ISSN: 0976-3031

Available Online at http://www.recentscientific.com

CODEN: IJRSFP (USA)

International Journal of Recent Scientific Research
Vol. 9, Issue, 6(E), pp. 27526-27530, June, 2018

International Journal of Recent Scientific Research

DOI: 10.24327/IJRSR

Research Article

CHANGING VIEWS ON VIRUSES

Raghavendra Rao M.V[1]., Sireesha Bala[1]., Sripada Pallavi.T[2]., KrishnaSowmya.M[3]., MahendraKumar[5]., Srinivasa Rao.D[4]., Jattavathu Madhavi [4]., Samir Fatteh[1]., Reshma Fateh[1] and Sateesh.Babu.A[1]

[1]Avalon University School of Medicine, Curacao, Central America
[2]Apollo Institute of Medical Science and Research Institute, Jubilee Hills, Hyderabad, Telangana, India
[3]Burjil Hospital, Abu Dhabhi, United Arab Emirates
[4]Acharya Nagarjuna University, Guntur, A.P, India
[5]Indian Institute of Science Education and Research, Bhopal

DOI: http://dx.doi.org/10.24327/ijrsr.2018.0906.2278

ARTICLE INFO

Article History:
Received 15th March, 2018
Received in revised form 27th April, 2018
Accepted 16th May, 2018
Published online 28th June, 2018

Key Words:

Transmission electron microscope (TEM), scanning electron microscope (SEM), Atomic force microscope (AFM), scanning near-field optical microscope (SNOM), cytopathic effect (CPE) Antiretroviral therapy (ART);

ABSTRACT

We are living in the viral world. Smallest the organism and greatest the disease. Virus is non living when present outside but living when present when present inside the cell. There are many types of viruses that cause a wide variety of viral diseases. The diagnosis of viral infection remains a major challenge in developing therapeutics. The clinical diagnosis provides state of the art for developing diagnostics for treatment (1). The main hurdle in developing diagnostics for the virus is their genome nature and high-level genome plasticity (2). To develop diagnostic for viral infection, there have been tremendous efforts have been over a period of several decades. The microscopy remains a major tool for morphological identification and validation of viruses. The advancement of cutting edge microscopy including electron microscopy, confocal microscopy, and scanning probe microscopy provide ease in identification and characterization of these intracellular pathogens (3). There have been continuous developments in Transmission electron microscope (TEM), scanning electron microscope (SEM), Atomic force microscope (AFM), scanning near-field optical microscope (SNOM), X-ray microscope, and ultrasonic microscope to enhance viral diagnosis (4).

INTRODUCTION

The morphological and physical characterization of viral particles provides only a basic knowledge about these intracellular pathogens (5). Hence, these techniques largely fail in identifying a molecular signature for viruses. As mentioned above viral genome possesses a great level of plasticity, and hence molecular identifications are essential to developing diagnostics (6). The molecular diagnosis also depends on the biochemistry of viral genetic material. The DNA and RNA viruses are entirely different in their genome and flow of their genetic information (7). Hence, it's very difficult to develop a common diagnostic for both the category. The nature of viral capsid is also an important parameter in developing diagnostics as each class of viruses' possesses different pattern and nature of protein expressing on the surface of viruses (8). Addition to molecular diagnosis, genome itself is being used as a potential target for the development of diagnostic devices. Now a day's genome sequencing and sequencing-based diagnostic devices are common not only in case of virus infection but also microbial infection as well. The genomic sequence-based diagnostics are accurate and effective and provide in-depth knowledge and information of pathogen (9). There are several variants in genomic-based diagnostics using PCR and Real-time PCR based technologies for the determination of viral load in the infected cell (10).

The microscopic and antibodies based assays are key diagnostic methods under conventional approaches (11). These approaches are effective and reliable in preliminary identification of viral infections and also act base for molecular and other advanced diagnostics (12).

Throughout the history of clinical diagnosis, microscopy remains a major analytical tool in biology and medical

Corresponding author:* **Raghavendra Rao M.V
Avalon University School of Medicine, Curacao, Central America

applications. The use of microscopy is versatile and had a wide range spectrum in microscopic examination of the virus and other microscopic pathogens associated with human diseases (13) Since most of the viruses are much smaller than normal microbes in their size and hence light microscope are used for preliminary studies (14).

The antibodies are raised against particular antigens. These raised antigens are conjugated with fluorescent tags and allow a reaction with virus-infected cells (15).

Electron microscopy is one of a most powerful microscopic tool for the microscopic organism and virus diagnosis. There is two major variants in electron microscopy one scanning electron microscopy (SEM) and second transmission electron microscopy (TEM). It detects virus particle, which is further characterized by their size and morphology (16).

Serological procedure for the laboratory diagnosis of Viruses help us to know the diagnosis. A rise in antibody titer to the virus can be used to diagnose viral infection. If the antibody titer in the convalescent phase serum sample is at least four-fold higher than the titer in the acute phase serum sample, the patient is considered to be infected (17). For the diagnosis of Hepatitis virus infection, HBsAg (Hepatitis viral surface antigen) or HBeAg (Hepatitis virus e antigens) can be detected (18). Detection of viral nucleic acids is one of the sensitive and rapid methods for the laboratory diagnosis of the virus (19). It requires the use of PCR (polymerase chain reaction) to amplify the viral genome present in the sample and the detection of the specific gene sequence of that particular virus by the use of a specific primer (while performing PCR) and probe (while detecting the specific sequence) (20). The growth of virus in the cell culture may produce a characteristic cytopathic effect (CPE) which helps us for presumptive diagnosis (21). If that particular virus does not produce the cytopathic effect, but,can be detected by several other techniques such as Immunofluorescence assay (e.g., DFA, IFA), Radioimmunoassay (RIA), Hemadsorption, decrease in acid production of infected cells, ELISA, Complement fixation, Hemagglutination inhibition method, Neutralization, etc (22).

History and Mechanism

The scientific study of viruses and the infections they cause – began in the closing years of the 19th century. Although Louis Pasteur and Edward Jenner developed the first vaccines to protect against viral infections, they did not know that viruses existed. The first evidence of the existence of viruses came from experiments with filters that had pores small enough to retain bacteria. In 1892, Dmitry Ivanovsky used one of these filters to show that sap from a diseased tobacco plant remained infectious to healthy tobacco plants despite having been filtered. Martinus Beijerinck called the filtered, infectious substance a "virus" and this discovery is considered to be the beginning of virology.

Louis Pasteur (1822–1895) was unable to find a causative agent for rabies and speculated about a pathogen too small to be detected using a microscope. In 1884, Dmitry Ivanovsky (1864–1920) studied tobacco mosaic virus. Paul Frosch (1860–1928) discovered the cause of foot-and-mouth disease.

From the 1950s to the 1960s, Chester M. Southam, a prominent virologist, injected malignant HeLa cells into cancer patients, healthy individuals, and prison inmates from the Ohio Penitentiary in order to observe if cancer could be transmitted. It was not until the invention of the electron microscope in 1931 by the German engineers Ernst Ruska (1906–1988) and Max Knoll (1887–1969), that virus particles, especially bacteriophages, were shown to have complex structures.

Significant Gap in Reseach

The major issue with conventional approaches in the detection of viral load in an infected cell is low resolution of microscopic techniques. The light and confocal microscopic techniques are efficient in microbial profiling but fail to profile viruses precisely. However, electron microscopy has an advantage over light and confocal microscopy as a higher resolution but still there are several viruses which are out of range of electron microscope as well (23). The antigen-based diagnostics are effective but in case of viruses the genome has a high probability of plasticity, and hence antigens remain changing. In such case, identification of suitable antigen and raising antibodies for ELISA and UV based microscopy find limitations. On several occasion, viruses remain in a dormant stage in the infected and hence there will not be any antigen for ELISA and other techniques. Additionally, viruses are highly dynamic in presenting their protein as antigens which further create a hurdle in ELISA based techniques (24). In case of HIV infection, it has been reported that for several years HIV viruses can live in a dormant stage based on host immune response and hence ELISA often seems negative though the patient is a carrier for HIV viruses. In the most of clinical laboratories still, we depend on ELISA based identification of HIV load in the infected in the case of blood transfusion (25). The serological protocols are quite complicated as viruses are quite difficult to grow and require a precise host for their replication. Similarly, the immunofluorescence assay depends on the growth of viral particle and their characteristic cytopathic effects (26). Often it has been reported due to many reasons viruses fail to grow even after providing recommend host and other growth supplements.

Major Advances and Discoveries

The modern approaches for diagnosis of viral infections associated with diseases to human largely depend on the molecular profiling and genome sequencing analysis. The polymerase chain reaction (PCR) and its applied variants are key tools in modern viral and other molecular diagnostics (27) and minimal exposure to infectious material, rapid turnaround time, and a high throughput (28). The real-time RT-PCR is the most widely used method due to moderate skill level requirement and relatively inexpensive and rapid sample screening. However, the final choice of AI detection method depends on laboratory capacity and set-up (29). The major drawback of all mentioned assays is the high start-up cost of equipment. The other disadvantage is that the reported methods were only validated with propagated AI strains. To ensure sufficient efficiency and reliability, all new assays must be thoroughly field validated. Nevertheless, a positive result obtained using molecular-based tools is very important for an immediate response, although must always be supported by virus isolation (30). These methods are effective in the

determination of viral load in the infected cell. In the category of modern approaches, PCR based methods and Genome sequencing methods are highly popular are in clinical use. The PCR based methods for virus diagnosis fall in the following categories

Multiplex PCR, Real time PCR, Reserve Transcriptase PCR PCR-based techniques are the most commonly used methods in avian influenza diagnostics. The conventional reverse transcriptase PCR (RT-PCR) assay and the real-time RT-PCR assay are used to identify influenza virus strains (31). The diagnostic application of real-time PCR technology offers advantages over the conventional RT-PCR method.

Numerous real-time PCR-based diagnostic assays have been reported to date. When validated with various samples, the assay showed higher sensitivity than a PCR assay with the TaqMan probes (33). By and large, RT-PCR-based techniques currently are the major diagnostic tool due to simplicity and reliability (34).

This method enables the detection of target proteins by ligated DNA strands, which are then amplified in a real-time PCR (35). Despite the fact that it is a recently established technique, it holds a promise as an alternative assay for protein measurement in complex biological samples with a detection limit in the low femtomolar range (36).

The early virus detection achieved by reliable, rapid techniques and viral DNA sequencing plays an important role in the successful prevention of the disease.

PCR reaction, followed by rapid sequencing that covers the cleavage site of HA gene (37). For example, a microarray with a clinical sensitivity of 95% and clinical specificity of 92% when validated against A/H5N1, A/H3N2, and A/H1N1 viral strains has been published. Another type of microarray, a low-density microarray, also deserves mention (38). However, the presence of multiple steps involved in these assays, like several amplification steps, probe labeling and incorporation of conjugated nucleotides into DNA makes them labor-intensive, time-consuming, and extremely costly (39). Additionally, the necessity of optimization of parameters and numerous primers design presents scientists with a challenge. Therefore microarray techniques come second to RT-PCR.

With the novel, cutting-edge technology in molecular and cell biology development of diagnostics gained a new momentum. The genome sequencing offers a complete information and data analysis to design new diagnostics for a candidate virus. There are several approaches for genome sequencing either conventional means, i.e., using chain termination or Sanger method. Further, next-generation sequencing (NGS); metagenomics has been established for complete genome sequencing and rapid methods for virus diagnosis as well (40). Efficient study design for accurate detection relies on the optimal amount of data representing a significant portion of a virus genome. The NGS has several advantages over conventional sequencing methods including ease in data analysis; complete genome sequencing, sequencing of lost and fragmented genome parts, etc. The NGS database is also helpful in comparison of genome sequence and study of phylogenetic analysis as well (41).

Here, clinical diagnosis is crucial not only in profiling and documenting infecting virus but also in making guidelines to minimize infections (42).

Human immune deficiency virus (HIV) infection that is widely distributed cause acquired immune deficiency syndrome (AIDS). It has been more than 30 years of HIV discovery and associated diseases to human we stand nowhere in a complete cure for AIDS. Hence giving more emphasis to prevention is more economical and effective (43). Similarly, in case of flu including influenza and swine flu prevailing and spreading rapidly are having a major concern. There is several other contagious viruses' infection affecting a large number of human populations causing a threat to global health and increasing diseases burden as well. In case of co-infection, it becomes more difficult to study and understand nature of virulence genes and factors for prevention protocols.

Ideas where the research go next?

Similar to the diagnosis of viral infections the management of diseases caused by viruses is quite difficult. There are several approaches to the management of viral diseases. The antiretroviral therapy is most advanced and effective way for management of diseases caused by RNA viruses. The management of viral diseases is based on many factors including nature of their genetic material, flow, and processing of genetic information, mechanism of infections, based on virulence gene/s and host-pathogen interactions. These factors are a key target for drug developments in case of virus-based diseases. In general, a virus has few common events in its life cycle and act as a target for drug development including attachment, injection, reserve transcription, lysis, and release. The available drugs are designed based on one and or multiple targets. The genomic mutations and target for molecular events including replication, transcription and translational.

Additionally, retroviruses have one additional molecular biology events reserves transcription also a key target for drug development (44). However, it's very difficult to target listed targets as viral genome and proteins are highly unstable due to constant mutations in the genome. Additionally, viruses are very difficult to predict and understand for their life cycle as well either lytic or lysogenic. The surface proteins are highly conserved and specific for host and finding a suitable target for these proteins is crucial in drug development. Viruses do not have their metabolism and utilize host cellular machinery for replication and hence targeting molecular events may affect host cell as well. There is another challenge in viral base disease management; drug delivery against the virus. As virus do not exist as a single cellular entity and in most of cases drugs are designed to target infected cells. There are difficulties to distinguish virally infected cell and healthy cell if the virus is in dormant stage (45). These are approaches used in the drug development and management of virus-based human diseases and disorders.

Current Debate

The clinical diagnosis of viruses remains a major challenge and rigorous task and challenge in modern diagnostics. Over a period of decades, several biological tools implemented in the diagnosis of viral load in a cell. In recent time, polymerase chain reactions based techniques have shown tremendous

promise in early diagnosis of viral load in an infected. The multiplex PCR and real-time PCR are most valuable and accurate tools in modern molecular biology not only viral load determination but also many other diseases as well. The nature of virus genome and its complex life cycle is a major challenge even in present time disable development of single and common diagnosis method.

References

1. Diagnostic methods in virology: Overview of diagnostic method. Avaible at: http://virology-online.com/general/Tests.htm
2. Boom R, Sol C J A, Salimans M M M, Jansen C L, Wertheim-van Dillen P M E, van de Noorda J. Rapid and simple method for purification of nucleic acids. *J Clin Microbiol.* 1990; 28:495–503.
3. Burke D G, Kalayjian R C, Vann V R, Madreperla S A, Shick H E, Leonard D G. Polymerase chain reaction detection and clinical significance of varicella-zoster virus in cerebrospinal fluid from human immunodeficiency virus-infected patients. *J Infect Dis*. 1997; 176:1080–1084.
4. Echeverria J M, Casas I, Tenorio T, de Ory F, Martinez-Martin P. Detection of varicella-zoster virus-specific DNA sequences in cerebrospinal fluid from patients with acute aseptic meningitis and no cutaneous lesions. *J Med Microbiol.* 1994; 43:331–335.
5. Guffond T, Dewilde A, Lobert P-E, Caparros-Lefebvre D, Hober D, Wattre P. Significance and clinical relevance of the detection of herpes simplex virus DNA by the polymerase chain reaction in cerebrospinal fluid from patients with presumed encephalitis. *Clin Infect Dis.* 1994; 18:744–749.
6. Jeffery K J M, Read S J, Peto T E A, Mayon-White R T, Bangham C R M. Diagnosis of viral infections of the central nervous system: clinical interpretation of PCR results. *Lancet*. 1997;349:313–317.
7. Koskiniemi M, Piiparinen H, Mannonen L, Rantalaiho T, Vaheri A. Herpes encephalitis is a disease of middle aged and elderly people: polymerase chain reaction for detection of herpes simplex virus in the CSF of 516 patients with encephalitis. J Neurol Neurosurg Psychiatry. 1996; 60:174–178.
8. Lakeman F D, Whitley R J the National Institute of Allergy and Infectious Diseases Collaborative Antiviral Study Group. Diagnosis of herpes simplex encephalitis: application of polymerase chain reaction to cerebrospinal fluid from brain-biopsied patients and correlation with disease. J Infect Dis. 1995; 171:857–863.
9. Meyer H M, Johnson R T, Crawford I P, Dascomb H E, Rodgers N G. Central nervous system syndromes of 'viral' aetiology. *Am J Med*. 1960; 29:334–347.
10. Mitchell P S, Espy M J, Smith T F, Toal D R, Rys P N, Berbari E F, Osmon D R, Persing D H. Laboratory diagnosis of central nervous system infections with herpes simplex virus by PCR performed with cerebrospinal fluid specimens. *J Clin Microbiol*. 1997; 35:2873–2877.
11. Mollaret P. La méningite endothélio-leucocytaire multirécurrente benigne: syndrome nouveau ou maladie nouvelle? *Rev Neurol* (Paris) 1944; 76:57–76.
12. Read S J, Jeffery K J M, Bangham C R M. Aseptic meningitis and encephalitis: the role of PCR in the diagnostic laboratory. *J Clin Microbiol*. 1997; 35:691–696.
13. Sawyer M H, Holland D, Aintablian N, Connor J D, Keyser E F, Waeker N J. Diagnosis of enteroviral central nervous system infection by polymerase chain reaction during a large community outbreak. *Paediatr Infect Dis J.* 1996; 13:177–182.
14. Schlesinger Y, Tebas P, Gaudreault-Keener M, Buller R S, Storch G A. Herpes simplex virus type 2 meningitis in the absence of genital lesions: improved recognition with use of the polymerase chain reaction. *Clin Infect Dis*. 1995; 20:842–848.
15. Shen S, Desselberger U, McKee T A. The development of an antigen capture polymerase chain reaction assay to detect and type human enteroviruses. *J Virol Methods*. 1997; 65:139–144.
16. Tanel R E, Kao S Y, Niemiec T M, Loeffelholz M J, Holland D T, Shoaf L A, Sturky E R, Burns J C. Prospective comparison of culture vs genome detection for diagnosis of enteroviral meningitis in childhood. *Arch Pediatr Adolesc Med*. 1996; 150:919–924.
17. Tedder D G, Ashley R, Tyler K L, Levin M J. Herpes simplex virus infection as a cause of benign recurrent lymphocytic meningitis. *Ann Intern Med*. 1994; 121:334–338.
18. Thoren A, Widell A. PCR for the diagnosis of enteroviral meningitis. *Scand J Infect Dis*. 1994; 26:249–254.
19. Wakefield A J J, Fox D, Sawyer A M, Taylor J E, Sweenie C H, Smith M, Emery V C. Detection of herpesvirus DNA in the large intestine of patients with ulcerative colitis and Crohn's disease using the nested polymerase chain reaction. *J Med Virol*. 1992; 38:183–190.
20. Yerly S, Gervaix A, Simonet V, Catflisch M, Perrin L, Wunderli W. Rapid and sensitive detection of enteroviruses in specimens from patients with aseptic meningitis. *J Clin Microbiol.* 1996; 34:199–201.
21. Flint SJ, Enquist LW, Ranciello VR, Skalka AM editors. Principles of Virology: Molecular Biology, Pathogenesis and Control of Animal Viruses. 2nd ed. Washington: American Society for Microbiology Press; 2003.
22. Lennet EH, editor. Laboratory Diagnosis of Viral Infections. 3rd ed. New York: Marcel Dekker; 1999.
23. Forbes BA, Weissfeld AS, Sahm DF, editors. Baily and Scott's Diagnostic Microbiology. 11th ed. St Louis: Mosby; 2002.
24. Lazarević I, Stanojević M, Ćupić M, Jovanović T. Značaj PCR metode u određivanju prisustva citomegalovirusa u krvi i urinu pa cijenata u post transplantacionom periodu. *Acta Chir Iugosl* 2006; 53(1):19-22.
25. Stabell EC, Olivo EC. Isolation of a cell line for rapid and sensitive histochemic assay for detection of herpes simplex virus. *J Virol Methods* 1992; 38(2):195-204.

26. Sewell DL, Horn SA, Dilbeck PW. Comparison of Cultureset and Bartels Immunodiagnostics with conventional tissue culture for isolation and identification of herpes simplex virus. *J Clin Microbiol* 1984; 19(5):705-6.
27. Hughes JH, Mann DR, Hamparian AA.Viral isolation versus immune staining of infected cell cultures for the laboratory diagnosis of herpes simplex virus infections. *J Clin Microbiol* 1986; 24(3):487-9.
28. Cann AJ editor. Virus Culture: A Practical Approach. Oxford: Oxford University Press; 1999
29. Ong H, Chandran V. Identification of gastroenteric viruses by electron microscopy using higher order spectral features. *J Clin Virol* 2005; 34(3):195-206.
30. Arcangeletti MC, De Conto F, Pinardi F, et al. Electron microscopy as a reliable tool for rapid and conventional detection of enteric viral agents: a five-year experience report. *Acta Biomed Ateneo Parmense* 2005; 76(3):165-70.
31. Ćupić M. Laboratorijska dijagnostika Herpes simplex virus infekcija. *Acta Ophthalmol Iug* 2002; 28:21-6.
32. Lazarević I, Jovanović T, Stanojević M, Ćupić M. Comparison of methods for CMV detection in urine of immunocompromised patients. 2nd European Comgress of Virology, Madrid, Spain, Abstract book, 2004. p.18.3.
33. Ranin J, Perović M, Ćupić M, Žerjav S, Salemović D, Jevtović Đ. Lumbosakralna poliradikulopatija izazvana citomegal ovirusom kod pacijenata sa sindromom stečene imunodeficijencije. Acta Infectologica Yugoslavica 2000; 5:173-8.
34. Storch GA. Diagnostic Virology. *Clin Infect Dis* 2000; 31(3): 739-51.
35. Wald A, Ashley-Morrow R. Serological testing for herpes simplex (HSV-1) and (HSV-2) infection. *Clin Infect Dis* 2002; 35(Suppl 2):173-82.
36. Brown ZA, Corey L, Ashley R, Zeh J, Selke S, Wald A. Effect of viral shedding, serologic status and abdominal deliveries on transmission rates of HSV from mother to infant. *JAMA* 2003; 289(2):203-9.
37. Emmons WW, Paparello SF, Decker CF, Sheffield JM, Lowe-Bey FH. A modified ELISA and western blot accurately determine anti-human immunodeficiency virus type 1 antibodies in oral fluids obtained with a special collecting device. *J Infect Dis* 1995; 171(6):1406-10.
38. Hogrefe W, Su X, Suong J, Ashley R, Kong L. Detection of herpes simplex virus type 2 – specific imunoglobulin G antibodies in Arrican sera by using recombinant gG2 Western blotting, and gG2 inhibition. *J Clin Micribiol* 2002; 40(10):3635-40.
39. Morrow RA, Friedrich D, Krantz E, Wald A. Development and use of a type-specific antibody avidity test based on herpes simplex virus type 2 glycoprotein G. *Sex Trans Dis* 2004; 31(8):508-15.
40. Jovanović T, Ćupić M, Stanojević M, Knežević A, Lazarević I. Osnove molekularne virusologije. Beograd: Medicinski fakultet Univerziteta u Beogradu; 2005.
41. Persing DH. In vitro nucleic acid amplification techniques. In: Persing DH, Smith TF, Tenover FC, White TJ, editors. Diagnostic Molecular Microbiology: Priciples and Applications. Washington DC: American Society for Microbiology; 1993.
42. Suvajdžić N, Čemerikić-Martinović V, Šaranović Đ, Petrović M, Popović M, Artiko V, Ćupić M, Elezović I. Littoria-cell angioma as a rare cause of splenomegaly. *Clin Lab Haem* 2006; 28:317-20.
43. Mazzulli T, Drew LW, Yen-Lieberman B, et al. Multicenter comparison of the Digene Hybrid Capture CMV DNA Assay (Version 2.0), the pp65 antigenemia assay, and cell culture for detection of cytomegalovirus viremia. *J Clin Microbiol* 1999; 37(4):958-63.
44. Chernoff DN, Miner RC, Hoo BS, et al. Quantification of cytomegalovirus DNA in peripheral blood leukocytes by a branched-DNA signal amplification assay. *J Clin Microbiol* 1997; 35:2740-4.
45. Lazarević I, Ćupić M, Delić D, Jovanović T. Genotipizacija hepatitis B virusa. V kongres medicinske mikrobiologije sa međunarodnim učešćem – MICROMED, Beograd, Zbornik radova; 2006. P.169-170.

How to cite this article:

Raghavendra Rao M.V *et al*.2018, Changing Views on Viruses. *Int J Recent Sci Res.* 9(6), pp. 27526-27530.
DOI: http://dx.doi.org/10.24327/ijrsr.2018.0906.2278

****