 to 11.1 ± 0.8, 16.2±1 to 10.1 ± 1.2 and 15.2 ±1.1 to 12.1 ±1.6 in 1, 5, and 10% of inoculums group, respectively. The highest reduction (37.6%) of lignin was found in the 5% level of inoculum group after 6 days of fermentation.

It showed the similarities with Syamsu et al. (2013), who found that the lignin value decreased from 8.13 to 4.96 when treated with microbial starter. Bansi et al. (2012) reported that the enzyme activity of cellulolytic microbes in probiotics caused degradation, reorganization, expansion and break of bonded lignin with the cell wall of rice straw.

Zainudin et al. (2013) found 27 cellulolytic bacterial strains of which 23 strains were closely related to *B. subtilis*, *Bacillus firmus*, *Thermomonospora* species, *Thermobifida fusca*, *Cellulomonas* species, *Ureibacillus thermosphaericus*, *Paenibacillus barengoltzii*, etc. These organisms were known as lignocellulose degrading bacteria and commonly involved in lignocellulose degradation.

Wang et al. (2008) showed that *B. subtilis* has the ability to decompose lignin, phenolic and non-phenolic lignin compounds having low molecular weight. The degradation rate was 9.47% in lignin and 38.8 and 41.84% degradation found in case of cellulose and hemicellulose, respectively.

The results indicated that the structure of rice straw was destroyed when treated with *B. subtilis* and degradation reaction was different in different groups. Several studies (Syamsu et al., 2013; Yoo et al., 2020) have interpreted that this fermentation synergistically and positively improved fermented material quality, which resulted in more soluble carbohydrates, and further improves feed efficiency.

The NDF, ADF and lignin are the least digestible fractions which represent the cellulose, hemicellulose and some ash content of rice straw. The quantity of these fractions reduced significantly when treated with *B. subtilis* strain CDB7 suggesting the availability of more nutrients for ruminants. Thus, this microbial treatment is expected to improve the quality of rice straw which in turn will help ruminants for better performances.

Conclusion

The present study was conducted to isolate the aerobic cellulolytic bacteria from the forest soils of Gazipur, Bangladesh. Out of 10 cellulolytic isolates only one showed the highest hydrolytic capacity and was identified as *B. subtilis* strain CDB7. The hydrolytic capacity suggests the ability of the isolate to produce enzymes that can utilize cellulose and hemicellulose as substrate when available in the media. The solid state fermentation of rice straw with the target isolate showed the significant reduction in ADF, NDF and lignin content which suggests more available sugars in treated rice straw. The cellulolytic bacteria obtained from this study not only degrade the cellulose but also lignin suggesting the diverse role of the isolate. Forest soil could therefore be a good source microbe for degradation of lignocellulosic materials degrading microbes and enzymes. The treatment of rice straw using such cellulolytic bacteria could be adopted by farmers to mitigate the scarcity of ruminant nutrition.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

ACKNOWLEDGEMENT

The authors thank the Department of Animal Science and Nutrition, Department of Dairy and Poultry Science, Bangabandhu Sheikh Mujibur Rahman Agricultural University, Gazipur-1706 for their technical support.

REFERENCES

Abdel-Rahman MA, El-Din MN, Refaat BM, Abdel-Shakour EH, Ewais EED, Alrefaey HM (2016). Biotechnological application of thermotolerant cellulose-decomposing bacteria in composting of rice straw. Annals of Agricultural Sciences 61(1):135-143.

Abou-Taleb KA, Mashhoor WA, Nasr SA, Sharaf MS, Abdel-Azeem HH (2009). Nutritional and environmental factors affecting cellulase production by two strains of cellulolytic Bacilli. Australian Journal of Basic and Applied Sciences 3(3):2429-36.

Akter M, Khan MJ, Akbar MA, Asaduzzaman M (2013). Improving quality of rice straw through treatment with urea and midden soil for large animals. Bangladesh Journal of Animal Science 42(1):29-34.

Alexander M (1978). Introduction to soil microbiology. Soil Science 125(5):331.

Association of Official Analytical Chemists (AOAC) (2000). Official methods of analysis. Association of Official Analytical Chemists Vol. 11, 17th edition, Washington DC, USA.

Bansi HA, Risiyanto R, Indriawaty RA (2012). Use of microbes to improve nutritional value of rice straw. In International Conference on Livestock Production and Veterinary Technology pp. 99-103.

Chanjula P, Petcharat V, Cherdthong A (2017). Effects of fungal (Lentinussajor-caju) treated oil palm frond on performance and carcass characteristics in finishing goats. Asian-Australasian Journal of Animal Sciences 30(6):811.

Conforti P (2011). Looking ahead in world food and agriculture: perspectives to 2050. Food and Agriculture Organization of the United Nations (FAO).

Ferbiyanto A, Rusmana I, Raffiudin R (2015). Characterization and identification of cellulolytic bacteria from gut of worker Macrotermes gilvus. Hayati Journal of Biosciences 22(4):197-200.

Hatami S, Alikhani HA, Besharati H, Salehrastin N, Afrousheh M, Yazdani ZJ, Jahromi Z (2008). Investigation on aerobic cellulolytic bacteria in some of north forest and farming soils. American-Eurasian Journal of Agricultural and Environmental Sciences 3(5):713-716.

Huque KS, Sarker NR (2014). Feeds and feeding of livestock in Bangladesh: performance, constraints and options forward. Bangladesh Journal of Animal Science 43(1):1-10.

Immanuel G, Dhanusha R, Prema P, Palavesam A (2006). Effect of different growth parameters on endoglucanase enzyme activity by bacteria isolated from coir retting effluents of estuarine environment. International Journal of Environmental Science and Technology 3(1):25-34.

Khianngam S, Akaracharanya A, Tanasupawat S, Lee KC, Lee JS

(2009). *Paenibacillus thailandensis* sp. nov. and *Paenibacillus nanensis* sp. nov., xylanase-producing bacteria isolated from soil. International Journal of Systematic and Evolutionary Microbiology 59(3):564-568.

Maki ML, Broere M, Leung KT, Qin W (2011). Characterization of some efficient cellulase producing bacteria isolated from paper mill sludges and organic fertilizers. International Journal of Biochemistry and Molecular Biology 2(2):146.

Min DY, Smith SW, Chang HM, Jameel H (2013). Influence of isolation condition on structure of milled wood lignin characterized by quantitative 13C nuclear magnetic resonance spectroscopy. Bio Resources 8(2):1790-1800.

Sariubang M, Ella A, Nurhayu A, Pasambe D (2002). Assessment of integrated farming system of beef cattle in South Sulawesi. WARTAZOA. Indonesian Bulletin of Animal and Veterinary Sciences pp. 24-28.

Selim ASM, Rahman M, Jahan M, Hoque SAM, Rabbi ME, Hossain MD, Fonseca M, Crossland WL, Tedeschi LO (2017). Assessment of the digestibility of probiotic-treated rice straw using the *invitro* gas production technique. Indian Journal of Animal Nutrition 34(4):389-395.

Sembiring H, Panjaitan T, Praptomo D, Muzani A, Sauki A, Nurul A. (2002). Prospects on cattle based crop-livestock systems for the irrigated paddy field in lombok. WARTAZOA. Indonesian Bulletin of Animal and Veterinary Sciences, pp. 9-17.

Syamsu JA, Ali HM, Yusuf M (2013). Application of technology for processing rice straw as feed for beef cattle. In International Conference on Agriculture and Biotechnology *IPCBEE* pp. 43-46.

Wanapat M, Kang S, Hankla N, Phesatcha K (2013). Effect of rice straw treatment on feed intake, rumen fermentation and milk production in lactating dairy cows. African Journal of Agricultural Research 8(17):1677-1687.

Wang Y, Liu YG, Xing-Mei XI (2008). Lignocellulose degrading ability of Bacillus subtilis and its enzyme production. Journal of Microbiology 28(4):1-6.

Wood TM, Bhat KM (1988). Methods for measuring cellulase activities. Methods in Enzymology 160:87-112.

Yoo D, Hamid MM, Kim H, Moon J, Song J, Lee S, Seo J (2020). Substitution effects of rice for corn grain in total mixed ration on rumen fermentation characteristics and microbial community *in vitro*. Journal of Animal Science and Technology 62(5):638.

Zainudin MH, Hassan MA, Tokura M, Shirai Y (2013). Indigenous cellulolytic and hemicellulolytic bacteria enhanced rapid co-composting of lignocellulose oil palm empty fruit bunch with palm oil mill effluent anaerobic sludge. Bioresource Technology 147:632-635.

Zhang YH, Lynd LR (2004). Toward an aggregated understanding of enzymatic hydrolysis of cellulose: noncomplexed cellulase systems. Biotechnology and Bioengineering 88(7):797-824.

Related Journals: