IJPSR (2017), Volume 8, Issue 10 (Research Article)

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 08 April, 2017; received in revised form, 08 September, 2017; accepted, 17 September, 2017; published 01 October, 2017

INCIDENCE OF HUMAN RHINOVIRUS COINFECTION WITH *STAPHYLOCOCCUS AUREUS* AMONG HIV PATIENTS SUFFERING FROM FLU LIKE ILLNESS

Ravish Katiyar [1], Vikas Agarwal [2], Sandeep Chowdhary [3], Neena Srivastava [4], Pooja Gaur [1] and T. N. Dhole [*1]

Department of Microbiology [1], Department of Immunology [2], Sanjay Gandhi Post Graduate Institute of Medical Sciences, Lucknow - 226014, Uttar Pradesh, India.
ART Centre [3], Dr. Ram Manohar Lohia Institute of Medical Sciences, Gomti Nagar, Lucknow - 226010, Uttar Pradesh, India.
Department of Physiology [4], King George's Medical University, Chowk, Lucknow - 226003, Uttar Pradesh, India.

Keywords:

Influenza like illness, Upper respiratory tract infections, Human rhinovirus, Coinfections, Mortality

Correspondence to Author:
Dr. T. N. Dhole

Professor and Head,
Department of Microbiology,
Sanjay Gandhi Post Graduate Institute of Medical Sciences,
Raebareli Road, Lucknow - 226014, Uttar Pradesh, India.

E-mail: tndhole@gmail.com

ABSTRACT: Background: Influenza and influenza like illness (ILI) is a common respiratory illness among HIV patients. ILI is caused by various respiratory viruses and bacteria other than influenza virus. The symptoms of ILI recover within a few days, but in HIV patients ILI with opportunistic coinfections may lead to serious complications and hospitalization. **Objective:** To determine the incidence of human rhinovirus (HRV) coinfection with *Staphylococcus aureus* among HIV patients suffering from flu like illness. **Materials and Methods:** In this study 92 throat swab samples of flu suspected HIV patients were collected from Department of Immunology, SGPGIMS, Lucknow and ART Centre, RMLIMS, Lucknow, during 2014 - 2015. Samples were cultured on mannitol salt agar (MSA) plates, *Staphylococcus aureus* were confirmed by the biochemical characterization. The confirmation of HRV was done by RT-PCR. HeLa cell lines were used for isolation of human rhinovirus. **Results:** The prevalence of HRV was found 17 (18.5%), while *S. aureus* were isolated from 40 (43.5%) samples. Coinfection of *S. aureus* was found in 11 HRV positive samples (64.7%), among them 5 (45.5%) isolates were found high colonizer. Only 2 HRV confirmed samples (11.8%) were found cell culture positive on HeLa cell lines. The significant enhancement in clinical symptoms such as coughing, sore throat and sputum was found during coinfection ($P < 0.05$). **Conclusion:** The high prevalence of HRV is found in flu suspected HIV patients in this region. *S. aureus* coinfection is found higher during HRV infection with higher rate of colonization. Respiratory complications were observed significantly increased during coinfection.

INTRODUCTION: Human immunodeficiency virus (HIV) increases susceptibility to numerous respiratory infections [1].

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.8(10).4441-46

Article can be accessed online on: www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.8(10).4441-46

Deficiencies of both humoral and cellular immunity during HIV infection can potentially alter the course and severity of respiratory infections [2].

In highly active antiretroviral treatment (HAART) era, a significant reduction in respiratory infections has been obtained due to improvement in immunity. Despite of HAART, HIV patients may remain at high risk of severe respiratory infections [3, 4]. Influenza or influenza like illness is one of the most common respiratory illness among HIV

patients. Common flu symptoms are fever, sputum, sweats, sneezing, sore throat, difficulty in breathing, weakness, *etc*. The symptoms are self limiting and resolve within a few days, but in immune compromise patients it may cause severe complications [5, 6]. ILI is mainly caused by respiratory viruses such as human respiratory syncytial virus, human rhinoviruses (HRVs), adenoviruses, coronaviruses and human parainfluenza viruses [7]. HRVs, belong to family picornaviridae are known causes of common cold and flu like illness. In immune compromised patients it may cause serious illness and hospitalization [8, 9].

Various bacteria, such as *Streptococcus pneumoniae, Haemophilus influenza and Staphylococcus aureus* are commensals in upper respiratory tract, these may cause coinfections or superinfections [10, 11]. *S. aureus*, a common resident of human respiratory tract is known cause of invasive and secondary staphylococcal infection. It also found associated with respiratory viral infections and flu like illness. The emergence of methicilline-resistant *S. aureus* (MRSA) is a new challenge for the medical world. Development of MRSA pneumonia after a respiratory viral infection is fatal in immune compromised individuals [12]. The bacterial co-infections during respiratory virus illness is a leading cause of morbidity and mortality, especially among immune deficient people [13].

In the HAART *era*, there is virtually no sufficient information available on the causes, complications and outcomes of respiratory coinfections. The involvement of *Staphylococcal* coinfection during respiratory viral illness in the HIV infected population remains to be elucidated [3]. The aim of this study is to analyze the incidence of human rhinovirus coinfection with *Staphylococcus aureus* among HIV patients suffering from flu like illness in the Northern India region.

MATARIALS AND METHODS:

Sample Collection: 92 throat swab samples of Influenza suspected HIV patients were collected from Department of Immunology, Sanjay Gandhi Post Graduate Institute of Medical Sciences, Lucknow and ART Centre, Dr. Ram Manohar Lohia Institute of Medical Sciences, Lucknow during 2014 - 2015. For each throat sample two swabs were collected in two separate collection tubes, one tube was containing viral transport medium (VTM), while the other tube was containing normal saline. Samples were transported maintaining the cold chain (4 - 5 °C).

Isolation and Phenotypic Characterization of *S. aureus:* The swabs were streaked immediately on mannitol salt agar (MSA) plates and incubated at 37 °C for 24 hours. Colonies were counted and further analysis was performed. Gram staining and biochemical tests such as oxidase and catalase were performed for the confirmation of the genus Staphylococcus [14].

Gram positive cocci, showed catalase positive and oxidase negative reactions were considered as Staphylococcus strains. The isolates were further characterized by coagulase, DNase and mannitol fermentation tests. All mannitol fermenter, DNase and coagulase positive strains were confirmed as *Staphylococcus aureus* [14].

Viral RNA Extraction: The viral RNA extraction from swab samples was done by QIAmp® Viral RNA Mini Kit (Qiagen) as per manufacturer's instructions and extracted RNA were stored at -40^{o}C for further analysis.

Molecular Detection of HRV by Reverse Transcription-polymerase Chain reaction (RT-PCR): RT-PCR for HRV detection was done by one step RT-PCR Kit (AgPath-ID, Life Technologies) using 2 µl of viral RNA as per protocol provided with kit. The primer, Forward 5'-GGGACCAACTACTTTGGGTGTCCG-3' and reverse primer 5'-CACGGACACCCAAAGTAGT-3' was used to amplify within specific 5' non coding region [15]. The PCR conditions were as follows- Reverse transcription at 50 °C for 30 minutes, Initial denaturation 95 °C for 10 minutes, followed by 45 cycles of denaturation at 95 °C for 30 seconds, annealing at 53 °C for 30 seconds, and elongation at 72 °C for 45 seconds.

Gel Electrophoresis: Amplified PCR products were loaded on 1.5% agarose gel containing ethidium bromide (EtBr) with gel loading dye and run in TAE buffer at 90 volts. The Products were visualized by the use of ultraviolet illumination unit (Bio-Rad).

Cell Culture of HRV: Monolayer of HeLa cells was prepared in cell culture flasks (Corning), using the minimal essential medium (MEM) (Sigma-Aldrich) supplemented with Earle's salts, L-glutamine, Penicillin-Streptomycin (Sigma) and 10% Fetal Bovine Serum (Gibco-Life Technologies).

Washing (three times) of subconfluent cells was done by Dulbecco's phosphate buffered saline (PBS) (Sigma) and then PCR confirmed and filtered (0.22 µm filter by Millipore) HRV positive throat swab samples were inoculated in cell lines and incubated for 45 minutes at 35 °C. Cells were washed with PBS, overlaid with 2% MEM, then moved to an incubator (35 °C, 5% CO_2), under 5 days observation for confirmation of cytopathic effects (CPE) [16].

Statistical Analysis: Clinical characteristics were compared between single HRV infection and HRV with *S. aureus* coinfection by chi-square test using SPSS software (IBM SPSS statistics 20).

RESULTS: In this study, out of total 92 samples, 68 samples (73.9%) were successfully cultured in MSA media, while the prevalence of *S. aureus* was found 40 (43.5%) in HIV patients during flu like illness, among them 12 samples (30%) were showed low numbers of colonies (1 to10) on MSA plates, considered as Low colonizer, 19 samples (47.5%) were showed moderate numbers of colonies (10 to100) were considered as moderate colonizer, while 9 samples (22.5%) were found high colonizer (number of colonies >100) (**Fig. 1**). Detection of HRV in ILI samples was done by RT-PCR amplification of the 5'NCR region (**Fig. 2**). Among 92 samples, 17 samples (18.5%) were HRV positive. Only 2 HRV positive samples (11.8%) were cultured on HeLa cell lines showed cytopathic effects (**Fig. 3**).

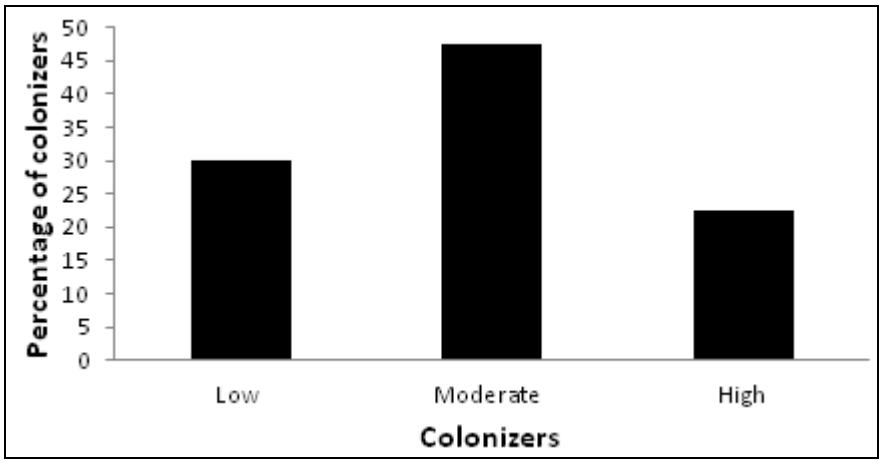

FIG. 1: GRAPH SHOWING PERCENTAGE OF COLONIZERS (LOW COLONIZER: 1 TO 10 COLONIES, MODERATE COLONIZER: 11 TO 100 COLONIES, HIGH COLONIZER: >100 COLONIES OF *S. AUREUS* ON MSA PLATE)

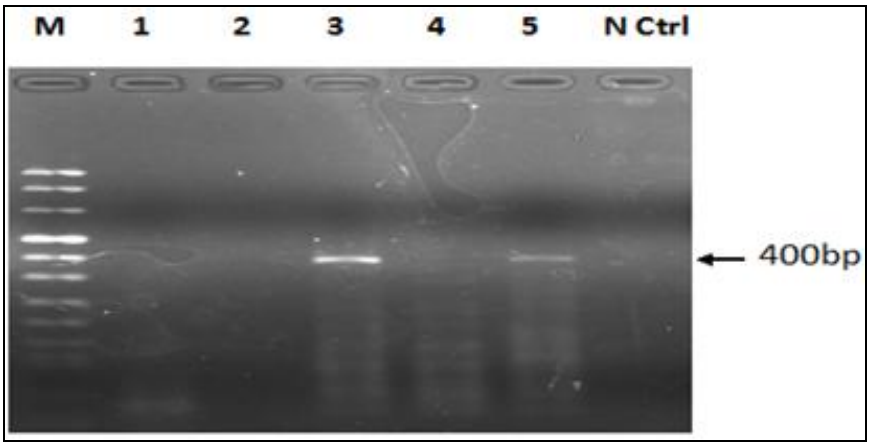

FIG. 2: THE GEL IMAGE SHOWING POLYMERASE CHAIN REACTION AMPLIFICATION OF THE 5' NON-CODING REGION OF VIRAL RNA. LANE M: MARKER 1000 BP. LANE 1 TO 5: LOADED SAMPLES (SAMPLES 3 AND 5 SHOWING AMPLIFIED PCR PRODUCT OF 400BP) AND LANE 6: NEGATIVE CONTROL

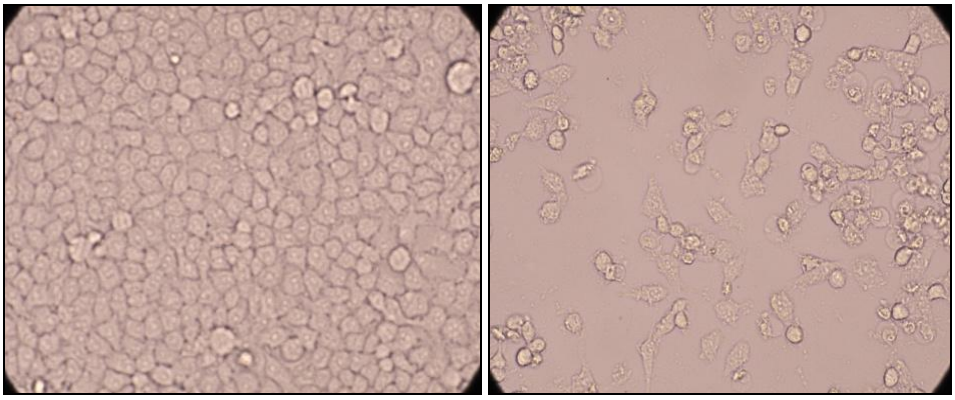

FIG. 3: HELA CELL LINES (400 X MAGNIFICATION); (a): HeLa CELL LINE (CONTROL) AND (b): HeLa CELLS SHOWING THE CYTOPATHIC EFFECT AFTER VIRAL INOCULATION

11 HRV positive samples (64.7%) were shown coinfection with *S. aureus*. Prevalence of high colonizer *S. aureus* was found higher 5 (44.5%) during coinfection. Clinical characteristics of patients were compared to single HRV infection and HRV with *S. aureus* coinfection.

The significant enhancement in the respiratory complications such as coughing (33.3% versus 91%; P < 0.05), sore throat (33.3 % versus 81.8%; P < 0.05) and sputum (16.6% versus 72.7%; P <0.05) were noticed during coinfection **Table 1**.

TABLE 1: CLINICAL CHARACTERISTICS OF PATIENTS

Clinical Characteristics	Single HRV Infection (N=6)	HRV with *S. aureus* Coinfection (N=11)	P Value*
Runny nose	5 (83.3%)	8 (81.8%)	0.622
Coughing	2 (33.3%)	10 (91%)	0.013
Sneezing	5 (83.3%)	7 (63.6%)	0.394
Sputum	1 (16.6%)	8 (72.7%)	0.027
Sore throat	2 (33.3%)	9 (81.8%)	0.046
Fever	4 (66.6%)	5 (45.5%)	0.402

*Chi-squere test was performed to compare the clinical characteristics between single HRV infection and HRV with *S. aureus* coinfection (p-value <0.05 was considered significant)

DISCUSSION: To the best of our knowledge this is the first study that provides analysis of the prevalence of human rhinovirus infection and its coinfection with *Staphylococcus aureus* among HIV patients showing flu like symptoms in the North India region. Respiratory coinfections among HIV patients have been well documented previously [11, 17].

Influenza associated respiratory illness is very common among adults and children infected with HIV [6, 18]. The involvement of other respiratory viruses with influenza like illness (ILI) in HIV positive is not well described in the HAART *era* [7]. HRV associated upper respiratory tract infections such as Influenza like illness in adults typically associated with mild course, but in immune compromised adults can cause more severe complications including lower respiratory tract infections with increased mortality [19 - 21]. In a previous study, severity of HRV infection was found similar to H1N1 Influenza 2009 in immune compromised adults with 12.6 % HRV prevalence, [8] while in the present study the prevalence of HRV is found 18.5%. Another study showed very high prevalence (31.7%) of HRV in South Africa under aged HIV patients hospitalized for lower respiratory tract infections [22]. Bacterial-viral coinfection in the same host may lead to high severity and pathology of illness. Superinfections caused by bacteria during respiratory viral infections found associated with significant cause of death [23]. In a previous study an increased mortality was observed in coinfection of *staphylococcus aureus* with Influenza in hospitals of Iowa and Maryland, USA during 2003 - 2009 [24].

The association of bacteria and viruses in the pathogenesis of respiratory illness have been well reported in the literatures. The mechanisms of bacteria-virus interactions are very diverse and not well defined [10].

The most common bacteria-virus interaction is found between influenza and *Streptococcus pneumoniae* [25]. However the HRV interactions with various bacteria in nasal epithelium have been studied previously, but the actual mechanisms of HRV and bacteria interactions are still unknown [26, 27]. In our study, 64.7% HRV positive samples were found coinfected with *S. aureus*, among them 44.5 % were high colonizer, that shows HRV infection enhances colonization of *S. aureus* in upper respiratory tract.

During coinfection the enhancement in the clinical chracteristics such as coughing, sore throat, sputum was observed. Same evidences of increased severity of complications during respiratory coinfections have been shown in previous studies [23, 28].

CONCLUSION: The study concludes that HRV might be a major cause of flu like illness in HIV patients other than Influenza virus. The RT-PCR method using amplification of 5'NCR region can be applied for the detection of HRV in clinical samples. Cell culture assay using HeLa cell line is not sensitive for HRV isolation. High prevalence of *S. aureus* is found during HRV infection among HIV patients. HRV infection enhances colonization of *S. aureus* in upper respiratory tracts. HRV coinfection with *S. aureus* enhances respiratory complications.

ACKNOWLEDGEMENT: We are thankful to Indian Council of Medical Research for financial supports.

CONFLICT OF INTEREST: Nil.

REFERENCES:

1. Davis JL, Fei M and Huang L: Respiratory infection complicating HIV infection. Curr Opin Infect Dis 2008; 21:c184-190.
2. Beck JM, Rosen MJ and Peavy HH: Pulmonary complications of HIV infection: report of the fourth NHLBI workshop. Am J Respir Crit Care Med 2001; 164: 2120-6.
3. Klein MB, Ying L, Balso LD, Cote SP and Boivin G: Influenza virus infection is a primary cause of febrile respiratory illness in HIV-infected adults, despite vaccination. Clinical Infectious Diseases 2007; 45: 234-40.
4. Lopez PC, Martin ZM, Benitez E, Fernandez GC, Guerrero F, Rodriguez IM and Giron GJA: Pneumonia in HIV-infected patients in the HAART *era*: incidence, risk, and impact of the pneumococcal vaccination. J Med Virol 2004; 72: 517-24.
5. Cohen JP and Macauley C: Susceptibility to influenza A in HIV-positive patients. JAMA 1989; 261: 245.
6. Sheth AN, Althoff KN and Brooks JT: Influenza susceptibility, severity and shedding in HIV-infected adults: A review of the literature. Clinical Infectious Diseases 2011; 52: 219-227
7. Khoo SH, Hajia M, Storey CC, Klapper PE, Wilkins EG, Denning DW, Dunbar EM, Corbitt G and Mandal BK: Influenza-like episodes in HIV-positive patients: the role of viral and 'atypical' infections. AIDS 1998; 12: 751-7.
8. Kraft CS, Jacob JT, Sears MH, Burd EM, Caliendo AM and Lyon GM: Severity of human rhinovirus infection in immune compromised adults is similar to that of 2009 H1N1 influenza. Journal of Clinical Microbiology 2011; 12: 1061-1063.
9. Finelli L, Fiore A, Dhara R, Brammer L, Shay DK, Kamimoto L, Fry A, Hageman J, Gorwitz R, Bresee J and Uyeki T: Influenza-associated pediatric mortality in the United States: increase of *Staphylococcus aureus* coinfection. Pediatrics 2008; 122: 805-811
10. Bosch AATM, Biesbroek G, Trzcinski K, Sanders EAM and Bogaert D: Viral and bacterial interactions in the upper respiratory tract. PLoS Pathog 2013; 9: e1003057.
11. Brundage JF: Interactions between influenza and bacterial respiratory pathogens: implications for pandemic preparedness. Lancet Infect Dis 2006; 6: 303-312.
12. Yu KOA, Randolph AG, Agan AA, Yip WK, Truemper EJ, Weiss SL, Ackerman KG, Schwarz AJ, Giuliano JS, Hall MW and Wardenburg JB: *Staphylococcus aureus* toxin response distinguishes respiratory virus-methicillin-resistant *S. aureus* coinfection in children. The Journal of Infectious Diseases 2016; 214: 1638-46.
13. Joseph C, Togawa Y and Nahoko S: Bacterial and viral infections associated with influenza. Influenza Other Respir Viruses 2013; 7: 105-113.
14. Rahimi F, Bouzari M, Katouli M and Pourshafie MR: Antibiotic resistance pattern of methicillin-resistant and methicillin sensitive *Staphylococcus aureus* isolates in Tehran, Iran. Jundishapur J Microbiol 2013; 6: 144-149.
15. Gaur P, Srivastava N, Awasthi S, Katiyar R, Srivastava NN, Singh DV, Kaistha S, Tripathi R, Misra VK, Prakash V, Kapoor P and Dhole TN. Molecular detection of human rhinovirus in respiratory samples of swine flu negative North Indian children with flu-like illness. Asian J Pharm Clin Res 2016; 9: 272-274.
16. Amineva SP, Aminev AG, Gern JE and Palmenberg AC: Comparison of rhinovirus a infection in human primary epithelial and HeLa cells. J Gen Virol 2011; 92: 2549-57.
17. Benito N, Moreno A, Miro JM and Torres A: Pulmonary infections in HIV-infected patients: an update in the 21st century. Eur Respir J 2012; 39: 730-745.
18. Legare CH and Gelman SA: South African children's understanding of AIDS and Flu: Investigating conceptual understanding of cause, treatment and prevention. Journal of Cognition and Culture 2009; 9: 357-370.
19. Ruuskanen O, Lahti E, Jennings LC and Murdoch DR: Viral pneumonia. Lancet 2011; 377: 1264 -1275.
20. Versluys AB, Rossen JW, Van EB, Schuurman R, Bierings MB and Boelens JJ: Strong association between respiratory viral infection early after hematopoietic stem cell transplantation and the development of life-threatening acute and chronic alloimmune lung syndromes. Biol. Blood Marrow Transplant 2010; 16: 782-791.
21. Winther B: Rhinovirus infections in the upper airway. Proc. Am. Thorac. Soc. 2011; 8: 79-89.

22. Nunes MC, Kuschner Z, Rabede Z, Madimabe R, Niekerk NV, Moloi J, Kuwanda L, Rossen JW, Klugman KP, Adrian PV and Madhi SA: Clinical epidemiology of bocavirus, rhinovirus, two polyomaviruses and four coronaviruses in HIV-Infected and HIV-uninfected South African Children. PLoS ONE 2014; 9: e86448.
23. Deng JC: Viral-bacterial interactions-therapeutic implications. Influenza and other respiratory viruses 2013; 7: 24-35.
24. McDanel JC, Perencevich EN, Storm J, Diekema DJ, Herwaldt L, Johnson JK, Winokur PL and Schweizer ML: Increased mortality rates associated with *Staphylococcus aureus* and influenza coinfection, Maryland and Iowa, USA. Emerg Infect Dis 2016; 22: 1253-1256.
25. McCullers JA: Insights into the interaction between influenza virus and *Pneumococcus*. Clin Microbiol Rev 2006; 19: 571-582.
26. Wang JH, Kwon HJ and Jang YJ: Rhinovirus enhances various bacterial adhesions to nasal epithelial cells simultaneously. Laryngoscope 2009; 119: 1406-1411.
27. Ishizuka S, Yamaya M, Suzuki T, Takahashi H, Ida S, Sasaki T, Inoue D, Sekizawa K, Nishimura H and Sasaki H: Effects of rhinovirus infection on the adherence of *Streptococcus pneumoniae* to cultured human airway epithelial cells. J Infect Dis 2003; 188: 1928-1939.
28. Murphy T, Bakaletz L and Smeesters P: Microbial interactions in the respiratory tract. Pediatr Infect Dis J 2009; 28: 121-126.

How to cite this article:
Katiyar R, Agarwal V, Chowdhary S, Srivastava N, Gaur P and Dhole TN: Incidence of human rhinovirus coinfection with *Staphylococcus aureus* among HIV patients suffering from flu like illness. Int J Pharm Sci Res 2017; 8(10): 4441-46.doi: 10.13040/IJPSR.0975-8232. 8(10).4441-46.