CLINICAL EFFICACY ASSESSMENT OF ANTIVIRAL-ANTIBIOTIC COMBINATION THERAPY FOR PREVENTION OF COMPLICATIONS ASSOCIATED WITH SEVERE INFLUENZA INFECTION

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LIST OF ABBREVIATIONS

AIDS	Acquired Immunodeficiency Syndrome
AKI	Acute Kidney Injury
AKIN	Acute Kidney Injury Network Criteria
AOR	Adjusted Odds Ratio
ARDS	Acute Respiratory Distress Syndrome
ARF	Acute Renal Failure
BMI	Body Mass Index
CA-MRSA	Community Acquired Methicillin-Resistant Staphylococcus aureus
CAP	Community-Acquired Pneumonia
CDC	Center for Disease Control and Prevention
CI	Confidence Interval
COPD	Chronic Obstructive Pulmonary Disease
CT	Computerized Tomography
DNA	Deoxyribonucleic Acid
ECMO	Extracorporeal Membrane Oxygenation
ESRD	End-Stage Renal Disease
FDA	Food and Drug Authority
HAP	Hospital-Acquired Pneumonia
HIV	Human Immunodeficiency Syndrome
ICU	Intensive Care Unit
ILI	Influenza-Like Illness
IPC	Infection Prevention and Control
JCI	Joint Commission International
LRTIS	Lower Respiratory Tract Infections
MODS	Multiple Organ Dysfunction Syndrome
MRSA	Methicillin-Resistant Staphylococcus aureus
NAAT	Nucleic Acid Amplification Test
OR	Odds Ratio
PCR	Polymerase Chain Reaction
Pdm09	Pandemic 2009
RIFLE	Risk, Injury, Failure, Loss, and End-Stage Criteria

RNA	Ribonucleic Acid
RRT	Renal Replacement Therapy
RSV	Respiratory Syncytial Virus
RTI	Respiratory Tract Infection
RT-PCR	Reverse Transcriptase Polymerase Reaction
RVIS	Respiratory Virus Infections
SCR	Serum Creatinine
SOFA	Sequential Organ Failure Assessment
TCAD	Triple-Combination Antiviral Drug
URTIS	Upper Respiratory Tract Infections

PENILAIAN KEBERKESANAN KLINIKAL KOMBINASI TERAPI ANTIVIRAL-ANTIBIOTIK DALAM PENCEGAHAN KOMPLIKASI BERKAITAN JANGKITAN INFLUENZA TERUK

ABSTRAK

Jangkitan bakteria sekunder, bantuan pernafasan mekanikal, dan kegagalan berbilang organ adalah komplikasi berkaitan dengan jangkitan Influenza yang teruk yang bertanggungjawab terhadap kadar penyakit dan kematian di seluruh dunia. Penggunaan antibiotik dalam rawatan jangkitan virus influenza masih lagi dipertikaikan. Kajian ini bertujuan untuk membuat perbandingan terhadap keberkesanan antara monoterapi ubat Antiviral dengan terapi kombinasi Antiviral-Antibiotik untuk mengelakkan komplikasi akibat jangkitan Influenza kepada pesakit-pesakit yang dimasukkan ke hospital. Kajian kohort retrospektif yang berbilang pusat ini dijalankan di dua buah hospital pendidikan yang mempunyai rawatan penjagaan pakar di Arab Saudi. Makmal telah mengesahkan bahawa pesakit Influenza-A (bukan H1N1), Influenza-B, dan Influenza-A (H1N1) yang dimasukkan ke hospital yang berada di bilik kecemasan selepas 48 jam menunjukkan gejala awal telah dikenalpasti dan dibahagikan kepada dua kumpulan; Kumpulan-1 adalah pesakit yang telah dimulakan dengan monoterapi ubat Antiviral sementara Kumpulan-2 adalah pesakit yang telah diperkenalkan dengan kombinasi terapi antiviral-antibiotik. Pesakit telah dinilai untuk hasil klinikal yang berbeza dalam kalangan pesakit kedua-dua kumpulan, seperti insiden jangkitan bakteria sekunder, keperluan alat bantuan pernafasan, tempoh kemasukan ke hospital, insiden kegagalan berbilang organ, kegagalan awal klinikal, dan tempoh untuk kestabilan klinikal. Analisis

perbandingan keberkesanan antara pesakit monoterapi Antiviral dengan pesakit terapi kombinasi Antiviral-Antibiotik bagi strain virus Influenza-A (bukan H1N1) (212 vs. 187 pesakit), Influenza-B (153 vs. 131 pesakit), dan strain Influenza-A (H1N1) (227 vs. 286 pesakit) menunjukkan bahawa insiden jangkitan bakteria sekunder, keperluan alat bantuan pernafasan, insiden kemasukan ke Unit Rawatan Rapi (ICU), tempoh kemasukan ke hospital dan tempoh untuk kestabilan klinikal dilihat berkurang secara signifikan bagi pesakit yang telah diperkenalkan dengan terapi kombinasi Antiviral-Antibiotik bagi ketiga-tiga strain Influenza. Kepantasan melegakan simptom telah tampak jelas bagi pesakit terapi kombinasi Antiviral-Antibiotik kerana markah min simptom Jangkitan Akut Pernafasan adalah lebih rendah secara signifikan pada Hari ke-4 kemasukan ke hospital bagi pesakit Influenza-A (H1N1) sementara Hari ke-3 kemasukan ke hospital bagi pesakit Influenza-A (bukan H1N1) dan Influenza-B. Kombinasi Oseltamivir-Azithromycin telah didapati sebagai terapi kombinasi paling berkesan terhadap kepantasan untuk melegakan simptom. Dalam kalangan pesakit warga emas (umur > 50 thaun), pesakit yang telah diperkenalkan dengan terapi kombinasi Antiviral-Antibiotik adalah tertakluk kepada signifikan secara statistiknya kurang jangkitan bakteria sekunder, kurang insiden keperluan bantuan pernafasan, dan tempoh kemasukan ke hospital yang lebih singkat bagi ketiga-tiga strain Influenza. Analisis *survival* menunjukkan bahawa kombinasi Antiviral-Antibiotik adalah berhubungkait dengan penurunan kadar kematian 90 hari dalam kalangan pesakit strain Influenza-A (H1N1) dan Influenza-A (bukan H1N1). Pengenalan awal terhadap terapi antibiotik yang dikombinasikan dengan Antiviral didapati lebih berkesan berbanding monoterapi Antiviral dalam mencegah komplikasi yang berkaitan dengan jangkitan Influenza yang teruk, terutamanya terhadap pesakit berisiko tinggi seperti pesakit warga emas,

pesakit yang tidak divaksin, dan pesakit yang mana rawatan antiviral dimulakan selepas 48 jam simptom kelihatan.

CLINICAL EFFICACY ASSESSMENT OF ANTIVIRAL-ANTIBIOTIC COMBINATION THERAPY FOR PREVENTION OF COMPLICATIONS ASSOCIATED WITH SEVERE INFLUENZA INFECTION

ABSTRACT

Secondary bacterial infections, mechanical respiratory support, and multi-organ failure are the complications associated with severe Influenza infection responsible for the mortalities and morbidities worldwide. The use of antibiotics in viral influenza infection is still debatable. The current study aimed to compare the efficacy of Antiviral drug therapy alone and Antiviral-Antibiotic combination therapy in prevention of complications associated with Influenza infection hospitalized patients. This two-center, retrospective cohort study was conducted in two tertiary care teaching hospitals in Saudi Arabia. Laboratory confirmed Influenza-A (non-H1N1), Influenza-B, Influenza-A (H1N1) hospitalized patients who presented in the emergency room after 48 hours of symptoms onset were identified and divided into two groups; Group-1 patients were initiated on Antiviral alone drug therapy while Group-2 patients were initiated on Antiviral-Antibiotic combination therapy. Both group patients were evaluated for different clinical outcomes, such as incidences of influenza associated secondary bacterial infections, the need for respiratory support, length of hospitalization stay, incidences of multi-organ failure, early clinical failure, and time to clinical stability. Comparative efficacy analysis of Antiviral alone therapy patients vs. Antiviral-Antibiotic combination therapy patients for Influenza-A (non-H1N1) strain (212 vs. 187 patients), Influenza-B (153 vs. 131 patients), and Influenza-A (H1N1) strain (227 vs. 286 patients) revealed that incidences of secondary bacterial infection, need of respiratory support, incidences

of ICU admission, length of hospitalization stay and time to clinical stability was statistically significant less for patients initiated on Antiviral-Antibiotic combination therapy for all three Influenza strains. The rapidity of symptoms relief was evident for Antiviral-Antibiotic combination therapy patients as the mean Acute Respiratory Infection symptom score was statistically significant low on hospitalization Day-4 (14.9 vs. 12.2; P<0.001) for Influenza-A (H1N1) patients while hospitalization Day-3 for Influenza-A (non-H1N1) and Influenza-B patients (12.9 vs 11.6, P= 0.039; 12.5 vs. 11.8, P= 0.007). Oseltamivir-Azithromycin combination was found to be the most effective combination therapy for the rapidity of symptoms relief. Among elderly patients (age >50 years), patients initiated on Antiviral-Antibiotic combination therapy were found to have statistically significant fewer secondary bacterial infections, fewer incidences of need for respiratory support, and shorter length of hospitalization stay for all three Influenza strains. Survival analysis revealed that the Antiviral-Antibiotic combination was associated with reduced 90-Day mortality among Influenza-A (H1N1) and Influenza-A (non-H1N1) strain patients (9.4% vs. 3.7%, P= 0.029; 6.6% vs. 2.8%, P= 0.044). Early initiation of Antiviral-antibiotic combination therapy was found to be more efficacious than Antiviral therapy alone in the prevention of severe Influenza infection-associated complications, especially in high-risk patients such as elderly patients, unvaccinated patients, and patients whom antiviral is initiated after 48 hours of symptoms onset.

CHAPTER 1:

INTRODUCTION

1.1 Overview of Influenza Infection

Respiratory tract infection (RTI) is characterized as any infectious disease of the lower or upper respiratory tract. Upper respiratory tract infection (URTI) includes acute rhinitis, acute otitis media, acute rhinosinusitis, laryngitis, common cold, and pharyngitis. Lower respiratory tract infection (LRTIs) includes tracheitis, bronchiolitis, pneumonia, and acute bronchitis (Ghebrehewet et al., 2016, Tan et al., 2008). RTI is the most frequent infectious disease in human beings. It is mainly due to high attack rate that RTIs are linked with substantial patient morbidity and associated mortality. In developing nations, morbidity owing to respiratory tract infections could be nevertheless as severe as that in underdeveloped nations (Fendrick et al., 2003). Even though most of the RTIs are self-limiting, still the high incidence rate of RTIs builds substantial health and monetary burden (Hollinghurst et al., 2008), particularly the time when away from everyday activities is taken into consideration (Lambert et al., 2004). Influenza infection and Respiratory Syncytial Virus (RSV) are the foremost contributors to this monetary burden of RTIs (Legand et al., 2013).

Influenza is a viral illness of worldwide apprehension, with a considerable degree of morbidity and mortality, that demonstrates both usual seasonal incidences of Influenza infection globally in addition to occasional Influenza infection pandemics (Moorthy et al., 2012). Each year, the Influenza virus spreads worldwide (Molinari et al., 2007), and frequently, novel strains of Influenza-A viruses arise and

trigger a pandemic (Brundage and Shanks, 2008, Chaves et al., 2015a). Influenza infection is clinically depicted by undifferentiated symptoms, which are usually observed in other RTIs such as sudden onset of fever, malaise, headache, as well as cough (Caini et al., 2018). Sickness due to Influenza infection is typically brief (3-5 days), and serious consequences are usually limited to high-risk patients, such as, elderly population, patients with comorbid illness (for example, chronic respiratory and cardiac disease, diabetes mellitus, and cancer) or immunocompromised individuals (Thompson et al., 2009).

1.1.1. Types of Influenza virus

Influenza viruses belong to the Orthomyxoviridae family of the virus, a Ribonucleic Acid (RNA) virus with distinct antigenic features (Moghadami, 2017). There are four types of Influenza viruses i.e., Influenza A, B, C, and D (Ghebrehewet et al., 2016, Ferguson et al., 2015). However, merely Influenza-A and Influenza-B viruses are clinically significant in humans (Memorandums, 1980, Webster et al., 1992, Olson et al., 2007, Ferguson et al., 2015, Organization, 2014). Influenza-A viruses are responsible for the utmost severe illness and are the usual trigger of seasonal epidemics and pandemics (Organization, 2014). The characteristics of different types of Influenza viruses are summarized in Table 1.1.

Table 1.1: Types of Influenza Viruses

Influenza type	At-risk groups
Influenza-A	
• Categorized into subtypes based on haemagglutinin (H) and neuraminidase (N) antigens on the surface of the viral envelope • 18 haemagglutinin subtypes and 11 neuraminidase subtypes have been identified. Three haemagglutinin types (H1, H2, and H3) are recognized to cause epidemic disease in humans.	People of all ages, but disproportionally causes severe disease in elders and individuals with underlying medical conditions.
Influenza-B	
Divided into lineages based on haemagglutinin glycoprotein.	Children are affected by Influenza-B infection at a disproportionally higher rate.
Influenza-C	
Influenza-C has only one glycoprotein.	Individuals of all ages.
Influenza-D	
Little is known, but it is believed to be linked to the Influenza C virus. Generally, infects pigs and cattle.	No human disease.

1.1.2. Epidemiology of Influenza Infection

Influenza infection is common throughout the year in tropical regions of the world (Viboud et al., 2006). However, in the Northern Hemisphere, the Influenza season usually starts from September-October, has its peak in December and, continue until mid of February (Alonso et al., 2015). The duration and severity of influenza epidemics could vary depending on the subtype involved (Caini et al., 2018). According to reports of the World Health Organization (W.H.O), around 3-5 million cases of severe Influenza illness appear every year, which causes around

290,000 to 650,000 casualties (Organization, 2018). The Centers for Disease Control and Prevention (CDC) estimates that during the Influenza season of 2019-2020 in USA, there were about 410,000 to 740,000 hospital admissions and around 24,000 to 62,000 deaths were related to flu-like illness (Prevention, 2020).

Epidemics due to seasonal Influenza infection from 1976 to 2004 have resulted in more than 200,000 hospital admissions annually, and more than 30,000 fatalities in the USA (Thompson et al., 2006). However, a distinct pattern has been observed in every season, such as with Influenza-A (H3N2) outbreak in 2002, the incidences of Influenza associated pneumonia and death were more common than Influenza-A (H1N1) infection (Thompson et al., 2003). Even though Influenza infection is common in children and elderly persons, around 90% of deaths are reported in elderly patients (Bautista et al., 2010).

Among recent Influenza seasons in recent years, the 2017-2018 Influenza season was reported as the longest and deadliest. The estimates indicate that more than 900 thousand people were hospitalized, and more than 80 thousand people died from Influenza infection. Out of these, around 58% of deaths occurred in elderly patients. The sick leaves of employees due to Influenza infection cause a loss of around 7 billion dollars every year, and the estimated overall cost of direct medical expenses is around 10.4 billion dollars every year (Prevention, 2020).

In Malaysia, the Influenza season usually present year-round. Variable periods of higher transmission occurred inconsistently, such as November to December, January to March, and July to September (Jamal and Sam, 2015, Sam et al., 2019). However, the use of Influenza vaccine is very low among Malaysian population, with a distribution rate of only 7.48 doses per 1000 people in 2013. The

incidence of seasonal influenza remains unknown. Seroprevalence rates of 22.3% for seasonal Influenza-A (H1N1) strain and 14.7% for seasonal Influenza-A (H3N2) strain were reported in Kuala Lumpur during Influenza-A (H1N1) pandemic in 2009 (Jamal and Sam, 2015, Sam et al., 2019).

Health authorities in Saudi Arabia kept a high level of alertness in monitoring the situation of pandemic Influenza-A (H1N1) all over the country especially during winter season, and around Hajj season which limits further spread of the virus locally, regionally, and internationally. A surveillance data of hospitals under Ministry of Health captured 113,588 suspected influenza cases, of which 17,094 (15%) cases tested positive for Influenza-A (H1N1), representing those who needed hospitalization. The compliance of reporting was questionable in some regions and at different levels, which resulted in under-reporting of suspected cases (Abdalla et al., 2020).

Epidemics caused by the Influenza-A virus are more common; however, Influenza-B epidemics can also occur (Sugaya et al., 2007, Kawai et al., 2005). Influenza epidemics are frequently presaged by the absence of students from the school, contemplating the significance of children as vectors of transmission to adults. Consequently, Influenza infections are spread throughout the population by way of outbreaks in community, schools, nursing homes, and hospitals. Outbreaks have also reported in varied locations such as cruise ships and aircraft (Belser et al., 2010). Low humidity and cold temperature facilitate the transmission of the Influenza virus (Lowen et al., 2007). Seasonal outbreaks thus can be explained by the culture of indoor gathering in the winter season. The higher concentration of Influenza virus in respiratory secretions cause efficient spread of Influenza infection among people in close contact through large droplets expelled out by coughing and

sneezing (Brankston et al., 2007). The airborne spread might also occur in the form of small particles generated in an infected patient (Fiore et al., 2011). These ways of transmission such as direct person-to-person contact or indirect transmission through contact with a contaminated surface contribute to the explosive nature of influenza outbreaks (Brankston et al., 2007). Deaths related to Influenza infection are relatively higher during pandemics in contrast to local epidemics (Osterholm, 2005), because of infection among masses and lack of defensive immunity against the latest circulating virus subtype. Pandemics are uncommon (11 pandemics in last 300 years) but could be disastrous (Morens et al., 2010).

1.1.3. Symptoms of Influenza

Influenza infection is characterized by the unexpected onset of dry cough, fever, headache, malaise, myalgia, nasal congestion, and/or sore throat (Figure 1.1) (Lam et al., 2016, Ohmit and Monto, 2006). Gastrointestinal signs/symptoms such as nausea, diarrhea, and vomiting are also common (Minodier et al., 2015). The incubation period (time from the moment of the exposure until the appearance of signs and symptoms) is 1 to 4 days (Lessler et al., 2009). Viral shedding typically starts from a day prior to the onset of symptoms until 5-7 days post-infection stage (Killingley et al., 2011, Lau et al., 2010). In the absence of an identified Influenza virus, this pattern of signs and symptoms are usually similar to other viral infections, and is frequently termed as Influenza-like illness (ILI). Symptoms of ILI closely resemble Influenza infection symptoms such that two previously have been undifferentiated. Hence, a symptom measure worthwhile in Influenza infection might also be valuable for assessing the existence and severity of symptoms of ILI (Powers III et al., 2018).

In the course of peak Influenza season, hospitals and emergency rooms might be overwhelmed by patients exhibiting ILI and other serious illnesses (Glaser et al., 2002, Rodriguez-Noriega et al., 2010). CDC trails Influenza infection activity utilizing fundamental markers such as the proportion of ILI visits in outpatient clinics, frequencies of Influenza infection related hospital admissions, and mortalities due to Influenza infection (Biggerstaff et al., 2017).

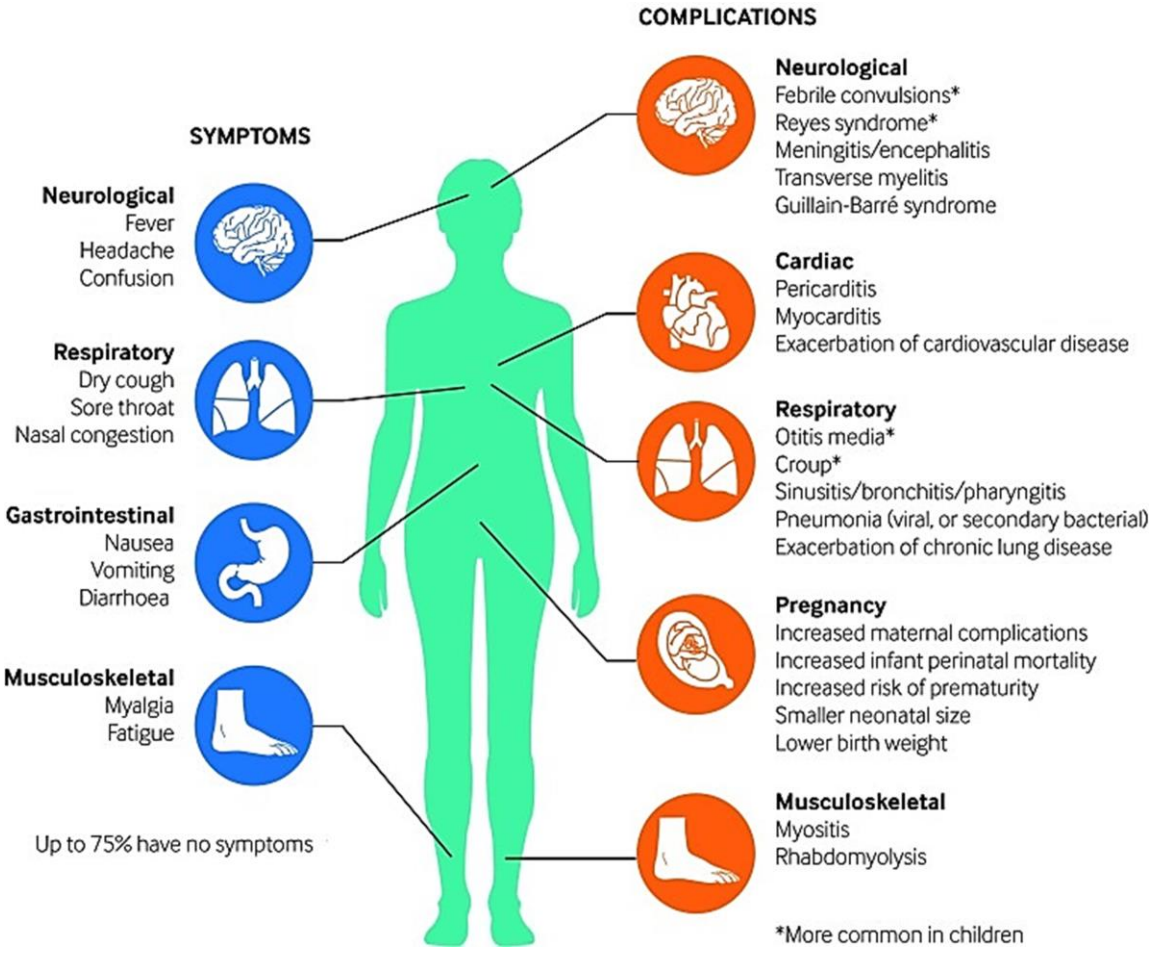

Figure 1.1: Symptoms and complications of Influenza
Adopted from; https://www.bmj.com/content/355/bmj.i6258.
Date Accessed: 24th August, 2020 (Ghebrehewet et al., 2016)

1.1.4. Complications of Influenza Infection

Generally, complications are mostly associated with high risk patients, i.e., age greater than 65 years or very young (age less than six months), and patients with underlying medical conditions. Every year, Influenza infection causes around 36,000 mortalities, and more than 200,000 hospital admissions in the United States (Thompson et al., 2003, Thompson et al., 2004). Not long ago, severe illness and 80% of Influenza infection associated mortalities were due to Influenza-A (H3N2) virus strain instead of Influenza-A (H1N1) virus strain infection (Thompson et al., 2004).

1.1.4 (a) Pulmonary Complications

Pulmonary complications are common in Influenza infection which includes exacerbations of chronic pulmonary diseases, pneumonia due to infrequent pathogens, primary viral pneumonia, and secondary bacterial pneumonia. Increased sensibility to bacterial infections following viral Influenza is a world-wide concern, which is one of the reasons for several hospitalizations as well as fatalities, predominantly in the course of Influenza infection pandemics (Shirey et al., 2019).

Influenza-associated bacterial pneumonia were reported in 1918 pandemic and throughout consecutive epidemic and interepidemic phases, causing the majority of mortalities associated with Influenza infection (Morens et al., 2008, Brundage and Shanks, 2008, Morens and Fauci, 2007, Louria et al., 1959). Clinical demonstration of bacterial pneumonia after seasonal Influenza infection closely resembles community-acquired pneumonia. Sometimes, patients develop bacterial pneumonia after 4 to 14 days post-Influenza virus infection, and characterizes with reappearance of symptoms such as fever, dyspnoea, productive cough, and abnormal chest radiographic reports (Bennet et al., 2015).

Influenza virus produces harm to the epithelial layer of upper and lower respiratory routes, causing increased contact of attachment locations essential for adherence bacteria such as pneumococcus (McCullers, 2006). Leucocytosis with left shift persisted length of fever and raised erythrocyte sedimentation rate are farther observed in bacterial superinfection (Jarstrand and Tunevall, 1975). More frequent isolated bacteria include *Streptococcus pneumoniae, Staphylococcus aureus,* and *Haemophilus influenzae* as well as some gram-negative bacteria (Schwarzmann et al., 1971). The most frequent reported pathogen involved is *Streptococcus pneumoniae*, followed by *Staphylococcus aureus, Streptococcus pyogenes, Streptococcus mitis* and *Haemophilus influenzae*. Around 9% to 20% of severe Influenza infection hospitalized patients were complicated by ventilator-associated pneumonia due to *Pseudomonas aeruginosa, Acinetobacter baumannii, MRSA, Achromobacter xylosoxidans,* and *Escherichia coli.* However, *Staphylococcus aureu*s and *MRSA* pathogens were found to be the most frequent in paediatric mortalities from Influenza-A (H1N1) pandemic 2009 strain (pdm09) in the USA. Other reported pathogens includes *Streptococcus pneumoniae, Streptococcus pyogenes,* and *Streptococcus constellatus* (Shannon et al., 2009).

Throughout the inter-pandemic phase, secondary bacterial infection with atypical pathogens as well as fungal microorganisms may also arise in Influenza infected patients. Concurrent *Legionella pneumophila* infection was reported in one small retrospective research. Secondary invasive Aspergillosis has also been reported with a significantly elevated mortality rate (Lewis et al., 1985, Boots et al., 1999). Histologic examination of the lungs of Influenza-A (H1N1) patients disclosed that 29% of non-survivors had secondary bacterial infections (Louie et al., 2009).

Viral infections are the chief contributor to exacerbations of asthma and COPD. Previous studies of asthmatic children and adults, and adults revealed that the majority of individual asthma exacerbations were linked with URTI (Johnston et al., 1995). COPD associated respiratory viruses were found in 56% of acute exacerbations individuals (Rohde et al., 2003). Mechanism of respiratory virus-induced exacerbations of chronic respiratory diseases are partly understood; however, they are possibly dependant on multiple factors as well as linked with inflammatory mediators like cytokines, interleukins, and changes in the proportion of T-cell subsets resulting in augmented sensitivity to other allergens (Wedzicha, 2004, Lin et al., 1988). Previous researches related to Influenza-A (H1N1)pdm09 revealed that asthma and COPD were two main comorbid conditions among severe Influenza hospitalized patients (Kaufman et al., 2009, Rello et al., 2009).

1.1.4 (b) Extra-pulmonary Complications

Along with its respiratory consequences, the Influenza virus can (directly and indirectly) affect other organ systems of the body. Direct cardiac complications, i.e., pericarditis and myocarditis have also been reported but are infrequent. It has been reported that about 50% of patients with Influenza infection had no cardiac symptoms; however, they demonstrated distinctive electrocardiographic features (Ison et al., 2005). Moreover, most patients returned to a normal state by 28 days with no evidence of cardiomyocyte damage or lessened ejection fractions. Raise of creatine phosphokinase is also frequent; however, this seems of skeletal muscle origin (Greaves et al., 2003). Influenza-A (H1N1) infected patients have not demonstrated cardiac complications; however, a case of a formerly healthy young woman was reported who developed myopericarditis following Influenza viral infection (Davoudi et al., 2012).

Influenza infection indirectly affects patients suffering from heart diseases. Convincing data support the association of viral Influenza and acute myocardial infarction and death (Warren-Gash et al., 2009). Even though, the way by which cardiac complication arises is not clearly explained in previous researches. However, Influenza is a strong inducer of pro-inflammatory cytokines (Kaiser et al., 2001), and inflammation is now believed to be an essential element of atherosclerotic ailment (Ross, 1999).

Myositis as well as rhabdomyolysis has been linked with seasonal Influenza infection (Foulkes et al., 1990). More than 50% of Influenza-A hospitalized patients have been reported with increased creatine phosphokinase level. Critically ill Influenza-A (H1N1) patients may develop renal failure and challenges with ambulation (Napolitano et al., 2009). Incidences of influenza infection associated acute renal failure has also been observed among Intensive Care Unit (ICU) admitted patients, with some of them requiring continuous renal replacement therapy (Napolitano et al., 2009, Control and Prevention, 2009b, Kaufman et al., 2009).

Neurologic complications of Influenza infection include aseptic meningitis, encephalomyelitis, encephalopathy, focal neurologic disorders, Guillain–Barré syndrome, and transverse myelitis (Studahl, 2003). Although, the pathogenesis is ambiguous but direct viral invasion and the development of antigen/antibody complexes or excessive production of systemic cytokines have been reported in patients with neurologic complications (Maricich et al., 2004). The electroencephalogram is typically unusual (Studahl, 2003). The encephalopathy/encephalitis outbreak of 1999 in Japan was linked with Influenza-A. All the affected patients had varied consciousness, and the majority had seizures. (Morishima et al., 2002). Neurologic complications have also been reported in

Dallas, Texas, among Influenza-A (H1N1) infected children. The majority of affected patients had varied mental status, and few had encephalopathy and seizures (Evans et al., 2009).

1.1.4 (c) High-Risk Patients for Developing Influenza Complications

Evaluating risk can ease the objective of vaccination during the Influenza season specially during vaccine shortages where the priority must be given to high risk individuals (Rothberg, 2004). Chronic disorders such as dementia, diabetes, heart disease, lung disease, renal disease, rheumatologic disease, and stroke are risk contributors for Influenza complications. Irrespective of age, individuals with high-risk medical conditions have higher rates of hospital admission and mortality (Glezen et al., 1987, Izurieta et al., 2000). Consequently, high-risk individuals aged 45 to 64 years have a risk comparable to individuals aged >65 years (Glezen et al., 1987). The Advisory Committee on Immunization Practices has encompassed vaccination recommendation to include all individuals aged >50 years due to the elevated occurrence of occult cardiopulmonary disease (Harper et al., 2005). For patients aged >65 years, high-risk circumstances are exceptionally dangerous (Hak et al., 2004).

1.1.4 (d) Influenza associated Complications in Elderly Patients

Elderly persons bear the greatest burden of morbidity and mortality of any group, with 54% to 70% of seasonal influenza-related hospitalizations and 71% to 85% seasonal Influenza-related deaths. Older patients are less likely to display classic Influenza infection symptoms than younger patients. Fever is often not present. Instead, many older patients were presented with exacerbations of pre-existing comorbidities, such as dyspnoea or cough. In a survey of laboratory-documented Influenza infection patients older than 50 years with COPD, respiratory

symptoms such as cough, sputum production, and/or dyspnea were observed in more than 90% of patients, while fever was observed in 68% patients, and myalgias in 81% patients. The elderly population is afflicted by significant comorbidities which are also reported as risk factors for severe Influenza-related complications (Wilhelm, 2018). Among adults, complications, hospitalizations, and deaths due to influenza infection are generally most common in individuals aged >65 years old. However, adults aged >50 years old are a priority group for vaccination because this group may be more likely to have chronic medical conditions that put them at high risk of severe influenza illness (Control and Prevention, 2019b)

Figure 1.2 enlists of the health and age factors that are known to increase a person's risk of getting serious complications from Influenza infection (Control and Prevention, 2016, Control and Prevention, 2019b)

- Adults 65 years and older.
- Children younger than 2 years old.
- Asthma & chronic lung diseases (such as COPD).
- Neurologic and neurodevelopment conditions.
- Blood disorders (such as sickle cell disease).
- Endocrine disorders (such as diabetes mellitus).
- Heart disease (such as congenital heart disease, congestive heart failure and coronary artery disease).
- Metabolic disorders, Kidney diseases & Liver disorders.
- People who are obese with a body mass index [BMI] of 40 or higher.
- People younger than 19 years old on long-term aspirin- or salicylate-containing medications.
- People with a weakened immune system due to disease (such as people with HIV or AIDS, or some cancers such as leukemia) or medications (such as those receiving chemotherapy or radiation treatment for cancer, or persons with chronic conditions requiring chronic corticosteroids or other drugs that suppress the immune system).
- People who have had a stroke.

Figure 1.2: List of all the health and age factors at high risk for serious Influenza complications

Adopted From: https://www.cdc.gov/flu/highrisk/index.htm

Accessed: 24th September, 2020 (Control and Prevention, 2016, Control and Prevention, 2019b)

1.1.5. Laboratory diagnostic techniques

Several Influenza diagnostic approaches are available virus isolation, Nucleic Acid Amplification Test (NAAT), immunochromatography-based rapid diagnostic test (Bochenek et al.), etc. The techniques which are available and under development (Vemula et al., 2016) are briefly described in Table 1.2.

Table 1.2: Influenza Infection Diagnostic Techniques

Laboratory diagnostic test	Description	Drawbacks
Virus Isolation-Viral Culture	Inoculation of permissive cell lines or embryonated eggs with infectious samples, propagation for 7–10 days to monitor cytopathic effect and final confirmation by specific antibody staining.	Final results take very long time i.e. 7-10 days
Direct Fluorescent Antibody Test	Direct staining of respiratory epithelial cells derived from nasopharyngeal swabs or aspirates with Influenza virus-specific fluorescent antibodies	No sub-type detection of Influenza-A strains.
Rapid Influenza Diagnostic Tests	Uses antibodies that target viral nucleoprotein and employ either enzyme immunoassay or immunochromatographic techniques. Simple and takes short processing time.	Some techniques don't differentiate between Influenza-A & B strains.
Nucleic Acid-Based Tests (NAT)	NAT assays are based on Polymerase chain reaction (PCR) and detect virus-specific Deoxyribonucleic Acid (DNA) or Ribonucleic Acid (RNA) sequences/genetic material. Far more sensitive and can detect viruses much earlier.	Final results take long time i.e. 2-5 hours.
Hemagglutination Inhibition Assay	Widely used method to confirm the presence of Influenza virus HA-specific antibodies in serum but possibility of false-positive results limit use of this test in hospitals.	False-positive results due to antibodies produced after vaccination.

1.2 Overview of Prevention Strategies of Influenza Infection

1.2.1 Trivalent and Quadrivalent Seasonal Influenza Vaccines

Yearly vaccination is suggested for all with age >6 months (excluding individuals who are known to have an allergy to vaccination or have undergone anaphylactic reaction earlier to the Influenza vaccine or any of its content) is vital for averting Influenza infection as well as its complications. Hand sanitization, cough etiquette, and self-isolation are also crucial aspects of decreasing spread of Influenza virus. Antiviral drugs have minor role in prevention of Influenza infection and they should be used mainly for treatment of confirmed influenza infection (Robson et al., 2019).

Various types of Influenza vaccines are available (Robson et al., 2019). The trivalent Influenza vaccine usually contain two Influenza-A subtype strains, and one Influenza-B strain, while quadrivalent vaccines includes additional Influenza-B virus lineage subtype. It has been observed that quadrivalent Influenza vaccines confer advanced protection without any apparent rise in adverse reactions (Greenberg et al., 2013). The quadrivalent vaccine is an effective vaccination for children and adults along with pregnant women. The children aged >6months upto 9 years must receive two doses at least four weeks apart. Patients in the first year after receiving a solid organ or hematopoietic stem cell transplant must also receive two doses four weeks apart (Robson et al., 2019).

1.2.2 Antiviral Prophylaxis

There is a role for Neuraminidase inhibitors in prevention of Influenza infection. Oseltamivir and Zanamivir may decrease the risk of symptomatic Influenza infection (Heneghan et al., 2016, Jefferson et al., 2014b). However, they

must not be considered as an alternative to vaccination as a preventive strategy of Influenza infection (Fiore et al., 2011). Post-exposure prevention with neuraminidase inhibitors must be taken into consideration only for individuals at high risk of complications and cannot be vaccinated, or have not been vaccinated, or expected to have an unsatisfactory or ineffectual response from the Influenza vaccine (Harper et al., 2009).

In the course of the Influenza infection outbreak in residential care institutions, chemoprophylaxis must only be taken into consideration, along with other infection control approaches (Australia, 2017). Antiviral prophylaxis must be given within twenty-four hours to all asymptomatic residents (Australia, 2017, Harper et al., 2009). Chemoprophylaxis must be administered for ten days or till the outbreak is finished (Australia, 2017). There might be a part to expand this approach of antiviral 'ring prophylaxis' in closed or semi-closed locations (i.e., cruise ships, military barracks, boarding schools) where antiviral prophylaxis in close contacts might shorten the range of infection (Australia, 2017, Lee et al., 2010b). Chemoprophylaxis antiviral approach has also been effective in immunocompromised individuals (Yue et al., 2017).

Chemoprophylaxis does not totally eradicate risk and vulnerability to reoccurrences of Influenza when prophylactic antivirals are discontinued (Fiore et al., 2011). Neuraminidase inhibitors could be ineffectual at stopping asymptomatic Influenza infection. Furthermore, long-term use of neuraminidase inhibitors for Influenza prevention might result in the emergence of resistance (Jefferson et al., 2014b, Heneghan et al., 2016, Li et al., 2015).

1.3 Overview of Antiviral Drugs in Treatment of Influenza Infection

Antivirals for Influenza infection are divided into two categories, which are used for both treatments and prevention of infection. These categories are neuraminidase inhibitors and adamantanes (amantadine & rimantadine). Moreover, various novel antiviral agents for the management of Influenza are presently in development.

Neuraminidase inhibitors, i.e., Zanamivir, Baloxavir, Oseltamivir, and Peramivir, are the backbone for treating viral Influenza infection. Nevertheless, they must be administered within 48 hours of the onset of symptoms and are of utmost helpful when given within 24 hours. They hinder the neuraminidase enzyme of the virus, stopping the virus from escaping the host cell (Bennett et al., 2014).

Neuraminidase inhibitors are moderately effective for the treatment of susceptible viral infections, decreasing the duration as well as the severity of symptoms. Neuraminidase inhibitors are also useful in decreasing the duration of shedding and viral titer (Aoki and Boivin, 2009). The United States Food and Drug Authority (FDA) has officially recognized these agents to manage acute uncomplicated Influenza in individuals who have been symptomatic for ≤48 hours. Both Zanamivir and Oseltamivir have extensively investigated among critically ill Influenza infected patients (Zachary et al., 2017).

As per CDC recommendations, Antiviral treatment is recommended as early as possible, preferably within 48 hours of illness onset, in patients with confirmed or suspected Influenza who (a) have the severe, complicated, or progressive illness, (b) are hospitalized, or (c) are at higher risk of Influenza complications. Hospitalized patients and patients with severe or complicated illness should receive oral or

enterically administered Oseltamivir because of insufficient evidence for inhaled Zanamivir and Intravenous (IV) Peramivir. Previously healthy, symptomatic outpatients not at high risk may be considered for antiviral treatment, if it can be initiated within 48 hours of illness onset. Acute uncomplicated Influenza in an outpatient may be treated with oral Oseltamivir, inhaled Zanamivir, or IV Peramivir. Antiviral treatment may still be beneficial when administered after 48 hours in patients with severe, complicated, or progressive illnesses and hospitalized patients (Control and Prevention, 2017, Tamiflu(R), 2016). The doses of different neuraminidase inhibitor antiviral drugs are summarized in Table 1.3 (Control and Prevention, 2017).

Table1.3: Recommended Dosing of Antiviral Medications for Seasonal Influenza Infection in Adults (Fiore et al., 2011, Grohskopf et al., 2019, Control and Prevention, 2017)

Antiviral agent	Dose
Oseltamivir	
Treatment, influenza A and B	75 mg orally twice daily for five days.
Chemoprophylaxis, influenza A and B	75 mg orally once daily.
Zanamivir	
Treatment, influenza A and B	10 mg (two 5 mg inhalations) twice daily for five days.
Chemoprophylaxis, influenza A and B	10 mg (two 5 mg inhalations) once daily.
Peramivir	
Treatment, influenza A and B	600 mg intravenously as a single dose.
Baloxavir	
Treatment, influenza A and B	40 kg to <80 kg 40 mg orally as a single dose. ≥80 kg 80 mg orally as a single dose.

1.3.1 Oseltamivir

Oseltamivir phosphate is an ethyl ester prodrug requiring ester hydrolysis for conversion to the active form, Oseltamivir carboxylate. Oseltamivir carboxylate is an inhibitor of Influenza virus neuraminidase affecting the release of viral particles (Tamiflu(R), 2016). Oseltamivir exhibits activity against Influenza-A and Influenza-B viruses. Oseltamivir is indicated for the prophylaxis of Influenza infection in adults and children aged one year or older. When used for prophylaxis in adults, Oseltamivir reduced the duration of influenza infection symptoms (Jefferson et al., 2014b). Oseltamivir is also indicated for treating uncomplicated acute Influenza-A and Influenza-B virus infection, in adults and children aged two weeks or older who have been symptomatic for no longer than two days. A large meta-analysis of unpublished data from manufacturers and regulatory agencies reported that when used for the treatment of Influenza, time to first alleviation of symptoms was shorter with Oseltamivir as compared with placebo (Control and Prevention, 2014, Jefferson et al., 2014b).

The usual dose of Oseltamivir is 75 mg orally twice daily for five days. CDC recommends initiating Oseltamivir within two days of symptom onset; treatment may still be beneficial when administered after 48 hours in patients with a severe, complicated, or progressive illness and hospitalized patients. Extended duration of therapy may be considered in patients who remain severely ill after five days of treatment or in immunosuppressed patients (Control and Prevention, 2014, Tamiflu(R), 2016).

The common reported adverse effects of Oseltamivir includes; gastrointestinal nausea (8% to 10%), vomiting (adult and adolescent, 2% to 8%; pediatric, 8% to 16%); neurologic headache (influenza treatment, 2%; influenza

prophylaxis, 17%); cardiovascular cardiac dysrhythmia; dermatologic erythema multiforme (rare), facial swelling (Tamiflu(R), 2016).

Efficacy of Oseltamivir in Prevention of symptoms and complications:

Oseltamivir reduces the duration of Influenza symptoms by nearly one day (Jefferson et al., 2014a, Fry et al., 2014, Cooper et al., 2003, Nicholson et al., 2000, Dobson et al., 2015, Treanor et al., 2000, Burch et al., 2009, Hayden et al., 1999, Aoki et al., 2003a, Kawai et al., 2005, Kawai et al., 2006) and reduce the duration of viral shedding by nearly two days (Aoki and Boivin, 2009, Fry et al., 2014). Some studies have also shown that Oseltamivir reduces illness severity and complications (Dobson et al., 2015, Treanor et al., 2000, Kaiser et al., 2003), hospital admissions (Venkatesan et al., 2017, Dobson et al., 2015), and the length of hospitalization (Lee et al., 2007a, Chaves et al., 2015b).

Meta-analyses have reported inconsistent findings concerning the impact of Oseltamivir on Influenza-associated LRTI complications. A possible explanation is that few studies include patients with Influenza-like illness which leads to an underestimated benefit since Oseltamivir does not have activity against viruses other than Influenza. For example, a meta-analysis that analyzed the results from 11 randomized trials concluded that overall Oseltamivir treatment reduced the risk of lower respiratory tract complications by 28% and 37% among patients with confirmed viral Influenza (Hernán and Lipsitch, 2011).

In the year 2015, a meta-analysis assessed all published and unpublished randomized trials of Oseltamivir used in adults for viral Influenza infection that were sponsored by the manufacturer of Oseltamivir. This meta-analysis was funded by the manufacturer of Oseltamivir but was performed by an independent research group.

With the objective of treating the population of laboratory-confirmed Influenza infected patients, there was a significant reduction in time to alleviate all influenza infection symptoms by Oseltamivir compared with placebo. Oseltamivir conferred no benefit in patients without confirmed Influenza infection. In the intention-to-treat Influenza-infected population, patients initiated on Oseltamivir had fewer LRTI complications and fewer hospital admissions (Dobson et al., 2015).

Efficacy of Oseltamivir in Prevention of Mortality: Some observational studies have found a link between Oseltamivir use and decreased mortality rate among Influenza infected patients (Hsu et al., 2012, McGeer et al., 2007, Bowles et al., 2002, Muthuri et al., 2014). No randomized trials have assessed mortality because all such trials have been conducted in healthy individuals in which the mortality rate from Influenza infection was reported to be very low.

A cohort study conducted over eight Influenza seasons of 1330 critically ill patients treated with Oseltamivir for Influenza infection reported 622 (47 %) deaths in patients admitted in ICU. Among patients with Influenza-A (non-H1N1), early treatment (≤48 hours from symptom onset) was linked with a decrease in mortality rate than late treatment. No effect on mortality was observed in patients infected with Influenza-B or Influenza-A (H1N1) (Lytras et al., 2019).

1.3.2 Zanamivir

Zanamivir is given as oral inhalation. Inhaled Zanamivir is contraindicated in individuals with asthma or other chronic respiratory diseases. An intravenous formulation of Zanamiviris evaluated in clinical trials (Ong and Hayden, 2007, Calfee et al., 1999, Marty et al., 2013) and use should be considered only to treat critically ill adults and children having a life-threatening condition due to suspected

or confirmed Oseltamivir-resistant pandemic influenza virus infection (Dulek et al., 2010). Inhaled Zanamivir has been demonstrated in randomized trials to shrink the duration of symptoms by 1 to 3 days (Monto et al., 1999, Hayden et al., 1997, The, 1998). Subsequent meta-analyses have shown similar benefits (Burch et al., 2009, Jefferson et al., 2014a). Zanamivir did not decrease the risk of self-reported investigator-mediated pneumonia or radiologically verified pneumonia in adults. There was no reduction in otitis media or sinusitis, but there was a small reduction in bronchitis. There was no data to evaluate the effect of hospital admission. In an open-label, non-randomized, cohort study (n=1113) comparing Oseltamivir and Zanamavir, the duration of fever due to Influenza was shorter in Zanamivir-treated patients compared with Oseltamivir-treated patients; However, the differences were not clinically significant for Influenza-A and were less than one day for Influenza-B (Kawai et al., 2008).

1.3.3 Peramivir

Peramivir was officially recognized by the United States FDA in 2014 for the management of uncomplicated viral Influenza infection in adults (ill for $\leq$2 days) (Zachary et al., 2017). Peramivir has a long-term and robust affinity with neuraminidase; therefore, it is administered as a single intravenous dose of 600 mg (Kohno et al., 2010, Zachary et al., 2017). The efficacy of Peramivir was demonstrated in a trial that included 297 patients with laboratory-confirmed Influenza infection who were randomly assigned to receive a single dose of either placebo or Peramivir at a dose of 300 or 600 mg. Influenza patients who received Peramivir 600 mg demonstrated that Influenza symptoms improved at an average of 21 hours earlier and turned out to be afebrile about 12 hours earlier than those who

received placebo. Efficacy could not be established in patients with Influenza infection requiring hospitalization (Zachary et al., 2017).

Peramivir has also been compared with Oseltamivir. In a systematic review and meta-analysis of seven studies (two randomized and five observational studies) in patients with seasonal Influenza, IV Peramivir (n=956) compared with oral Oseltamivir (n=720) resulted in a significantly shorter time (-7.17 hours) to the alleviation of Influenza symptoms or fever; however, in a subgroup analysis, there was no significant improvement using only the two randomized trials. There was no significant between-group improvement in symptoms in hospitalized patients, in total mortality, in the length of hospital stay, or in the rate of serious adverse events in evaluable studies (Lee et al., 2017b).

In a trial of 121 patients hospitalized with laboratory-confirmed Influenza infection, the patients were randomly assigned to treatment with Peramivir (600 mg IV once daily for five days) or placebo. However, reduction in viral shedding was noticed in patients receiving Peramivir; although, the difference was statistically non-significant. However, it would be premature to conclude, based on one study, that Peramivir might not have a role to in treatment of hospitalized patients (De Jong et al., 2014).

1.3.4 Baloxavir

Baloxavir marboxil is a recently approved oral selective inhibitor of Influenza cap-dependent endonuclease which impedes Influenza proliferation by preventing the beginning of mRNA synthesis (Heo, 2018). Baloxavir was approved in Japan in February 2018 for the treatment of Influenza in adults and children aged ≥12 years old (Heo, 2018, Zachary et al., 2017). It was approved in the United States

in October, 2018 for treatment of uncomplicated acute Influenza in adults and children ≥12 years of age who have been symptomatic for ≤48 hours (Zachary et al., 2017). The median time to alleviate symptoms in intention-to-treat infected population was 53.7 hours with Baloxavir versus 80.2 hours with placebo. The time to the improvement of symptoms was similar to Baloxavir and Oseltamivir. Baloxavir was associated with more rapid declines in infectious viral load than placebo or Oseltamivir (Hayden et al., 2018).

Unanswered questions include whether Oseltamivir-Baloxavir combination therapy provides a more significant clinical benefit than Oseltamivir or Baloxavir monotherapy in hospitalized patients and severely immunocompromised patients, and whether Baloxavir can successfully treat patients with Neuraminidase inhibitors-resistant Influenza infections. The emergence of resistance after a single dose raises concerns about the long-term utility of this drug as monotherapy, mainly if it is used widely (Uyeki, 2018).

1.3.5 Antiviral Adjunct Therapies

There are several investigational adjunct treatment approaches for the treatment of Influenza (summarized in Table 1.4). These approaches include parenteral and/or long-acting formulations of Neuraminidase inhibitors, combination antiviral therapy with more than one agent, and antiviral agents with novel mechanisms of action. Some experts have called for the use of primary virologic endpoints to evaluate new Influenza infection treatment therapies (Ison et al., 2010).

Table 1.4: Adjunct Treatment Approaches for Treatment of Influenza Infection

Investigational approach	Description
Adjunctive therapies with Statins	Anti-inflammatory effects of statins could reduce the severity of illness. In a surveillance study, patients receiving a statin had a lower probability of dying than those who were not (Vandermeer et al., 2011).
Adjunctive therapies with Clarithromycin and naproxen	Work by immunomodulatory and/or direct antiviral effects of clarithromycin and/or naproxen and better treatment of bacterial pneumonia (Hung et al., 2017).
Adjunctive therapies with Intravenous immunoglobulin, convalescent plasma, and hyperimmune globulin	There is also interest in using intravenous immunoglobulin, convalescent plasma, or hyperimmune globulin as adjunctive therapy for severe Influenza infections. However, further study is necessary before these preparations can be recommended for Influenza (Zachary et al., 2017).

Where; NAI= Neuraminidase Inhibitor, RNA= Ribonucleic Acid

Adjunct therapy of antibiotics with antiviral drugs is examined in few researches. Administration of antibiotics is commonly continued after the diagnosis of viral RTI. One hypothesis that could explain antibiotic use after viral diagnosis is clinician anxiety over the possibility of concurrent or developing bacterial RTI. Radiographic evidence that caused concern for pneumonia was the strongest predictor for continuation of antibiotic therapy after a diagnosis of viral RTI (Shiley et al., 2010b). Influenza may be complicated by pneumonia, which has two recognized types: primary viral and secondary bacterial infection. The prognosis of patients presenting with bacterial pneumonia rapidly worsens with delay in treatment, and effective empirical treatment (before or in the absence of a specific