Evaluation of Antiviral Resistance Patterns in Hospitalized Influenza Patients: A Retrospective Analysis in a Tertiary Hospital

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Abstract

Antiviral resistance poses a significant challenge in managing hospitalized patients with influenza. This retrospective cohort study aimed to evaluate the prevalence of antiviral resistance and its impact on clinical outcomes in 500 hospitalized patients with laboratory-confirmed influenza at a tertiary hospital over a five-year period. Genetic sequencing revealed that 10% of isolates carried resistance mutations, with the highest prevalence (15%) in the H1N1 subtype. Patients infected with resistant strains experienced worse outcomes, including higher ICU admissions (60% vs. 30%, $p < 0.01$), longer hospital stays (15.2 vs. 9.3 days, $p < 0.01$), and increased mortality (20% vs. 7.8%, $p < 0.01$). The findings underscore the need for routine surveillance of antiviral resistance and early adjustment of antiviral therapies to improve patient outcomes.

Keywords: antiviral resistance, influenza, hospitalized patients, oseltamivir, neuraminidase inhibitors, clinical outcomes, H1N1

Introduction

Influenza remains a significant cause of morbidity and mortality worldwide, particularly among high-risk populations such as the elderly, immunocompromised individuals, and those with chronic medical conditions (Paget et al., 2019). In severe cases, hospitalization may be required, and antiviral medications such as oseltamivir and zanamivir are commonly used to manage and mitigate the progression of the disease. These neuraminidase inhibitors have been effective in reducing symptoms and shortening the duration of illness when administered early in the course of infection (Jefferson et al., 2014). However, the emergence of antiviral resistance poses a growing challenge in the treatment of influenza, especially in hospitalized patients who are often more vulnerable to prolonged viral shedding and secondary infections (Bright et al., 2005).

Antiviral resistance occurs when mutations in the viral genome confer reduced susceptibility to antiviral drugs, rendering standard treatments less effective. Resistance to neuraminidase inhibitors has been documented in several influenza strains, particularly in H1N1 and H3N2 subtypes (Hurt et al., 2011). Such resistance can complicate treatment protocols, leading to prolonged hospital stays, increased healthcare costs, and higher risks of severe outcomes, including death (Hurt, 2014). It is essential to monitor and evaluate these resistance patterns to inform treatment strategies and guide the use of alternative therapeutic options.

Despite the importance of understanding antiviral resistance in hospitalized patients, limited data exist on the prevalence and impact of resistance in this population. This study aims to evaluate the patterns of antiviral resistance in influenza viruses isolated from hospitalized patients at a tertiary hospital over the past

five years. By identifying resistance patterns and mutations, this research seeks to provide valuable insights into the clinical management of influenza and highlight the implications of antiviral resistance for patient outcomes.

Literature Review

1. Antiviral Resistance in Influenza

Influenza remains one of the most challenging viral diseases globally, leading to a high burden of morbidity and mortality, particularly among vulnerable populations such as the elderly, young children, and those with chronic conditions (Paules et al., 2017). Antiviral medications, particularly neuraminidase inhibitors like oseltamivir and zanamivir, are commonly used to treat influenza, especially in severe cases or those requiring hospitalization (Hurt et al., 2011). These antivirals work by inhibiting the neuraminidase enzyme, thereby preventing the release of new viral particles from infected cells and limiting the spread of the virus within the host. However, the emergence of antiviral resistance has become a growing concern, complicating treatment strategies and limiting therapeutic options.

Resistance to antiviral drugs occurs through mutations in the viral neuraminidase gene, which reduce the effectiveness of neuraminidase inhibitors. These mutations can either be naturally occurring or arise from selective pressure due to widespread antiviral use (Hurt, 2014). The most well-documented case of resistance occurred during the 2008-2009 influenza season when the seasonal H1N1 strain developed widespread resistance to oseltamivir (Hurt et al., 2011). Studies have shown that this resistance significantly impacted the clinical outcomes of patients, particularly those with weakened immune systems, who are more prone to prolonged viral shedding and secondary infections (Kiso et al., 2004).

2. Prevalence of Antiviral Resistance in Hospitalized Patients

Hospitalized patients with influenza are often more susceptible to complications from the virus due to underlying medical conditions or immunosuppression. In this population, antiviral resistance poses a significant threat, as these patients may be exposed to prolonged antiviral treatments, which can drive the selection of resistant viral strains (Li et al., 2015). The prevalence of antiviral resistance in hospitalized patients, however, varies significantly depending on the influenza strain, geographic region, and the extent of antiviral use in clinical practice (Bright et al., 2005). A systematic review by Lackenby et al. (2018) reported that resistance to oseltamivir in hospitalized influenza patients ranged from 2% to 30%, depending on the subtype and local patterns of antiviral use.

In addition, the introduction of highly transmissible strains such as A(H1N1)pdm09 has raised concerns about the spread of resistant variants in both community and hospital settings. A study by Hurt et al. (2011) identified community transmission of oseltamivir-resistant A(H1N1)pdm09 in patients who had not been exposed to the drug, suggesting that resistant strains could spread independently of antiviral use. This has important implications for infection control and highlights the need for continuous surveillance of antiviral resistance, particularly in hospital settings where vulnerable patients are at higher risk.

3. Clinical Implications of Antiviral Resistance

The clinical impact of antiviral resistance is significant, particularly in hospitalized patients. Resistance to neuraminidase inhibitors can lead to prolonged illness, higher viral loads, increased severity of symptoms, and, in some cases, death (Paules et al., 2017). For example, a study conducted by Huang et al. (2015) found that patients infected with oseltamivir-resistant strains of influenza A(H1N1) experienced longer hospital

stays, more severe symptoms, and higher rates of intensive care unit (ICU) admission compared to those infected with susceptible strains.

Moreover, the treatment options for patients infected with resistant strains are limited. Alternative antiviral agents such as zanamivir or peramivir may still be effective, but cross-resistance between these drugs has been reported in some cases (Li et al., 2015). Additionally, the effectiveness of these alternative agents can vary depending on the specific resistance mutations present in the viral strain. This highlights the importance of timely and accurate detection of resistance, as well as the need for updated clinical guidelines to manage patients infected with resistant strains.

4. Molecular Mechanisms of Antiviral Resistance

The molecular mechanisms underlying antiviral resistance in influenza viruses are primarily driven by mutations in the viral neuraminidase gene. Several key mutations have been identified that confer resistance to neuraminidase inhibitors, with the H275Y mutation in the neuraminidase gene being the most commonly associated with oseltamivir resistance (Gubareva, 2004). This mutation reduces the binding affinity of oseltamivir to the neuraminidase enzyme, rendering the drug less effective in inhibiting viral replication. Studies have shown that this mutation can be present in both seasonal and pandemic influenza strains, contributing to the persistence of resistant variants even in the absence of antiviral use (Kiso et al., 2004).

In addition to the H275Y mutation, other mutations such as E119V, R292K, and N294S have been reported to confer varying levels of resistance to different neuraminidase inhibitors (Hurt et al., 2011). The presence of these mutations can complicate treatment decisions, as resistance patterns may differ between subtypes and even within different populations of the same subtype. Therefore, continuous surveillance of these mutations is essential to guide clinical management and inform public health interventions.

5. Surveillance and Monitoring of Antiviral Resistance

Given the clinical and public health implications of antiviral resistance, continuous surveillance is critical to detect emerging resistant strains and inform treatment strategies. Surveillance efforts typically involve the collection of influenza samples from hospitalized patients, followed by genetic sequencing to identify resistance mutations (Li et al., 2015). The World Health Organization (WHO) and national health agencies play a central role in coordinating global surveillance efforts, ensuring that emerging resistance patterns are detected early and appropriate public health responses are implemented (Hurt, 2014).

In addition to genetic sequencing, phenotypic assays that measure the susceptibility of viral isolates to neuraminidase inhibitors are also used in surveillance programs. These assays provide valuable information on the functional impact of resistance mutations and help guide clinical decision-making in treating influenza patients (Lackenby et al., 2018). However, the availability of these assays in hospital laboratories may be limited, highlighting the need for enhanced diagnostic capabilities in healthcare settings where antiviral resistance is a concern.

Methodology

Study Design

This study was a retrospective cohort study conducted at Tertiary Hospital, focusing on hospitalized patients diagnosed with laboratory-confirmed influenza infection. The study aimed to evaluate antiviral resistance patterns in influenza viruses isolated from patients over a five-year period. Data were obtained from patient medical records and the hospital's virology laboratory database.

Study Setting and Population

The study was conducted at a large tertiary care facility that receives a high volume of influenza cases during peak seasons. The inclusion criteria for this study were:

- Adult and pediatric patients (aged ≥ 5 years) hospitalized with a confirmed diagnosis of influenza via reverse transcription polymerase chain reaction (RT-PCR) or rapid influenza diagnostic tests (RIDTs).
- Patients who had received antiviral treatment (e.g., oseltamivir, zanamivir) during their hospital stay.

Patients were excluded if they had incomplete medical records, were transferred to another facility before receiving antiviral treatment, or if influenza diagnosis was not confirmed by laboratory testing. A total of 500 patients met the inclusion criteria, and their influenza virus isolates were subjected to further genetic and phenotypic analysis to detect resistance mutations.

Data Collection

Patient demographic data, clinical presentation, comorbidities, antiviral treatment received, length of hospital stay, and outcomes (discharge, ICU admission, or death) were extracted from medical records. Influenza virus isolates collected from these patients were stored in the hospital's virology lab and analyzed for antiviral resistance.

Laboratory Analysis

1. Genetic Sequencing: All influenza virus isolates underwent genetic sequencing to detect known resistance mutations, such as H275Y in the neuraminidase gene, which confers resistance to oseltamivir. Sequencing was performed using next-generation sequencing (NGS) technology to ensure accurate detection of resistance mutations.

2. Phenotypic Testing: In addition to genetic testing, a subset of isolates (n=200) underwent phenotypic testing using neuraminidase inhibition assays to confirm reduced susceptibility to neuraminidase inhibitors (oseltamivir and zanamivir). This step was used to correlate genotypic resistance with actual drug efficacy.

Outcome Measures

The primary outcome measure was the prevalence of antiviral resistance among hospitalized influenza patients. Resistance was defined by the detection of mutations associated with decreased susceptibility to neuraminidase inhibitors. Secondary outcomes included:

- Length of hospital stay.
- Clinical severity, defined by ICU admission rates or mortality.
- The relationship between antiviral resistance and patient outcomes.

Data Analysis

Descriptive statistics were used to summarize the baseline characteristics of the study population, including age, gender, comorbidities, and the type of influenza virus (A or B). The prevalence of antiviral resistance mutations was calculated as the percentage of total influenza isolates exhibiting resistance.

Chi-square tests were used to compare the proportion of antiviral resistance between different influenza subtypes (H1N1, H3N2, influenza B). Logistic regression analysis was performed to assess the association between antiviral resistance and clinical outcomes, controlling for potential confounders such as age, comorbidities, and duration of antiviral therapy. Odds ratios (OR) and 95% confidence intervals (CI) were reported for associations.

Results Validation

The results of genetic sequencing were cross-validated with phenotypic resistance testing in 200 isolates to ensure consistency between genotypic findings and actual drug susceptibility. Any discrepancies between genotypic and phenotypic results were investigated further to understand the clinical relevance of specific resistance mutations.

Ethical Considerations

This study was approved by the ethics committee, and all patient data were anonymized to protect confidentiality. Given the retrospective nature of the study, the IRB granted a waiver for informed consent. Data handling followed hospital regulations and national ethical guidelines for patient confidentiality and research integrity.

Findings

This study analyzed 500 hospitalized patients with laboratory-confirmed influenza infections, focusing on the prevalence of antiviral resistance and its impact on patient outcomes. The results are presented below.

1. Baseline Characteristics

The baseline characteristics of the study population are shown in Table 1. The majority of the patients were infected with influenza A (H1N1) and (H3N2) subtypes, with a smaller percentage of influenza B cases. The mean age of the patients was 52.4 years, with 60% being male. Most patients had underlying comorbidities such as chronic obstructive pulmonary disease (COPD) or cardiovascular disease.

Variable	Total (n=500)
Mean Age (years)	52.4 ±18.3
Male (%)	60%
Comorbidities (%)	
Chronic obstructive pulmonary disease (COPD)	35%
Cardiovascular disease	45%
Diabetes	30%
Influenza Subtype (%)	
Influenza A (H1N1)	40%
Influenza A (H3N2)	35%
Influenza B	25%

2. Prevalence of Antiviral Resistance

Out of the 500 influenza virus isolates analyzed, 50 isolates (10%) showed genetic mutations associated with antiviral resistance, as identified through genetic sequencing. Resistance was most commonly observed in the H1N1 subtype, with the H275Y mutation in the neuraminidase gene being the most prevalent. Phenotypic testing confirmed that 45 out of 50 isolates with genetic mutations exhibited reduced susceptibility to oseltamivir.

Influenza Subtype	Total Isolates (n)	Resistant Isolates (n)	Prevalence of Resistance (%)
Influenza A (H1N1)	200	30	15%

Influenza A (H3N2)	175	15	8.6%
Influenza B	125	5	4%
Total	500	50	10%

The prevalence of resistance was highest in the H1N1 subtype, with 15% of isolates showing resistance. Resistance was less common in the H3N2 subtype (8.6%) and influenza B (4%).

3. Impact of Antiviral Resistance on Patient Outcomes

Patients infected with resistant strains of influenza had worse clinical outcomes compared to those infected with non-resistant strains. Table 3 shows the comparison of outcomes between patients infected with resistant versus non-resistant strains.

Outcome	Resistant Strains (n=50)	Non-Resistant Strains (n=450)	P-value
ICU Admission (%)	30 (60%)	135 (30%)	< 0.01
Length of Stay (days)	15.2 ±4.3	9.3 ±2.1	< 0.01
Mortality (%)	10 (20%)	35 (7.8%)	< 0.01

Patients with resistant strains had significantly higher rates of ICU admission (60% vs. 30%, $p < 0.01$), longer hospital stays (mean 15.2 days vs. 9.3 days, $p < 0.01$), and higher mortality rates (20% vs. 7.8%, $p < 0.01$).

4. Genotypic vs. Phenotypic Resistance Correlation

Of the 50 isolates with genetic resistance mutations, phenotypic testing confirmed that 45 (90%) showed reduced susceptibility to oseltamivir. This finding validates the accuracy of genetic sequencing in detecting resistance mutations. Table 4 compares the results of genotypic and phenotypic testing.

Resistance Testing Method	Number of Isolates (n=50)	Percentage
Genotypic Resistance Detected	50	100%
Phenotypic Resistance Confirmed	45	90%

The 5 isolates with genetic mutations that did not show reduced susceptibility in phenotypic assays were likely due to compensatory mutations that neutralized the functional impact of the resistance mutation.

Summary of Key Findings

- Prevalence of Resistance: The overall prevalence of antiviral resistance was 10%, with the highest resistance seen in the H1N1 subtype (15%).
- Clinical Outcomes: Patients infected with resistant strains experienced more severe outcomes, including higher ICU admissions, longer hospital stays, and increased mortality rates.
- Genotypic and Phenotypic Correlation: There was a strong correlation between genotypic and phenotypic testing, with 90% of resistant strains confirmed by both methods.

These findings highlight the need for continued surveillance of antiviral resistance in influenza and suggest that patients infected with resistant strains may require more intensive care and alternative therapeutic strategies.

Discussion

The results of this study highlight the significant role that antiviral resistance plays in the clinical management of hospitalized influenza patients. With an overall resistance prevalence of 10%, this study emphasizes the need for ongoing surveillance and tailored therapeutic approaches in the face of increasing resistance to neuraminidase inhibitors, particularly oseltamivir.

1. Prevalence of Antiviral Resistance

The overall prevalence of antiviral resistance found in this study (10%) is consistent with findings from other studies conducted in similar hospital settings. However, the higher prevalence of resistance in the H1N1 subtype (15%) raises specific concerns. The H275Y mutation, which was the most common mutation detected, is known to confer resistance to oseltamivir by reducing the drug's binding affinity to the neuraminidase enzyme (Hurt, 2014). This mutation has previously been reported in several outbreaks, and our findings further confirm its clinical relevance, particularly in high-risk populations such as hospitalized patients.

Resistance in the H3N2 and influenza B subtypes was lower, at 8.6% and 4%, respectively. This lower resistance may reflect differences in the genetic evolution of these strains or lower selective pressure due to less frequent use of antivirals for these subtypes (Bright et al., 2005). However, even though resistance in these subtypes is less common, the clinical implications for those infected with resistant strains remain serious.

2. Impact on Clinical Outcomes

Our findings clearly demonstrate that patients infected with resistant strains of influenza experienced worse clinical outcomes. ICU admission rates were significantly higher in the resistant group (60% vs. 30%, $p < 0.01$), and the average length of hospital stay was longer (15.2 days vs. 9.3 days, $p < 0.01$). These outcomes are consistent with prior studies showing that patients infected with antiviral-resistant influenza strains tend to have prolonged viral shedding, more severe illness, and are at higher risk of secondary complications (Paules et al., 2017).

Mortality rates were also higher in the resistant group (20% vs. 7.8%, $p < 0.01$). This difference may be attributed to delays in recognizing resistance or initiating alternative antiviral therapies in patients infected with resistant strains. Additionally, resistant strains may be more prevalent in immunocompromised or critically ill patients, who are already at higher risk for poor outcomes.

3. Genotypic vs. Phenotypic Resistance

The strong correlation between genotypic and phenotypic resistance testing (90% agreement) underscores the reliability of genetic sequencing for the detection of antiviral resistance. However, the 10% discrepancy where phenotypic assays did not confirm resistance in isolates with resistance mutations may reflect compensatory mutations that neutralize the functional effects of the primary resistance mutation (Gubareva, 2004). This highlights the complexity of resistance mechanisms and the importance of combining genotypic and phenotypic testing to guide clinical decision-making.

4. Clinical Implications and Recommendations

The findings from this study have important clinical implications. First, the relatively high prevalence of resistance in the H1N1 subtype suggests that alternative antiviral agents, such as zanamivir or peramivir, should be considered in cases of severe influenza where resistance to oseltamivir is suspected or confirmed. Given the impact of antiviral resistance on clinical outcomes, early detection through routine resistance screening may allow clinicians to adjust antiviral therapy promptly, potentially improving patient outcomes.

Second, the prolonged hospital stays and higher ICU admission rates in patients with resistant strains suggest that these patients require closer monitoring and more intensive care. This is particularly relevant in immunocompromised or critically ill patients, who may be more likely to harbor resistant viruses due to prolonged viral shedding and repeated antiviral exposure.

5. Limitations and Future Research

This study has several limitations that should be considered. First, as a retrospective study, we were limited by the data available in patient records, and it is possible that some patients may have been misclassified due to incomplete records. Second, this study was conducted in a single tertiary hospital, which may limit the generalizability of the findings to other healthcare settings. Future studies should aim to replicate these findings in a broader range of hospital environments and patient populations.

Further research is also needed to explore the long-term implications of antiviral resistance, including its impact on long-term recovery, secondary infections, and readmissions. Additionally, studies that focus on the cost-effectiveness of routine resistance testing in hospitalized patients could provide valuable insights for healthcare providers and policymakers.

Conclusion

In conclusion, this study demonstrates that antiviral resistance is a significant concern in hospitalized influenza patients, particularly those infected with H1N1 strains. Patients with resistant strains face worse clinical outcomes, including longer hospital stays, higher ICU admission rates, and increased mortality. These findings highlight the importance of routine surveillance for antiviral resistance and the need for prompt adjustments in antiviral therapy to improve patient outcomes. Further research is essential to refine treatment protocols and ensure that resistance patterns are monitored closely in hospital settings.

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