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REVIEW

Pandemism of swine flu and its prospective drug therapy

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Abstract Swine flu is a respiratory disease caused by influenza A H1N1 virus. The current pandemic of swine flu is most probably due to a mutation—more specifically, a reassortment of four known strains of influenza A virus subtype H1N1. Antigenic variation of influenza viruses while circulating in the population is an important factor leading to difficulties in controlling influenza by vaccination. Due to the global effect of swine flu and its effect on humans, extensive investigations are being undertaken. In this context, Tamiflu is the only available drug used in the prophylaxis of this disease and is made from the compound shikimic acid. Due to the sudden increase in the demand of shikimic acid, its price has increased greatly. Thus, it is necessary to find an alternative approach for the treatment of swine flu. This review presents the overall information of swine flu, beginning from its emergence to the prevention and treatment of the disease, with a major emphasis on the alternative approach (bacterial fermentation process) for the treatment of swine flu. The alternative approach for the treatment of swine flu includes the production of shikimic acid from a fermentation process and it can be produced in large quantities without any time limitations.

Introduction

Swine influenza has emerged as the primary public health concern of the 21st century. Although various strains of avian influenza have been recognised for decades, the lethality and mutability of the H1N1 subtype of the influenza virus has served as the source of the human influenza pandemic—swine flu. A highly lethal but non-human-to-human-transferable influenza A subtype, H1N1, swine flu emerged and raged through South-East Asian countries, Egypt and other countries, after the preparation of the world for the 2008 pandemic influenza, i.e. H5N1, bird flu [1].

In the last decade, there have been many outbreaks of avian influenza, which has affected the poultry industry all over the world. The largest and most severe outbreak of avian influenza began in South-East Asia in mid-2003 and cases are sporadically continuing till the present day. Outbreaks have also been reported in other parts of the world, such as in Europe (Netherlands, Turkey, Romania, Italy, Germany, France, Kazakhstan, Russia, Croatia and Ukraine), Africa (Egypt) and Asia (Malaysia, Mongolia, Iraq and India). The outbreak affected domesticated as well as wild migratory birds. The possible zoonotic nature of the disease has caused major panic, leading to the killing of millions of poultry birds, affecting the meat industry [2].

Influenza A virus is the genus of the Orthomyxoviridae family of viruses [3]. Multipartite, negative-sense, single-stranded RNA genome and a lipid envelope are the characteristics of influenza viruses [4].

According to the antigenic properties of the viral nucleoprotein, the influenza viruses are divided into three genera, i.e. influenza A viruses, influenza B viruses and influenza C viruses [5]. Mainly, humans are infected by influenza B and C viruses. Low-level sporadic diseases are also caused by influenza B and C viruses. In addition to this, these viruses cause limited outbreaks and can never cause a pandemic [6, 7]. In contrast to this, most of the seasonal influenza and all known pandemics are caused by influenza A viruses [7].

The causative agent of swine flu was first identified as influenza A H1N1 virus in pigs. This strain remained predominant for 60 years till the emergence of a new strain,

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H3N2, in America in 1997–1998, having three different subtypes and five different genotypes [8].

The 1918 flu pandemic (Spanish flu pandemic) caused by the H1N1 subtype (type A influenza) is the most famous and most lethal outbreak, and lasted from 1918 to 1919. An estimated 20–100 million people were killed during this flu outbreak [8, 9]. Spanish flu is famous as "the greatest medical holocaust in history" because it killed many more people than the Black Death Bubonic Plague. In this pandemic, there was an extreme severity of symptoms and an extremely high infectious rate of up to 50 % [9]. This flu was initially misdiagnosed as dengue, cholera or typhoid due to the unusual symptoms. These symptoms include haemorrhage from mucous membrane, especially from the nose, stomach and intestine. Bleeding from the ears and petechial haemorrhages in the skin were also observed [8]. Most of the deaths were caused by bacterial pneumonia, which was the result of a secondary infection caused by influenza [10].

Even the Arctic and remote Pacific islands were affected by 1918 flu pandemic. The mortality rate reached up to 0.1 % due to the death of 20 % of infected people [8, 10]. Most of the pandemic influenza deaths (99 %) occurred in the people under the age of 65 years [11], while normal influenza is more deadly to the very young (under 2 years of age) and the very old (over the age of 70 years) [8].

Several pandemic threats, such as the pseudo-pandemic of 1947, the 1976 swine flu outbreak and the 1977 Russian flu, were caused by the H1N1 subtype [12]. Further, the level of preparedness increased and resulted in the advent of the H5N1 avian flu outbreaks due to the high fatality rate of the H5N1 strain, which has limited human-to-human transmission [13].

In 1997, H5N1 emerged as a human threat in Hong Kong. This outbreak among poultry resulted in the death of six people and 18 people became infected. However, the prompt culling of poultry eradicated the disease from Hong Kong [14]. Further, in 2003, some other cases of flu were also presented in Hong Kong, followed by the severe outbreaks of the "Z-strain" of H5N1 in Thailand, Vietnam, Indonesia, Cambodia and China in 2004. The H5N1 virus spread along migratory pathways to Turkey and Russia, leading to a human outbreak which, later on, reached Europe and several African nations [15, 16].

The faeces of healthy-appearing water fowls contain avian influenza A virus and has the ability to infect chickens and other poultry according to the level of contact. Highly pathogenic strains can result in the devastating mortality rates of chickens and other birds. Asia is the ideal place for transmission and breaching species barriers, as poultry, ducks, pigs and humans live in crowded conditions there [17].

The later flu pandemics, such as 1957 Asian flu (type A, H2N2 strain) and the 1968 Hong Kong flu (type A, H3N2 strain), were not so devastating, yet, they killed millions of people. The secondary infections were controlled during later pandemics due to the availability of antibiotics, which helped in the reduction of the mortality rate as compared to that of Spanish flu of 1918 [10]. Table 1 [18–20] represents the major known flu pandemics throughout the years.

Different strains of influenza viruses keep on changing as the years pass by. These changes in the viral strains are due to the high mutation rate. Figure 1 represents the formation of different important influenza virus strains in different years [21].

Current status of influenza virus H1N1

Emergence of the H1N1 virus

Emergence of the H1N1 strain of influenza A virus was taken into serious consideration when the news of swine flu started appearing in the regional newspapers of America. Due the rapid global spread of the H1N1 strain, the World Health Organization (WHO) announced a global pandemic alert to phase 5 on April 29, 2009. Phase 5 indicates sustained human-to-human transmission of a novel influenza strain of animal origin in one WHO region of the world and exported cases detected in other regions [22]. Immediately after that, in response to the available information on sustained human-to-human transmission in multiple parts of the world, the WHO raised the level of influenza pandemic alert to phase 6 on June 11, 2009 [23]. Swine-origin 2009 A (H1N1) influenza virus was further recognised as the causative agent of this influenza-like illness [24].

The initial assumption was that the virus apparently circulated in the swine population without detection and

Table 1 Known flu pandemics [18–20]

Name of pandemic	Date	Deaths	Subtype involved	Pandemic severity index
Asiatic (Russian) flu	1889–1890	1 million	Possibly H2N2	Not applicable
1918 flu pandemic (Spanish flu)	1918–1920	20–100 million	H1N1	5
Asian flu	1957–1958	1–1.5 million	H2N2	2
Flu	1968–1969	0.75–1 million	H3N2	2
2009 flu pandemic	2009–present	10,000 up to December 6	H1N1	Not applicable

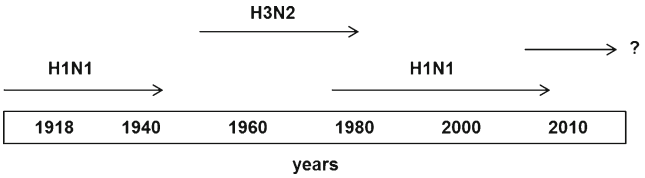

Fig. 1 Main types of influenza viruses in humans

crossed the species barrier to humans, which was later confirmed by Dr. Oliver Pybus of Oxford University and his team [25]. They further attempted to reconstruct the origins and time scale of the 2009 flu pandemic using computational methods. According to their research, this strain has been circulating among pigs, possibly on multiple continents, for many years prior to its transmission to humans. It was also believed that H1N1 was derived from several viruses circulating in swine, and that the initial transmission to humans occurred several months before the recognition of outbreak. The team concluded that, despite widespread influenza surveillance in humans, the lack of systematic swine surveillance allowed for the undetected persistence and evolution of this potentially pandemic strain for many years [26].

This strain of influenza virus contains a previously unseen combination of gene segments of Eurasian and North American swine influenza virus lineages [27]. However, there are uncertainties about the outbreak, including the transmissibility and origin of the virus. In this outbreak, the earliest affected country may have been Mexico, with many cases in other nations associated with travels to and from that country [22]. On the contrary to this assumption, U.S. federal agricultural officials believed that it emerged in pigs in Asia, but then travelled to North America in humans [28]. One of the unusual characteristics of the 2009 A (H1N1) influenza virus as compared with other recent zoonotic influenza viruses is sustained human-to-human transmission with a basic reproduction ratio (R_0) estimated to be in the range of 1.2–1.6, which is higher than that reported for seasonal human influenza A viruses [25].

Genetic reassortment is one of the major reasons for a pandemic outbreak which takes place between viruses from different hosts so that a new virus is produced, capable of infecting a third host [29–32]. Similarly in the case of swine flu, interspecies transmission of influenza A virus and human influenza virus took place. Here, pigs, which are considered to be the most logical candidate for the reassortment, were involved, as they can be infected by either avian or human viruses [33, 34]. The H1N1 virus is a combination of the swine, human and avian flu genes drawn from different strains that infect pigs [35]. The current H1N1 swine flu virus is a ‘quadruple reassortant’ virus, with six of its genes from flu viruses that were circulating in North American pigs and two genes of Eurasian origin [36, 37]. The process is described later in Fig. 3.

Structure of influenza virus

Influenza A virus, causing influenza in birds and some mammals, is of the Orthomyxoviridae family of viruses. Severe disease can be caused in both domestic poultry and rarely in humans by some strains of influenza A [38]. Furthermore, the transmission of viruses from wild aquatic birds to domestic poultry may lead towards an outbreak, followed by human influenza pandemic [39].

The structure of influenza viruses A, B and C are quite similar to each other [40]. The virus is spherical (very rarely filamentous) and is 80–120 nm in diameter [41]. The influenza virus is an enveloped virus having an outer lipid layer membrane, which is taken from the host cell. The viral envelope consists of glycoproteins (proteins linked to sugars), named haemagglutinin (HA) and neuraminidase (NA). The main factors to determine the type of influenza virus (i.e. A, B or C) and the subtype are the glycoproteins HA and NA [3]. RNA genome and other viral proteins are contained in the central core of the viral particle. The other viral proteins function as the protector of the genome RNA. Single-stranded RNA has been generally reported in influenza virus, while in some special cases, there is double-stranded RNA [41].

Generally, a viral genome contains seven or eight pieces of segmented negative-sense RNA, where each piece of RNA contains either one or two genes [42]. The influenza A genome contains 11 genes on eight pieces of RNA, encoding for 11 proteins: HA, NA, nucleoprotein (NP), matrix protein 1 (M1), M2, NS1, NS2 (NEP), PA, PB1, PB1-F2 and PB2 [43].

However, the virion of influenza B virus consists of an envelope, a matrix protein, a nucleoprotein complex, a nucleocapsid and a polymerase complex. In addition to this, it contains surface projections which are made of HA and NA [44]. In contrast to this, influenza virus C has seven RNA segments and encodes nine proteins [45].

The HA, mediating binding of the virus to target cells and entry of viral genome into the target cells, is a lectin. In addition, NA is responsible for the release of progeny virus from the infected cells, by cleaving sugars which bind the mature viral particles [46]. Thus, the glycoproteins (HA and NA) are the targets for antiviral drugs and are antigens to which antibodies can be raised [19, 47, 48]. The structure of influenza virus is described in Fig. 2.

Evolution of new strains of influenza virus

The genetic material of all the organisms can mutate, resulting to changes in the nucleic acids. Mutations are generally

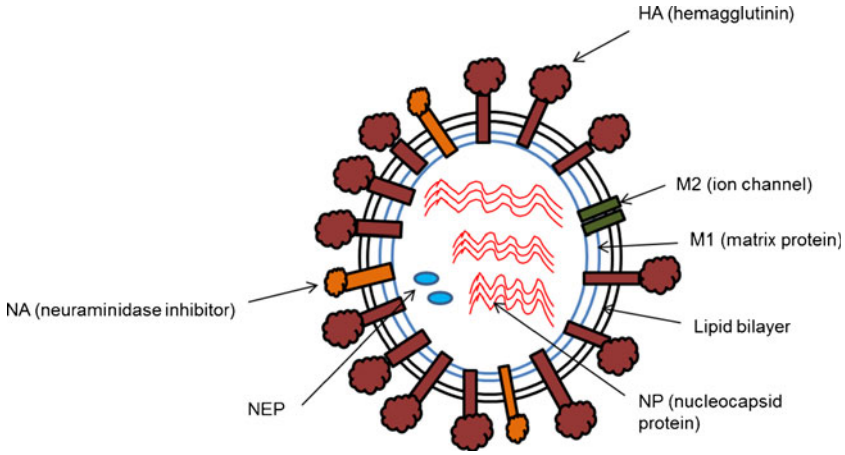

Fig. 2 Structure of influenza virus [3]

random, while, in contrast to this, their selection is not random. The survival and selection of viruses is termed as 'selection'. Selection ensures that, in the next generation, increase in the virus' ability to survive and reproduce through mutation will be represented in large numbers. Providing genetic variation for the action of selection, mutations are the base of evolution. RNA is the genetic material in all influenza viruses (orthomyxoviruses). The replication of RNA provides more errors than that of DNA replication. Selection acts on the extra mutations provided by these extra errors. Due to different replication processes, the mutation rate and the ability to evolve quickly is greater in the case of all RNA viruses and is greater than that of DNA viruses. The accumulation of all these mutations eventually evolves a new viral strain. Major antigenic shifts can occur in influenza virus by genetic reassortment [49–54].

Just like the emergence and spread of a new virus around the world, the morbidity and mortality of influenza virus initially increased and, later on, decreased because the causative agent underwent a progressive antigenic drift and then the human population also acquired some degree of immunity against the causative agent [5]. An accidental laboratory release caused the reappearance of a seasonal variant of the H1N1 virus in 1977, which has continued to circulate both in humans and in pigs, along with the H3N2 virus [55].

The antigenic variation of influenza viruses while circulating in the population is an important factor leading to the difficulties of controlling influenza by vaccination [56–58]. The analysis of the entire genome has been permitted by the development of sensitive biochemical techniques, along with providing tools for the determination of underlying molecular mechanisms which are responsible for these changes. For example, the fact that the H3N2 viruses arise from H2N2 strains by a recombination event is the result of the peptide mapping and hybridisation studies of that virus [49, 51, 59]. There is evidence indicating the surprising the rise of H2N2 strains by recombination events [59], but recombination cannot be held responsible for the emergence of the 1977 pandemic strain [59, 60].

There are two different types of antigenic variation in influenza viruses [61]:

1. Antigenic drift: causes a number of point mutations of the gene which codes for the viral surface glycoproteins, i.e. HA and NA, as well as the immune response of the host.
2. Antigenic shift: the sudden appearance of a completely new human influenza virus strain due to the replacement of the total HA and/or NA genes of a human strain for the corresponding genes of an animal influenza virus (Fig. 3)

Mutation and reassortment result in the constant evolution of new influenza viruses [63]. Small changes occurring in the HA and NA antigens on the surface of the virus is termed 'antigenic drift'. It is responsible for the creation of a variety of strains until the evolution of the one infecting people immune to the pre-existing strains. The older strains are being replaced by newer ones which rapidly sweep through the human population, causing an epidemic [64]. Due to the similarity of the new strains produced by antigenic drift to the older strains, people are still immune to them.

In contrast to this, the reassortment of influenza virus results in the formation of completely new antigens—for example, reassortment between human and avian strains (antigenic shift). Thus, the presence of new antigens increases the susceptibility rate towards the virus in humans and causes the uncontrollable spread of the disease, resulting in a pandemic [65].

Further, a different approach was proposed to follow the evolution of the virus where interactions of a fixed set of viral strains with a human population over a constantly changing set of immunities to different viral strains resulted in periodic pandemics [66]. Antigenic shift, or reassortment,

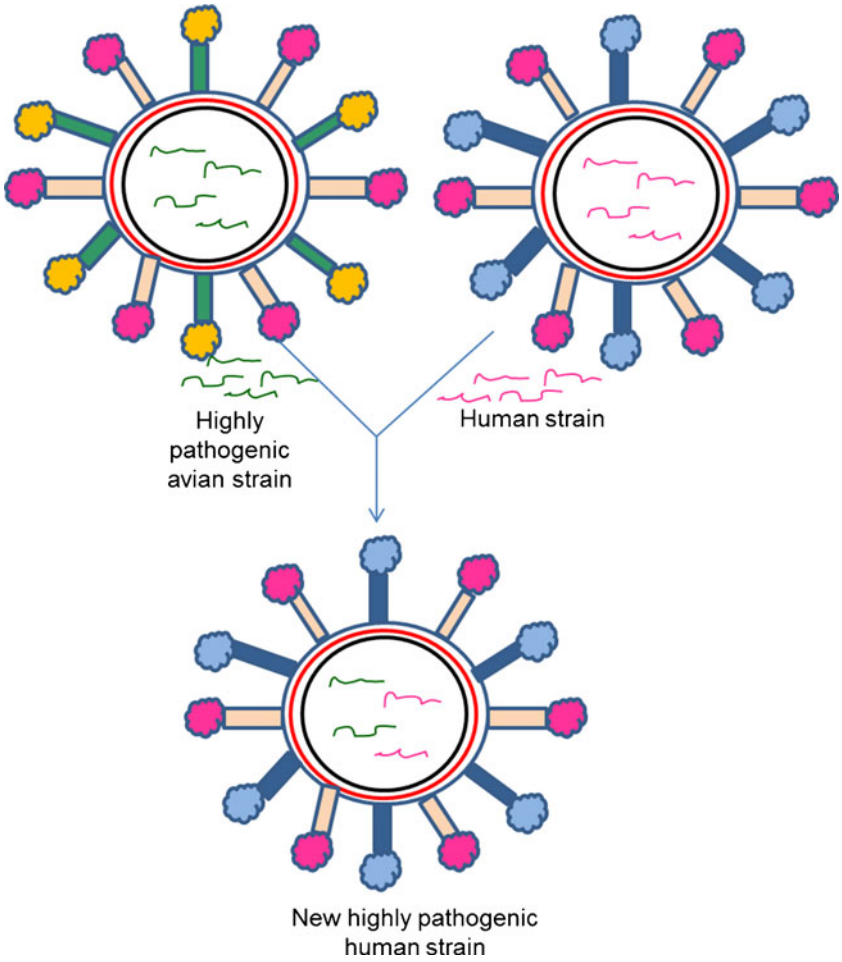

Fig. 3 Antigenic shift or reassortment [62]

can result in novel and highly pathogenic strains of human influenza.

It is very difficult to quantitate the inherent virulence of an influenza virus. Interest has been shown towards correlates of virulence at a molecular level by the development of live attenuated influenza vaccines [67]. Virulence is the increased host mortality resulting from viral infections [68].

Factors other than the virulence of the virus (communicability) like relatedness to previously circulating strains and environmental factors play an important role in the process of determining the impact of an influenza epidemic [69].

The most virulent virus with the ability to reproduce and spread to new cells are always selected during the process of natural selection. The mutant virus utilises the resources very quickly and makes a lot copies through replication while the total resources of the body are limited. The virulence is increased due to the selection between genetic variants or mutants within the same organism. In contrast to this, selection between the hosts decreases virulence. Further, the selective disadvantage associated with physical incapacitation is reduced by transport. There is a selective disadvantage to highly virulent deadly strains due to the rapid long-distance transportation between large and dense populations [70].

Symptoms of swine flu

The early signs and symptoms in human patients suffering from swine flu are non-specific and indistinguishable from seasonal and A/H3N2 influenza virus, making early diagnosis and treatment significantly delayed, causing hysteria in the masses. The most frequent symptoms of swine flu are fever (94 %), cough (92 %), headache (80 %), chills (60 %), rhinorrhoea, body aches and sore throat, which are also common in swine flu [23]. However, a variety of other clinical symptoms unusual for seasonal influenza have also been reported, including vomiting (25 %) and diarrhoea (25 %), in a relatively large proportion of cases [71, 72]. While seasonal flu is not normally associated with gastrointestinal symptoms like diarrhoea and vomiting, with fever rarely above 101 °F [73]. In contrast to this, symptoms of swine flu come suddenly with much greater intensity. This causes weakness and fatigue for up to 2 or 3 weeks, including muscle aches and period of chills and sweats (as fever comes and goes). Abortion and pre-term birth have also

been reported among pregnant women, especially those with pneumonia [74]. Moreover, some patients have required hospitalisation because of severe pneumonia and respiratory failure. The starting of seasonal flu leads to the sudden increase in the number of school-aged children getting sick with flu-like illness, followed by similar infection in other age groups, especially adults. In case of emergency among adults, symptoms needing urgent medical attention include breathing difficulty, pain or pressure in the chest or abdomen, sudden dizziness, confusion, severe or persistent vomiting and dehydration [75]. Infection is highest in young people between 12 and 17 years of age, but the risk of hospitalisation and death is higher in pregnant women, people with diabetes, asthma and heart disease in elderly patients. In contrast to seasonal influenza, a substantial proportion of the cases of severe illness and death have occurred among young and previously healthy adults [76]. Differences between seasonal and swine flu symptoms are shown in Table 2

Thus, it is clear from the Table 2 that swine flu is always detected at a very late stage as people generally misunderstand it to be the seasonal flu. Therefore, people infected with swine flu when travelling around the world unknowingly transfer it to other people.

As the preliminary clinical presentation of swine flu is similar to seasonal flu, doctors treating this disease encounter many problems in the diagnosis, unless a high level of suspicion is adopted. Due to the delay in diagnosis, mass hysteria can be created, which can lead to immense workloads at laboratories and in hospitals, indirectly affecting the economy of any nation [77]. In addition, the panic and overreaction created by media-manufactured mass hysteria further led to the level where one has to seriously think about whether the symptoms are of swine flu or of seasonal flu (common cold). Therefore, it has become necessity to create awareness among doctors and communities about the differences between swine flu and seasonal flu symptoms for an early diagnosis and effective treatment [73].

Hence, a specific detection kit for swine flu should be developed so that anyone showing early symptoms of flu can get themselves tested and, if found positive, they can be quarantined to avoid transmission.

Transmission of swine flu

Epidemics of any infectious diseases among humans and other animals are the result of the transmission of a pathogen either directly between hosts or indirectly through the environment or intermediate hosts. Similarly, the infectiousness of swine flu in the infected host (or hosts) and the susceptibility of uninfected individuals (who are exposed to infection) decides the efficiency of transmission. Infectiousness is basically of three different types: biological, behavioural and environmental [78].

There are three main ways in which influenza can spread [79, 80]: (a) direct transmission, when an infected person sneezes, mucus directly enters into the eyes, nose or mouth of another person; (b) the airborne route: the aerosols (0.5–5 μm in diameter) produced by coughing, sneezing or spitting by an infected person are small enough to cause an infection. A single sneeze releases up to 40,000 droplets [81], most of these droplets are quite large and will quickly settle out of the air [80]. The survival of influenza virus in airborne droplets is influenced by the levels of humidity in the atmosphere and UV radiation. Low humidity and lack of sunlight in winters are the factors aiding the survival of this virus [80]; (c) hand-to-eye, hand-to-nose or hand-to-mouth transmission: influenza virus can persist outside the body and it can also be transmitted by contaminated surfaces, such as bank notes [82], door knobs, light switches and other household items. The persistence time of the virus on a surface varies, with the virus surviving for 1 to 2 days on hard, non-porous surfaces such as plastic or metal, for about 15 min on dry paper tissues and only 5 min on skin [83]. However, in mucus, the virus can be protected for longer periods (up to 17 days on banknotes). Moreover, avian influenza viruses can survive indefinitely in frozen conditions. A

Table 2 Difference in symptoms of seasonal and swine flu

Symptoms	Seasonal flu	Swine flu
Aches	Slight body ache and pain	Severe body ache and pain
Chest discomfort	Mild to moderate chest discomfort	Severe chest discomfort
Chills	Chills are uncommon	60 % of people experience chills
Coughing	A hacking, mucous-producing cough	A non-mucous-producing cough
Fever	Fever is rare	Fever is usually present, with a temperature of 100 °F or higher for 3–4 days
Headache	Uncommon	Very common
Stuffy nose	Present	Commonly not present
Sudden symptoms	Symptoms tend to develop over a few days	Rapid onset within 3–6 h
Tiredness	Very mild	Moderate to severe

heating of 56 °C (133 °F) for a minimum of 60 min and the use of acids causes their inactivation at pH <2 [80].

The relative importance of these three modes of transmission is unclear, but they may all contribute to the spread of the virus [84, 85]. The infectiveness is greatest in the people transferring influenza between the second and third days after infection, and infectivity lasts for around 10 days [86]. Children shed virus from just before they develop symptoms until 2 weeks after infection and are much more infectious than adults [87]. The transmission of influenza can be modelled mathematically, which helps predict how the virus will spread in a population [78].

Prevention of swine flu

Infection control

There are different ways of reducing the transmission of influenza virus, including good health and hygiene habits. These habits include frequent hand washing with soap and water or with alcohol-based hand rubs and the habit of not touching the eyes, nose or mouth [88]. Furthermore, covering coughs and sneezes along with avoiding close contact with sick people and spitting [89] and the use of face masks play important roles in preventing the transmission of this disease [89–91]. The risk of transmitting influenza, as well as producing more severe disease symptoms, increases with smoking. According to different laws of mathematical modelling of infectious diseases, the exponential growth rate of influenza epidemics can be increased among smokers. Thus, smokers are indirectly responsible for a large number of influenza cases [92–95].

Surface sanitising also acts as a prevention technique against some infections, as influenza can also be spread through aerosols and contact with contaminated surfaces. Alcohol acts as an effective sanitiser against the influenza virus. The sanitising effect can last for a longer time period when quaternary ammonium compounds are used with alcohol [96]. Quaternary ammonium compounds are used to sanitise the hospital rooms or equipments used by patients suffering with influenza. Diluted chlorine bleach can also be used to sanitise households [97].

The rate of spread of the virus was slowed by the closure of schools, churches and theatres, but the overall death rate was not affected by this [98, 99]. The movement of influenza-infected people from one place to another is the main cause of no effect in the transmission of the disease by reducing people gatherings [89].

Vaccination

In 1944, Thomas Francis Jr. developed a heat-killed virus for influenza. Furthermore, the Australian virologist Sir Frank Macfarlane Burnet showed in his experiments that the virus loses virulence on being cultured in fertilised hen's eggs [8, 100]. The development of anti-influenza drugs is slower than the development of vaccines, as it takes a less amount of time [48].

Drifting of the predominant circulating strains of influenza virus has resulted in the requirement of influenza vaccination each year. It is necessary to review the composition of influenza vaccines annually and the vaccine constituents should be changed in order to maintain protection against the drifted influenza virus strains. The success of vaccine strain selection by monitoring seasonal influenza vaccine effectiveness is checked as part of a publicly funded programme by most countries around the world. Vaccine effectiveness is an estimate from an observational study, while vaccine efficacy is an estimate derived from a trial. The percentage reduction of cases among vaccinated individuals is defined as vaccine efficacy. It is done efficiently by using routinely collected data which is available from sentinel surveillance networks [101, 102].

In the case of influenza, the high-risk groups such as children and the elderly or people having asthma, heart disease, diabetes and who are immune-compromised are recommended for vaccination against the disease with an influenza vaccine. Of the different methods for producing influenza vaccines, the most common is to grow the virus in fertilised hen's eggs. The virus usually gets inactivated after purification and helps to produce an inactivated virus vaccine. On the other hand, the virus can be grown in eggs till the time it loses its virulence and a live vaccine is produced from that avirulent strain. Due to the high mutation rate of the virus, a particular influenza vaccine usually confers protection for no more than a few years and results in the variability of the effectiveness of these influenza vaccines [19].

Prediction of the new strains of virus which are likely to be circulated in the following year is done by the WHO each year. It also allows the pharmaceutical companies to develop the best immunity-providing vaccines against these strains [103]. There are vaccines to protect poultry from avian influenza. These vaccines show effectiveness against multiple strains of the virus and can be used as a prevention technique [104].

Formulation of vaccines is done every year, but it is not possible to cover all the strains which actively infect people around the world. Due to this, individuals can get infected even after vaccination. Approximately 6 months are required by manufacturers to formulate and produce the millions of doses of the vaccine required to deal with the seasonal epidemics (such as the H3N2 Fujian flu in the 2003–2004 flu season) [105]. As the vaccine takes about 2 weeks to become effective against the disease, it is possible that an individual can be infected just before vaccination and become sick [106].

According to the Centers for Disease Control and Prevention (CDC) recommendations in the year 2006–2007, children younger than 59 months of age receive the influenza vaccine [107]. Due to these vaccines, the immune system reacts in the same way as if the body is actually infected and can cause general infection symptoms which are not as long lasting as influenza. A severe allergic reaction is seen as a dangerous side effect of this vaccine but is very rare [106].

Research is currently being undertaken to develop vaccines against a possible influenza pandemic, along with finding vaccinations against seasonal influenza. If a vaccine is produced which is effective against the influenza pandemic, then it can be helpful in saving millions of lives. There is little time between the identification of a pandemic strain and the need for vaccination. Thus, non-egg-based options for vaccine production are the area of research nowadays. The egg-based or cell-based technology and the recombination technologies are able to provide better 'real-time' access, along with being produced more affordably, giving the best reason for the increased access for people in low- and moderate-income countries [23]. The U.S. Food and Drug Administration (FDA) approved four vaccines against H1N1 influenza virus in September 2009 [108].

Treatment of swine flu

Plenty of rest, intake of large amount of liquids, along with the avoidance of alcohol and tobacco is recommended to people suffering from swine flu. An intake of paracetamol is also advised to relieve fever and muscle aches associated with the flu. Aspirin should not be taken by small children and teenagers during an influenza infection because it can lead towards Reye's syndrome, a potentially fatal disease of the liver [109].

Antibiotics have no effect on swine flu infection, as it is caused by a virus. Antibiotics can only work in the case of secondary infections, such as bacterial pneumonia. Antiviral drugs are effective against the disease until the strains of influenza are not resistant towards them [110].

Antiviral drugs are taken in case of becoming sick with swine flu. Use of these drugs make the illness milder, making the patients feel better in a faster way [111].

Antiviral drugs for swine flu

Prevention from swine flu viruses or treatment of the disease can be done using antiviral drugs. Only a healthcare professional can prescribe these medications [111]. Two types of inhibitors are used as drugs for the treatment of swine flu, i.e. neuraminidase inhibitors and M2 inhibitors.

Neuraminidase inhibitors

Oseltamivir (Tamiflu) and Zanamivir (Relenza) are the neuraminidase inhibitors used in the treatment of swine flu. These antiviral drugs can halt the spread of the virus in the body [112]. These antivirals are effective against both influenza A and B [113]. There are different degrees of resistance of different strains of influenza viruses against these antiviral drugs. It is very difficult to predict the resistance of the future pandemic strain [114].

Oseltamivir

The spread of non-resistant strains of the influenza virus between cells in the body is slowed down by the antiviral drug oseltamivir (Tamiflu). It is a neuraminidase inhibitor and is used in the treatment and prophylaxis of influenza A and influenza B infection. It can also act as a transition-state analogue inhibitor of influenza neuraminidase which can prevent the progeny virions from detaching from the infected cells. It is known as the first commercially developed, orally active neuraminidase inhibitor [111]. The chemical structure of oseltamivir is given in Fig. 4.

Dosage Oseltamivir is recommended for persons aged 1 year and above. Usually, 75 mg twice daily for 5 days is the recommended dosage for adults. Treatment should be started within 2 days of the appearance of the symptoms. Further, in case of children, the dose ranges from 30 to 75 mg twice daily, depending upon the body weight [115].

Mechanism of action Oseltamivir becomes hepatically hydrolysed to the active metabolite (free carboxylate of oseltamivir). It is a competitive inhibitor of sialic acid, which is found on the surface proteins of the host cells. It prevents new viral particles from being released from the infected cells by blocking the activity of viral neuraminidase [116].

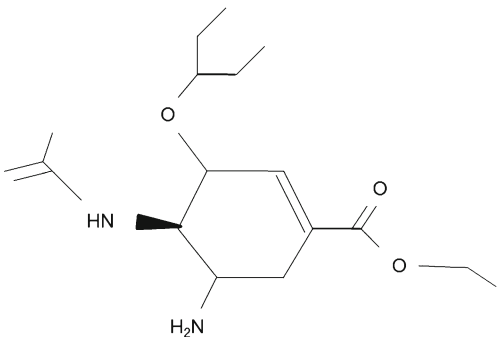

Fig. 4 Oseltamivir {ethyl (3R,4R,5 S)-5-amino-4-acetamido-3-(pentan-3-yloxy)cyclohex-1-ene-1-carboxylate}

Side effects Common adverse drug reactions include nausea, vomiting, diarrhoea, abdominal pain and headache. In rare cases, hepatitis and rashes are also reported [117].

Zanamivir

Zanamivir is a neuraminidase inhibitor and is used in the treatment and prophylaxis of influenza virus A and B. It is currently marketed under the trade name of Relenza by GlaxoSmithKline. Patients with breathing difficulties are not recommended for treatment with zanamivir [118]. The structure of zanamivir is given in Fig. 5.

Dosage The normal dosage of Relenza is 10 mg once every 12 h for 5 days. In general, two dosages are recommended [119].

Mechanism of action It binds to the active site of neuraminidase protein and the influenza virus is unable to escape from the host cells and infecting other individuals [118].

Side effects Relenza usage may cause the constriction of airways, leading to the shortness of breath, severe allergic reactions, seizures, hallucinations and delirium [119].

M2 inhibitors

The viral ion channel (M2 protein) is blocked by the antiviral drugs named amantadine and rimantadine. This blockage helps in preventing the virus from infecting cells [120]. These drugs are effective against influenza A viruses but are ineffective against influenza B virus because of the absence of M2 proteins [113]. A high level of resistance has been seen in the virus strains against these drugs (adamantine and rimantadine), which is due to the easy availability of amantadine all over the world and their use to prevent outbreaks of influenza in farmed poultry [121–123].

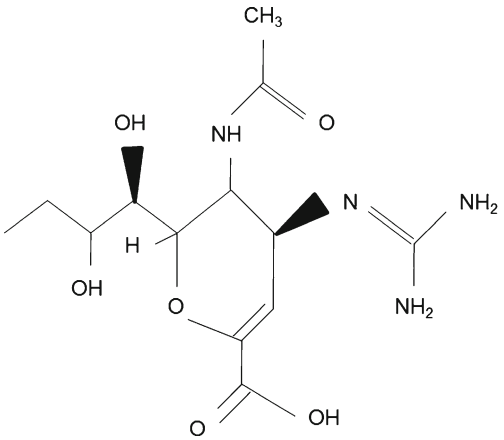

Fig. 5 Zanamivir {(2*R*,3*R*,4*S*)-4-[(diaminomethylidene)amino]-3-acetamido-2-[(1*R*,2*R*)-1,2,3-rihydroxypropyl]-3,4-dihydro-2H-pyran-6-carboxylic acid}

Amantadine

Amantadine is an organic compound in which an amino group is substituted at one of the four methylene positions of the adamantane backbone. It is generally known as 1-aminoadamantane and is marketed under the trade name of Symmetrel. Along with being used as an antiviral drug, it can also be used as an antiparkinson drug [124].

Dosage It should be given 100 mg once daily to influenza B virus-infected people [125]. Figure 6 depicts the chemical structure of amantadine.

Mechanism of action Viral protein M2 is required for the uncoating of the viral particle inside a cell by endocytosis. Amantadine inhibits the viruses by interfering in the uncoating process inside the cell. Further, the drug blocks the ion channel formed by the M2 protein [126].

Side effects Nervousness, anxiety, agitation, insomnia and difficulty in concentrating are the general side effects. Livedo reticularis is the other potential side effect, which is a dermatological reaction that results in skin mottling and purpurish mesh network of blood vessels [117].

Rimantadine

Rimantadine is an orally administered antiviral drug to prevent influenza virus A infection. It is able to shorten the duration of the disease and moderate the severity of influenza, if taken within 1–2 days of developing symptoms. It is commercially known as Flumadine in the market [127]. Figure 7 describes the chemical structure of rimantadine.

Dosage The recommended rimantadine dose for treating flu in adults and adolescents of 16 years of age and more is

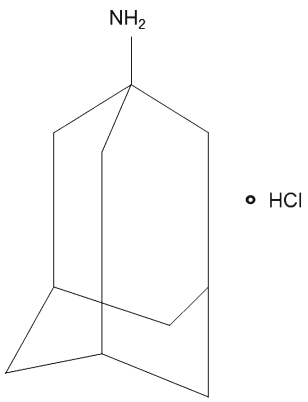

Fig. 6 Amantadine {adamantan-1-amine}

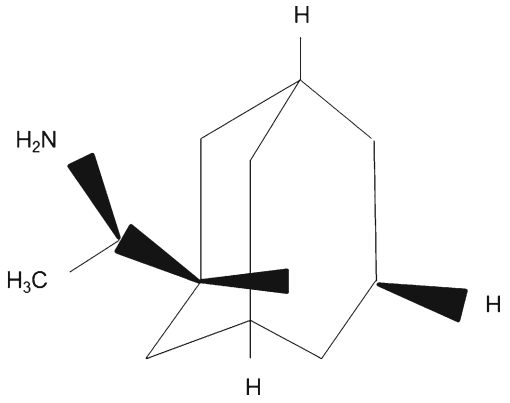

Fig. 7 Rimantadine {(RS)-1-(1-adamantyl)ethanamine}

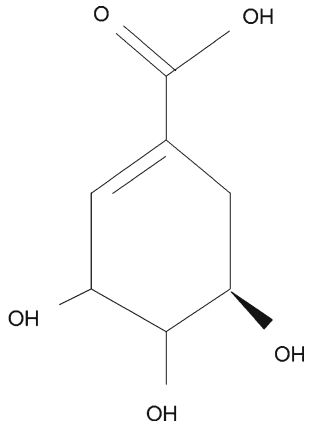

Fig. 8 Structure of Shikimic acid

100 mg twice daily for 7 days after the appearance of the first symptom. It is not recommended for children below the age of 16 years. Furthermore, people with kidney failure or liver disease and the elderly are recommended to have a lower dose of rimantadine, i.e. 100 mg once daily [4].

Mechanism of action Rimantadine inhibits the M2 ion channel and, later on, inhibits the replication of influenza virus [125].

Side effects Nausea, upset stomach, nervousness, tiredness, lightheadedness, trouble sleeping and difficulty in concentrating are the common side effects known in this case [111].

Finding an ultimate cure of the disease

Thus, it is clear from the above discussion that it is very important to find an ultimate cure of this disease. Researchers suggest that shikimic acid is the basic compound for the production of the swine flu drug Tamiflu and is found in the seeds of an evergreen Chinese plant Star anise (*Illicium verum*) [128]. Nowadays, shikimic acid plays an important role against swine flu by reducing the severity of the symptoms. It is obtained from the seeds of Star anise and, further, is converted to epoxide. In the process of the formation of Tamiflu, the most dangerous part involved is the azide chemistry, as it is very explosive [129].

Shikimic acid

Shikimic acid occurs in a variety of compounds naturally. It is an intermediate compound in the formation of several aromatic compounds [130]. It is a white crystalline compound having two types of functional groups in the same molecule, three hydroxyl groups and a carboxylic group (Fig. 8). It is widely used as a chiral building block for the synthesis of pharmaceuticals [131].

Importance and uses of shikimic acid

Shikimic acid is widely used as a chiral building block for the synthesis of pharmaceutical drugs such as oseltamivir (a neuraminidase inhibitor), which is used in the treatment and prophylaxis of both influenza A and influenza B viruses [132, 133]. Shikimic acid serves as the starting material for the production of Tamiflu [134, 135]. Thus, it acts as an important compound against swine flu.

Limitations of shikimic acid production

The bulk production of Tamiflu is a difficult task, as it not only takes months to complete but is hazardous too. Careful handling and relatively mild reaction conditions are required for the synthesis of the drug, as potentially explosive azide chemistry is involved. The production of large amounts of the drug requires a sufficient amount of starting material (shikimic acid). The major harvesting period for Star anise is from March to May, which is not enough to supply the large amount of starting material to meet the drug requirements worldwide. Besides Star anise, *Ginkgo biloba* can also be a source of shikimic acid [136]. Since both raw materials are in short supplies, the pharmaceutical industry needs to find an alternative sustainable supply.

What can be the alternative approach?

Recently, researchers have reported that shikimic acid can also be obtained from a few microbes, as it exists as an intermediate in the pathway synthesising amino acids (Fig. 9).

During the production of shikimic acid, avoiding the formation of shikimate pathway byproduct is the main issue, as it reduces the yield and quality [135, 137–139]. Carbon-limited growth conditions increase byproduct formation, while carbon-rich conditions favour shikimic acid production compared to that of byproducts [139–141].

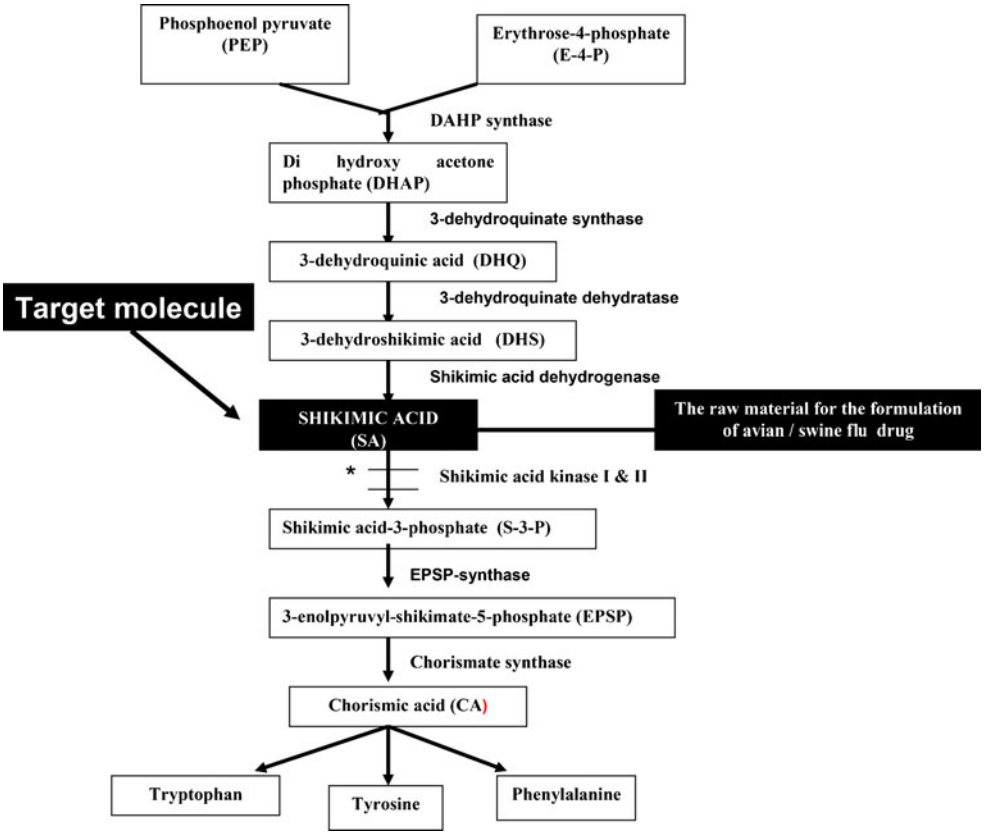

Fig. 9 Shikimic acid pathway

The use of a recombinant strain of *Escherichia coli* under fermenter-controlled conditions has resulted in the synthesis of shikimic acid from glucose. In the present experiment, along with shikimic acid, quinic acid and dehydroshikimic acids were also synthesised as byproducts [137].

Iomantas et al. [142] carried out an alternative approach for shikimic acid production by EPSP synthase-deficient *E. coli* strains by blocking the aromatic amino acid pathway after the production of shikimate-3-phosphate (S3P). Bacterial phosphatases converted shikimate-3-phosphate to shikimic acid. According to Draths et al. [137], the microbial production of shikimic acid by metabolic engineering is most advanced. Through metabolic engineering, the aromatic amino acid pathway is blocked soon after the formation of shikimic acid. The whole process of blocking is done by the transduction of disrupted *aro K* and *aro L* genes encoding shikimate kinase I and II.

At present, Roche, the Swiss pharmaceutical company, produces shikimic acid by fermenting *E. coli*. As per the present requirements, it claims that 0.13 g of shikimic acid is needed per Tamiflu capsule. So, 10,000 t should provide enough feedstock for more than 20 million courses of treatment, a drastic increase from 55 million in the year 2005 and it is planned to charge 12 Euros in less developed countries for a 5-day treatment as compared to 15 Euros in developed countries.

Scientists in India have not yet attempted to research the production of shikimic acid and its applications. A survey of the literature shows that there are no research publications or patents directly addressing the production and application of shikimic acid in India. In this context, only Cipla, the Indian generics company, claims to produce a version of Tamiflu within months, since they have experience of making the HIV drug AZT, which relies on a similar chemistry. Currently, the market price of shikimic acid is $1,000 per kg, increased from the usual price of $40 per kg due to a huge demand in the world market for Tamiflu.

Conclusion

It can be concluded that shikimic acid has immense importance in drugs formulation for swine flu, which is a serious threat to the human population. However, the availability of shikimic acid is of great concern. Currently, the worldwide demand of shikimic acid is fulfilled by plant sources. In the conventional method, shikimic acid can be extracted from the Chinese plant only at a particular time each year (March to May). Such a limited and time-bound supply of shikimic acid is overshadowing the ability of drug scientists to fulfil the demands on shikimic acid, which can be elevated without any prior warning. Therefore, researchers are currently

undertaking serious searches for alternative and reliable sources of this important molecule.

In this context, we would particularly like to mention a technique based on our studies on the production of shikimic acid from microbial sources. Shikimic acid is being produced by a wild strain of microorganism which can be more stable than other modified strains. Around a 30-fold increase in the levels of shikimic acid is achieved through process optimisation (containing a one-variable-at-a-time approach) and a statistically designed approach (i.e. Plackett–Burman and response surface methodology). Vacuum distillation was used for the concentration of the broth. The concentrated broth was subsequently purified through ion-exchange chromatography. Thus, it clear that the microbial production of shikimic acid is a better approach to prepare the base material of the drug Tamiflu in a shorter time period without any seasonal limitation (data not shown).

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