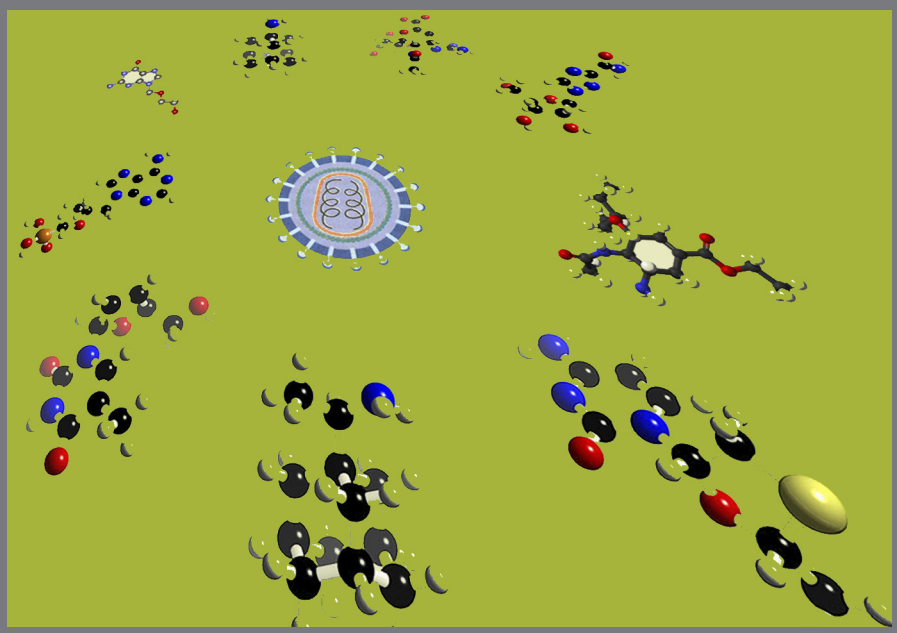
rapport 2015:5

Usage of Antivirals and the Occurrence of Antiviral Resistance in Norway 2013-2014



RAVN

Resistensovervåking av virus i Norge

Resistance against Antivirals in Norway

Norwegian Institute of Public Health

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Introduction

The number of available antiviral drugs for treating viral infections is increasing. The emergence of drug-resistant viruses is well documented as a cause of treatment failure. Especially immuno-compromised patients are regarded as an vulnerable group in this respect. Drug-resistant viruses may carry the potential for transmission. This potential is well described for HIV and influenza. Resistance to the new generation hepatitis C virus inhibitors is also likely to become a cause of concern. It is therefore important to conduct continious surveillance in order to detect any emergence or change in drug resistance and to develop optimal treatment regimen based on such information.

It is a pleasure to present the second report from the surveillance system Resistance against Antivirals in Norway (RAVN). This report presents new data on resistance against agents for the treatment of influenza, HIV infection, hepatitis B infection and human herpes virus infections from the years 2013 and 2014. The surveys have been conducted by the Norwegian Institute of Public Health and the Oslo University Hospital. It is our hope that the report contains valuable data for those developing treatment regimens and strategies to prevent transmission of viral infection.

John-Arne Røttingen

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Any use of data from RAVN 2013-2014 should include specific reference to this report.
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Contents

Sammendrag

Det finnes i dag en rekke tilgjengelige antivirale medikamenter i Norge, og antallet er raskt stigende. Med den økende bruken av slike medikamenter har man sett en markant økning i virusresistens mot medikamentene, slik man opplevde for bakterier etter inntoget av antibiotika på 40- og 50-tallet. Overvåking av denne utviklingen vil gi oss helt nødvendig kunnskap om utbredelse og forekomst av resistens for å kunne etablere forebyggende tiltak og gi grunnlag for behandlingsstrategier skreddersydd den enkelte pasient. Det langsiktige målet er å få utviklet medikamenter som effektivt behandler og utrydder kroniske virusinfeksjoner.

Overvåking av virusresistens hos influensa og HIV-1 har foregått systematisk i Norge siden 2005–2006. I 2011 startet implementering av disse dataene inn i registeret RAVN (Resistensovervåking av virus i Norge). Samtidig i denne perioden har resistensdata for heptatitt B-virus (HBV) og cytomegalovirus (CMV) blitt innsamlet fra de respektive referanselaboratoriene og registrert i RAVN.

Influensa

- Resistensovervåking av influensavirus utføres ved Nasjonalt Folkehelseinstitutt (FHI) og er viktig for fortløpende å kunne gi kunnskapsbaserte råd om empirisk antiviral behandling ved årlig influensasesong, samt ved pandemi. Overvåkingen har avslørt nye resistenstrender som senere har vært påvist også i andre land.

HIV-1

- Overvåkingen har vist at resistens finnes hos nylig diagnostiserte HIV-1 tilfeller som ikke står på antiviral behandling. Dette må følges nøye videre for å kunne oppdage en eventuell økende trend. Dette vil kunne ha betydning for legers valg av medikamenter ved oppstart av behandling.
- Siden starten av HIV-1 resistensovervåkingen har i underkant av halvparten av nydiagnostiserte tilfeller blitt sendt til resistensundersøkelse, men i løpet av de siste årene har det vært et større antall undersøkte. Det er viktig å øke denne andelen ytterligere, samt tilstrebe at prøvene er representative for alle pasientgruppene. Insidensen av HIV-1 infeksjon i de senere år har økt i gruppen med menn som har sex med menn, og det er derfor spesielt viktig at denne gruppen er godt representert i HIV-1 resistensovervåkingen.

HBV

- Virusresistens hos kroniske HBV-pasienter ser for tiden ut til å være et mindre problem i Norge. Pasientene gis effektiv førstelinje-behandling som undertrykker virusreplikasjonen slik at antiviral resistens motvirkes.
- Antall pasienter som behandles med disse midlene er mye lavere enn forventet ut i fra det estimerte tallet på 20 000 tilfeller av kronisk HBV-infeksjon i Norge.
- Det finnes ingen oversikt over total antall av pasienter som får behandling, herunder informasjon om behandlingsregime, varighet av behandling og behandlingssvikt. I overvåkingsammenheng er det viktig å innhente og systematisere slik informasjon i tilknytning til tilgjengelige resistensdata.

CMV

- Alvorlig behandlingstrengende CMV-infeksjoner ser en først og fremst hos pasienter med nedsatt infeksjonsforsvar. Det er også i denne gruppen at de fleste tilfellene av behandlingssvikt forekommer.
- Ved behandlingssvikt vil omlag en fjerdedel av tilfellene skyldes at CMV utvikler resistens.

Anbefaling fra RAVNs fagråd

Influensavirusresistensovervåking fortsetter som før, HIV-1 resistensovervåking av primærresistens bør intensiveres, og HBV-, HSV- og CMV-resistensovervåking fortsetter som før. Det bør lages en tilrådning for systematisk overvåking av resistensutvikling ved HCV-infeksjoner.

Summary

To date, there are numerous antiviral drugs available in Norway and this total is rapidly increasing. With the increasing usage of these antivirals, a marked rise of antiviral resistance against these drugs has been observed, as seen in the 1940s and 1950s with the flood of antibiotics used against bacteria. Surveillance of this development will give us the necessary knowledge on prevalence and spread of viral resistance to be able to establish preventative measures, thereby providing a solid basis for individual clinical treatment strategies. The long term goal is to develop drugs that effectively eradicate chronic virus infections.

The surveillance of influenza and HIV antiviral resistance has been conducted continuously in Norway from 2005 and 2006 respectively, and the process of implementing this surveillance into the register RAVN (Resistance against Antivirals in Norway) started in 2011. At the same time, resistance data for HBV and CMV has been collected from the national reference laboratories for inclusion into RAVN.

Influenza

- Surveillance of influenza antiviral resistance is conducted at the NIPH and is vitally important to continuously be able to provide evidence-based advice on the empirical antiviral treatment during annual influenza season and pandemics.
- Monitoring has revealed new susceptibility trends that have subsequently been identified in other countries.

HIV-1

- The surveillance has shown that viruses with resistance mutations can be found among newly diagnosed HIV-1 patients, and this must be monitored closely to follow any increasing trend. This might give an impact on treatment regime at start of therapy.
- Resistance surveillance was carried out in less than half of the newly diagnosed HIV-1 cases during the first years of implementation, but in the last years there has been an increase in the percentage of samples tested. It is a necessity to improve the surveillance even further, ensuring a representative number of samples from all patient risk groups. The incidence of HIV-1 has increased among MSM in recent years and it is therefore important that the surveillance of this group is well covered.

HBV

- Antiviral drug resistance seems to be a minor health problem in Norway among chronic HBV (CHB) patients at the present time. Patients in Norway are increasingly given first-line therapy that effectively suppresses the virus replication and limits the development of drug resistance.
- The number of patients on nucleos(t)ide analogue (NA) therapy appears to be lower than expected, given the estimated number of 20 000 cases of CHB infections in Norway.
- There is no overview of the total number on treatment for HBV-infection in Norway, including type and duration of treatment used and treatment failure. For surveillance it is important to obtain and systemise these data.

CMV

- Serious CMV-infections that require antiviral treatment are mainly seen in severely immunosuppressed patients. Most treatment failures are seen in this group of patients.
- Upon treatment failure about one forth is caused by development of resistant CMV

Recommendation from RAVN council

Influenza virus-, HBV-, HSV- and CMV resistance monitoring continue as before, HIV resistance monitoring of primary infections is to be intensified, and a program for monitoring the development of HCV resistance is recommended.

Background

In the beginning of 2010, the NIPH published a report "Utredning om nasjonalt overvåkingssystem for virusresistens" resulting in the establishment of RAVN (Resistensovervåking av virus i Norge, Resistance against Antivirals in Norway) a national register recording antiviral susceptibility surveillance.

The RAVN Centre has been set up and is run by physicians and scientists at the Department of Virology at NIPH. The RAVN Council has been appointed to make recommendations for the annual surveillance and holds meetings twice a year. Representatives from both RAVN Council and RAVN Centre at the NIPH are participating in the European Society of Antiviral Resistance (ESAR). Department of Virology and the RAVN council leader have been working on the rules and guidelines for RAVN in close collaboration with the lawyers at the Ministry of Health.

In the following report all national data on viral resistance is presented. The data has been collected and processed by the RAVN Centre and Council. ■

The organization of RAVN

RAVN stands for Resistance against Antivirals in Norway ("Resistensovervåking av virus i Norge"), and was established in accordance with the Ministry of Health's (MoH) "National Strategy for the prevention of infections and antibiotic resistance in health care (2008–2012)". RAVN consists of a Centre located at the Department of Virology at NIPH, and a Council who work together to plan and manage the annual surveillance of viral resistance in cooperation with participating regional laboratories. The regulations governing RAVN came into effect 1st July 2014. ■

The usage of antivirals in Norway

During the last 15 years, the development of new specific antivirals has been accelerated due to research into HIV medicines (1) and hepatitis C medicines. The prescribed amount of antiviral drugs has been increased every year. According to The Norwegian Drug Wholesales statistics database, antiinfectives for systemic use cost increased by 20% in 2014 (3). The increase is mainly due to increased sale of antivirals. Figure 1 shows the sales of direct acting antiviral drugs (DAA)(ATC group J05A), during the past five years.

The usage of anitivirals for the treatment of influenza is shown in Table 1. The variation betweeen the years are probably linked to the size of the yearly influenza epidemic.

There are currently 36 approved antivirals for HIV in Norway. The usage of these drugs has increased more than 50 % from 2010 to 2014, as indicated in figure 2 showing the number of patients given at least one prescription per year. In addition there is an increase in prescriptions of combination products including more than one active entity. The total number of patients on HIV therapy indicates a maximum number of patients since a few substances are also used in treatment of HBV. The patients might have been treated with different drug regimens during the period. The largest increase for single substances measured in number of patients since 2010 is seen for ritonavir and tenofovir disoproxil (3) Ritonavir is exclusively used as a protease-inhibitor enhancer and is always used

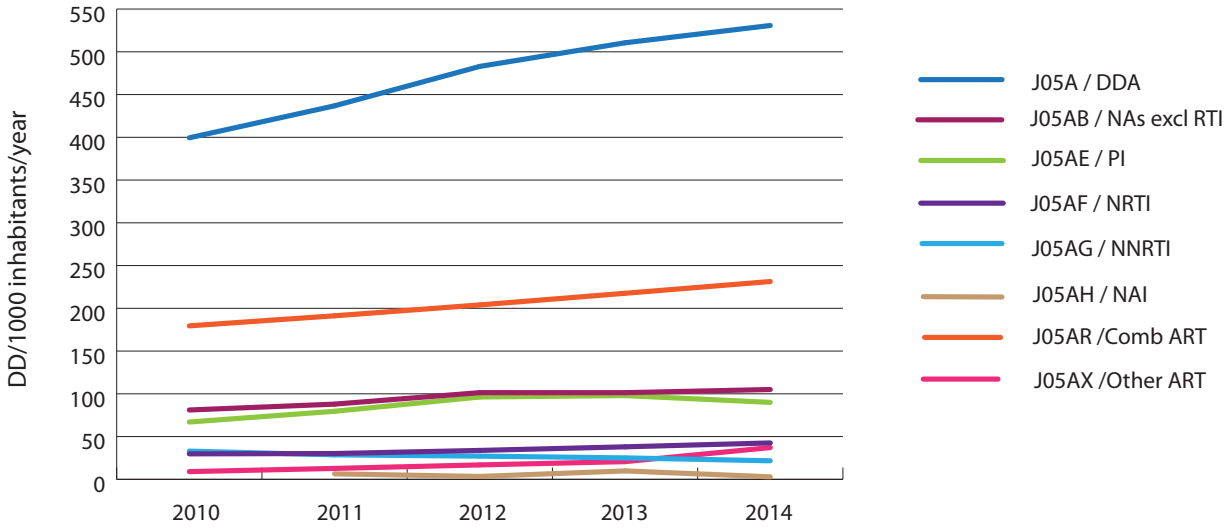

Figure 1. Sales of direct acting antiviral drugs (DAA), ATC group J05A for 2010–2014 given in DDD/1000 inhabitants/year. Source: The Norwegian Drug Wholesales statistics database.

Table 1. Number of individuals with at least one prescription of neuraminidase inhibitor (NI) drug according to year.

NI drug	**Number of individuals with one or more prescription per annum**				
	2010	**2011**	**2012**	**2013**	**2014**
Zanamivir	35	36	34	85	18
Oseltamivir	3 829	2 612	1 776	3 911	1 076

in combination with other HIV drugs, decreasing pill burden and frequency of dosing. Trends in usage may be due to new combinations of drugs. The usage of combination therapy emtricitabine and tenofovir disoproxil increases, whereas triple combination with emtricitabine, tenofovir disoproxil and rilpivirin is increasing even more. Since 2012 two new fixed combinations of three active substances has been introduced and even one fixed combinations with four substances (emtricitabine, tenofovir disoproxil, elvitegravir and cobicistat). These combinations are all increasingly used.

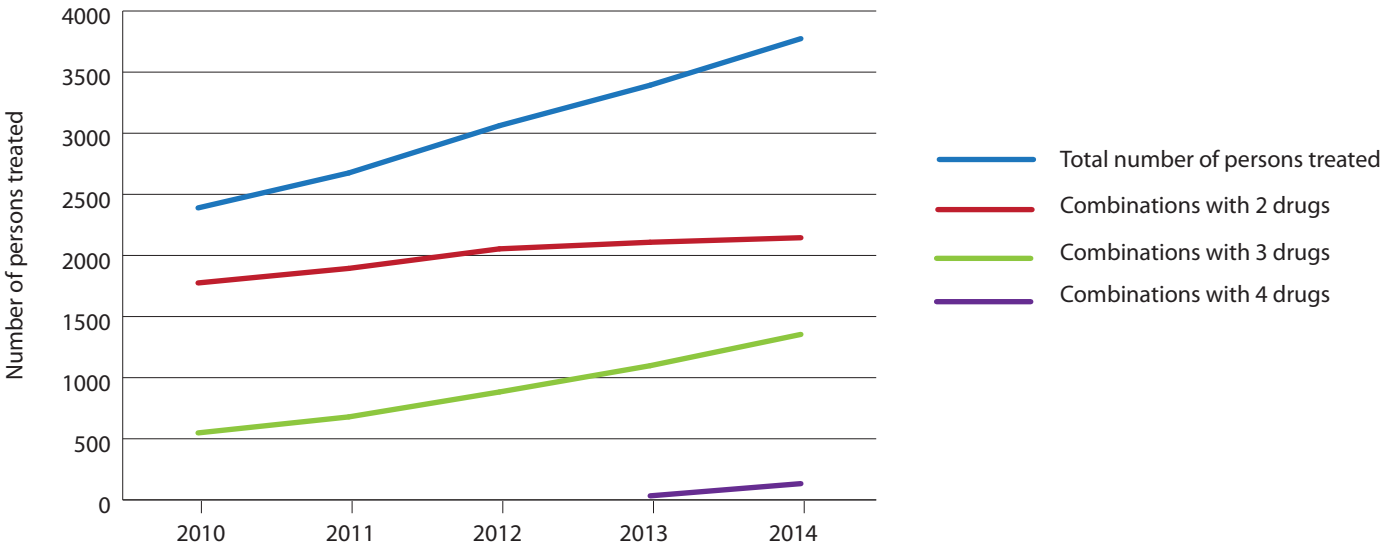

Figure 2. Trends in use of antivirals for treatment against HIV from 2010–2014. Source: The Norwegian Prescription Database (NorPD), Norwegian Institute of Public Health

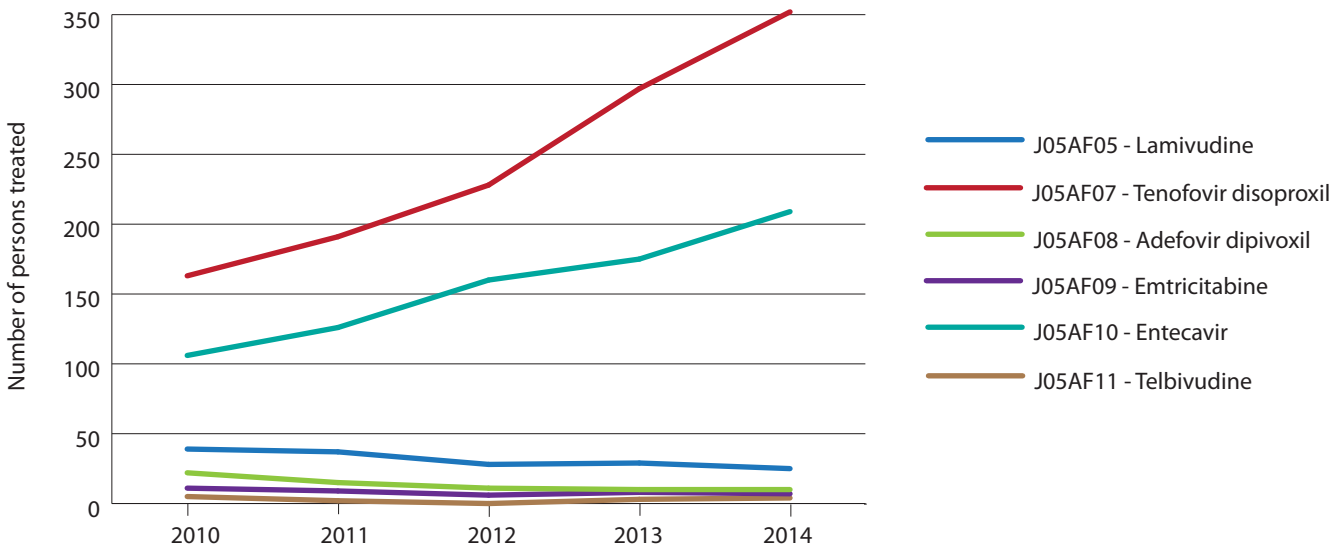

Figure 3. Patterns of prescriptions for HBV-treatment from 2010–2014 based on the number of patients given at least one prescription per year. Source: The Norwegian Prescription Database (NorPD), Norwegian Institute of Public Health

There are currently 8 approved therapies for HBV infection including three interferon based and six nucleoside/nucleotide analogues (NA) (lamivudine, adefovir dipivoxil, emtricitabine, entecavir, telbivudine and tenofovir disoproxil). Treatment of HBV with antivirals is generally given as mono-therapy. The use of these NA-drugs is shown in figure 3. The data is based on the annual number of patients given at least one subscription per year for the period 2010– 2014(3). Lamivudine, adefovir dipivoxil, tenofovir disoproxil and emtricitabine are drugs that are approved for both HBV and HIV, while entecavir and telbuvidine are approved for HBV only. An estimate of patients treated for HBV with antivirals in Norway will therefore be in the range of 213–590 in 2014 based on the patients that used drugs approved for HBV only and the total number of patients treated with the six NA-drugs (excluding lamivudine for HIV). First-line therapy (entecavir and tenofovir disoproxil) has been increasingly used for several years and account for over 90% of the six NA treatments given in 2014.

Until 2011 HCV-therapy was based on a combination of pegylated interferon and ribavirin for a given period depending on HCV-genotype. In 2011 two new protease inhibitors (PI), telaprevir and boceprevir, were licensed for combination therapy with ribavirin and interferon in HCV genotype 1 infection. In 2014 three new antiviral drugs targeting HCV entered the market: Sofosbuvir; a pangenotypic polymerase inhibitor, Simeprevir; a second-wave protease inhibitor and Daclatasvir; a pangenotypic NS5A inhibitor. With these new direct-acting antivirals (DAA) the therapy for chronic HCV-infection has greatly improved.

In 2014 more than 90% of patients were given combination therapy with ribavirin and DAA. The overall number of patients on treatment have increased during the last five years with the new drugs on the market (figure 4). There are a number of new substances that are ready for release from 2015–2016. The usage of antivirals is expected to increase further the coming years in connection with the introduction of these new drugs.

Figure 5 shows the two most prescribed drugs for herpes virus infections over the last five years. The following drugs ganciclovir, famciclovir, cidofovir and foscarnet have been prescribed very rarely in this period. The use of valganciclovir is increasing (table 2).

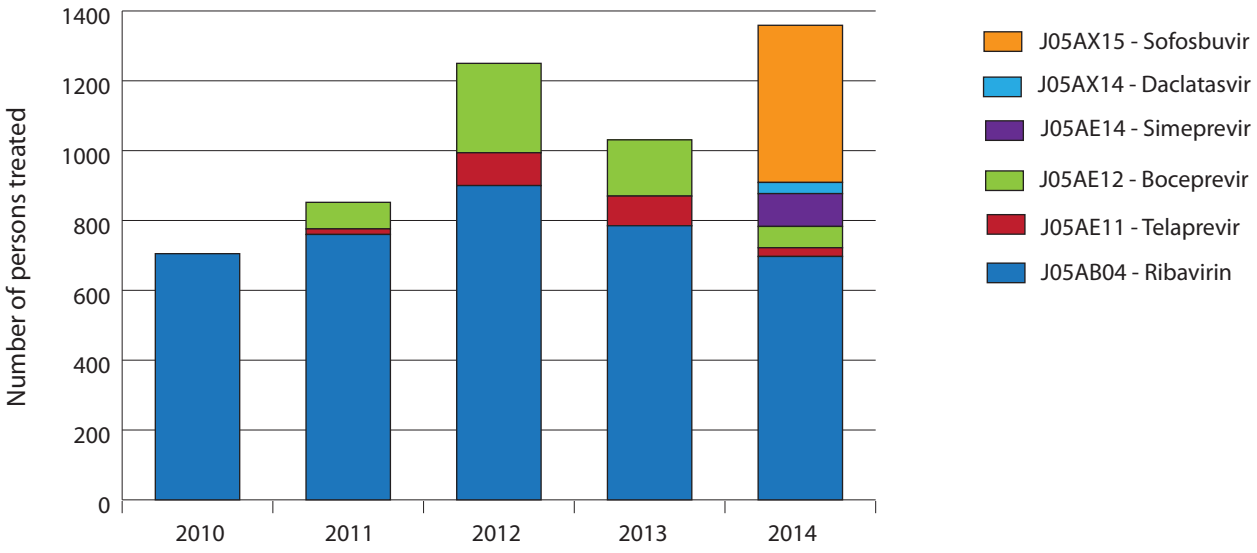

Figure 4. Patterns of prescriptions for HCV-treatment from 2010–2014 based on the number of patients given at least one prescription per year. Source: The Norwegian Prescription Database (NorPD), Norwegian Institute of Public Health

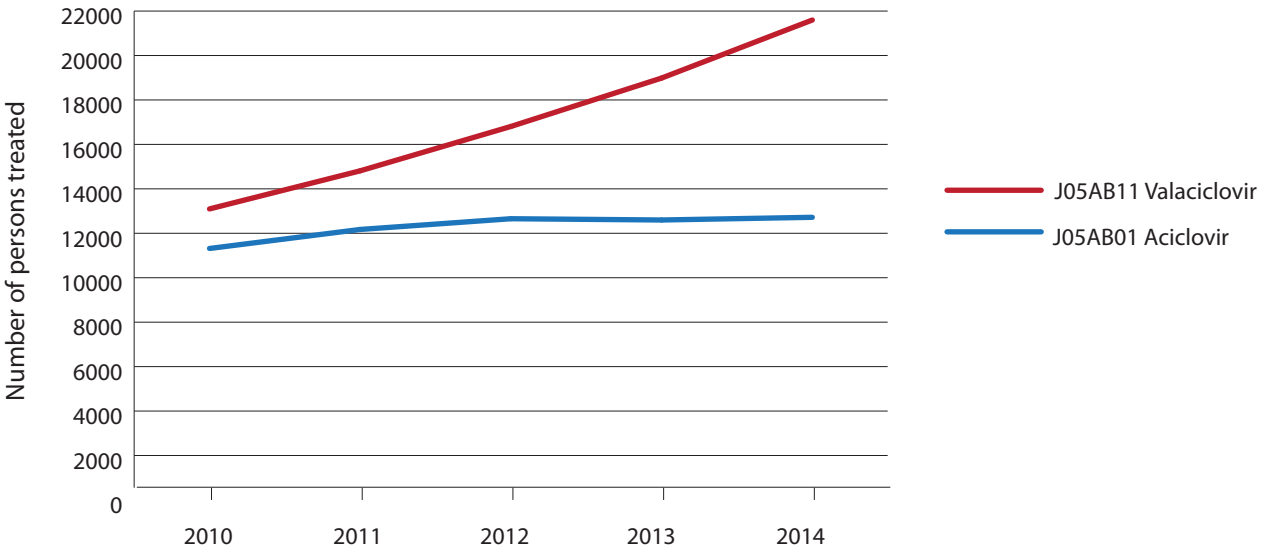

Figure 5. Number of individuals with at least one prescription of acyclovir and valaciclovir per year for the periode 2010–2014.

Table 2. Number of patients given prescription for herpes virus infections per year for the periode 2010-2014. Source: The Norwegian Prescription Database (NorPD), Norwegian Institute of Public Health.

	2010	2011	2012	2013	2014
Aciclovir	11 316	12 172	12 655	12 598	12 719
Ganciclovir	3	1		1	2
Famciclovir			1	2	4
Cidofovir					
Valaciclovir	13 096	14 811	16 807	18 985	21 597
Valganciclovir	283	319	347	365	378

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Influenza virus drug resistance

During the annual winter epidemics influenza illness has a large effect on society. For the affected individual, the illness is troublesome, although most people recover without medication. What make influenza a major public health problem are cases with serious complications, hospitalisation and death. People with certain medical conditions are more likely to develop severe influenza infections and are highly recommended to be vaccinated against influenza every season (1).

Influenza virus is recognised by rapid evolution and annual global spread. The rapid evolution of the influenza viruses also has an impact on the susceptibility against antiviral drugs.

Antivirals against influenza

There are two classes of antiviral drugs available against influenza in Norway, the M2-inhibitors amantadine and rimantadine, and the neuraminidase inhibitors, oseltamivir and zanamivir (3). M2-inhibitors block viral replication of influenza A virus, but are ineffective against influenza B- or C-virus which do not possess M2 proton channels. Studies have shown that both drugs can prevent influenza A illness in 70–90 % of cases, and that they can reduce the duration and severity of influenza A if treatment is started within the first two days after the symptom debut (4). Usage has been limited due to side effects from the central nervous system, especially in the case of amantadine, but less so for rimantadine (4). For reasons that remain unclear, the frequency of M2 blocker resistance in human A(H3N2) viruses gradually rose from almost nil to 100 per cent during the first decade of this century, and the pandemic A(H1N1) virus that emerged in 2009 has been uniformly resistant to M2 blockers since the start.

The norwegian pandemic stockpile of anti-influenza medicines includes oseltamivir, zanamivir and rimantadine meant for prophylactic use (2). Zanamivir (an inhalation drug) and the oral oseltamivir are the only medicines licensed in Norway for both chemoprophylaxis and treatment of influenza type A and B infections. The clinical effect of these two medicines is almost identical. When oseltamivir is used profylactically, it is proven to be effective in up to 89 % of healthy adults (5), zanamivir similarly in up to 84 % (6). It has been shown that the drugs reduce the duration of symptoms by two days and the degree of severity in healthy adults and children with laboratory confirmed influenza (5), but the the clinical usefulness is being debated.

Development of resistance

Resistance can develop in different ways. The resistant form may occur by de novo mutation, by exchange of genetic material between different influenza strains or it can be present initially as a rare variant. Selection can occur when an appropriate antiviral substance is present at suboptimal concentrations, or when the virus is not fully sensitive. It is also possible that resistance is 'hitch-hiking' on another advantageous feature that promotes this variety over other viruses. Resistance can thus grow in the absence of antiviral agents as long as the mutation which confers resistance does not cause any significant evolutionary disadvantage for the virus.

Occurrence of resistance to anti-influenza agents

The high mutation rate in RNA viruses such as influenza provides an opportunity for selection of resistant viruses. Currently all circulating human influenza A viruses are resistant to amantadines. During winter 2007–2008 resistance to oseltamivir (substitution H275Y in the NA protein) was observed in an unexpectedly high proportion of influenza A (H1N1) viruses in Norway. The mutant viruses were still fully susceptible to zanamivir and the M2 blockers. Previously, this particular resistance generating mutation had been known for some years, but the virus viability was reduced and the mutation had not been observed in circulating A (H1N1) virus. The emergence of resistance occurred in almost complete absence of oseltamivir use in Norway. The resistant virus spread to the entire world and all H1N1 viruses circulating uptil the 2009 pandemic H1N1 were resistant to oseltamivir. Currently all, but very few, pandemic H1N1 (H1N1pdm09) viruses are sensitive towards both oseltamivir and zanamivir. Few insidenses have occurred in persons that have used the neuraminidase inhibitor as a prophylactic agent or immunocompromised patients being treated

with oseltamivir during protracted infection. Two outbreaks of oseltamivir resistance have however been observed both in Okaido, Japan (2013-2014) and in Australia (2011). Toward the end of the 2011 influenza season in Australia, local spread of oseltamivir resistant H1N1pdm09 viruses was observed (11). Apparently, these viruses did not spread beyond the initial area and ceased to circulate with the ending of the season there.

Zanamivir resistance has been reported in sporadic cases with influenza B (8). The structural similarity between the natural substrate and zanamivir, and high concentration of the drug in the respiratory tract where virus replication occurs, help to reduce the risk of resistance development. Different influenza viruses show varying sensitivity to neuraminidase (NA) inhibitors (7).

Oseltamivir has been the drug of choice, mainly because of its ease of administration in tablet form. The alternative, zanamivir, is inhaled and has not been used nearly as extensively as oseltamivir. Two other recently developed NA inhibitors, peramivir and laninamivir, are currently approved for use in Japan. Peramivir is also approved in USA. Other anti-influenza drugs that target different stages of viral replication such as favipiravir (T-705) and nitazoxanide (Alinia) are also in late-stage clinical trials (9).

The clinical significance of resistant influenza virus

Severe influenza infection requires specific antiviral therapy. In cases with immunodeficiency, resistance could affect the course of the disease as these patients often have prolonged duration of infection and higher viral load, factors which in turn contribute to the development of resistance (10).

Normally in influenza infection, susceptibility testing will not be possible before the start of the treatment, as the window of opportunity for efficient treatment is very narrow. Even laboratory confirmation of influenza infection can be too time-consuming, leaving empirical treatment as the only option. Choice of medicine should therefore be evidence based, by using knowledge from resistance surveillance and cross resistance. Active and timely sentinel surveillance for antiviral drug resistance is therefore important and evidence of community spread of resistant viruses should be reported rapidly. It is important for patient care that clinicians are aware of emerging resistance so that alternative drugs are considered in the event of a poor response to oseltamivir. Special care should be taken to minimize the risk of virus transmission from hospitalized patients undergoing oseltamivir treatment.

Surveillance of influenza resistance

Surveillance of influenza resistance in Norway

The WHO national reference laboratory for influenza in Norway is located at the NIPH and monitors the occurrence of influenza viruses in Norway. A volunteer network of sentinel physicians in all parts of the country provide samples taken from patients with influenza-like illness, and the medical microbiology laboratories submit confirmed influenza strains. These samples are analysed by virus cultivation, sequencing and other methods. Resistance monitoring is performed using both genotypic and phenotypic susceptibility testing of virus isolates. Since 2007, the influenza reference laboratory has made annual reports of influenza resistance surveillance, and has published a number of research results in international journals. During the influenza season the results from resistance surveillance are published weekly on the NIPH's website www.fhi.no/influensa.

Surveillance of influenza resistance through WHO / European Influenza Surveillance Network

The WHO European Regional Office, in coordination with the European Centre for Disease Prevention and Control, conducts surveillance of seasonal influenza in the Region and publishes a weekly regional bulletin on seasonal influenza . The data are collected by clinicians' networks and laboratory networks, consisting primarily of WHO-recognized National Influenza Centres (NICs). In Norway this is the national reference laboratory for influenza located at the NIPH

The regional surveillance network also participates in the WHO Global Influenza Surveillance and Response System (GISRS). This enables WHO to recommend the composition of the influenza vaccine for the following season for the northern and southern hemispheres.

In the EU/EEA, the European Centre for Disease Prevention and Control (ECDC) coordinates the European Influenza Surveillance Network (EISN) which consists of contact points for influenza surveillance nominated by the Competent Bodies for surveillance of the Member States. Epidemiological, virological and resistance surveillance data on influenza are collected through The European Surveillance System (TESSy).

Surveillance findings in the 2013/14 and 2014/15 influenza season

All viruses from 2013 to 2015 were as expected resistant towards adamantanes, M2-ion blockers. Findings from the Norwegian influenza resistance surveillance are summarised in table 1. All of the pandemic A(H1N1)pdm09 and the A/H3N2 viruses analysed in 2013-2014 were 100% susceptible to the neuramidase inhibitors oseltamivir and zanamivir in the phenotypic assay (MUNANA)

Among the nearly 400 viruses analysed for resistanse at FHI season 2014-2015 one H1N1 (0,74%) from week 19 in 2015 was found to be highly resistant towards oseltamvir (Tamiflu™) with the substitution H275Y in the virus neuraminidase. The virus had a 400-fold reduction in sensitivity towards oseltamivir. The virus was from a polyclinical patient that had not recieved antiviral treatment or been travelling. The resistant virus does not seem to have spread further in the community. All other viruses analysed were sensitive to both oseltamivir and zanamiviru (Relenza™)

All influenza B viruses that were analysed were susceptible to both oseltamivir and zanamivir.

Table 1. Norwegian influenza viruses resistant to the NIs oseltamivir and zanamivir and M2 blockers (adamantanes), during the influenza seasons 2005/6 through 2014/15.

Season	Oseltamivir resistance			Zanamivir resistance			Adamantane resistance	
	A(H1N1)	A(H3N2)	B	A(H1N1)	A(H3N2)	B	A(H1N1)	A(H3N2)
2005/06	0% (n=6)	0% (n=13)	0% (n=21)	0% (n=6)	0% (n=13)	0% (n=21)	Nd	75% (n=4)
2006/07	0% (n=5)	0% (n=10)	nd	0% (n=5)	0% (n=10)	Nd	0% (n=6)	90% (n=10)
2007/08	67,8% (n=272)	0% (n=2)	0% (n=59)	0% (n=114)	0% (n=2)	0% (n=59)	0% (n=112)	100% (n=2)
2008/09	100% (n=33)	0% (n=13)	0% (n=1)	0% (n=5)	0% (n=12)	0% (n=1)	0% (n=5)	100% (n=65)
2009-pdmH1	0% (n=884)	nd	0% (n=11)	0% (n=36)	nd	0% (n=9)	100% (n=258)	100% (n=2)
2010/11	1.6% (n=244)	0% (n=1)	0% (n=30)	0% (n=2)	0% (n=1)	0% (n=24)	100% (n=54)	100% (n=10)
2011/12	0% (n=27)	0% (n=72)	0% (n=5)	nd	0% (n=60)	0% (n=4)	100% (n=21)	100% (n=56)
2012/13	0% (n=256)	0% (n=22)	0% (n=24)	0% (n=20)	0% (n=22)	0% (n=19)	100% (n=11)	100% (n=5)
2013/14	0% (n=183)	0% (n=43)	0% (n=27)	0% (n=32)	0% (n=43)	0% (n=27)	100% (n=77)	100% (n=67)
2014/15	0,74% (n=136)	0% (169)	0% (n=92)	0% (n=136)	0% (n=166)	0% (n=92)	nd	100% (n=30)

Conclusion

It is exceedingly important to have national antiviral susceptibility monitoring systems that can deliver timely data to inform public health and clinical recommendations for antiinfluenza drug use.

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Human immunodeficiency virus

HIV is a retrovirus that infects cells of the human immune system and destroys them or impairs their function. Infection results in progressive deterioration of the immune system leading to immune deficiency. Immunodeficient patients are more susceptible to a wide range of infections. Acquired immunodeficiency syndrome (AIDS) is a condition recognised by either the occurrence of specific diseases associated with HIV infection or a $CD4^+$ T cell count below 200 cells per µL. HIV can be found in the bodily fluids of infected people (blood, semen, vaginal fluids and breast milk) and may be transmitted through unprotected sex, sharing of contaminated needles or other sharp instruments, from mother to child during pregnancy, childbirth or breast feeding, or through blood transfusion with contaminated blood.

There are two main types of HIV, HIV-1 and HIV-2, and since the 1980s HIV-1 has spread worldwide and accounts for the pandemic. In 2014 it was estimated that 36.9 million people live with HIV throughout the world, and that approximately 1.2 million people died of AIDS related causes worldwide (1). Further, as of March 2015, 15 million people living with HIV-1 had access to antiretroviral therapy.

By 2014 there were 5622 diagnosed cases of HIV-1 in Norway, 3 803 men and 1 819 women (2). Immigrants represent half of all newly diagnosed individuals every year (all transmission routes). The situation the last two decades has been characterized by a continuous increase in the number of diagnosed HIV-1 cases. The increase is among men having sex with men (MSM) and immigrants infected in their home countries before arrival in Norway (Table 1). The trend, with increasing HIV-1 prevalence among MSM, started around 2000 and is now spreading from MSM communities in Oslo to the larger cities and urban areas elsewhere in Norway(3). Similarly, the number of reported cases of syphilis and gonorrhoea in this group has also increased dramatically in recent years and underlines the extent of unsafe sex. The same trend is seen in most Western countries.

Antiretroviral drugs and development of resistance

The introduction of an effective antiretroviral therapy has resulted in a significant reduction in HIV-1-related morbidity and mortality. There are currently five different drug classes targeting different phases of HIV's lifecycle; CCR5 blockers prevent binding between

Table 1. Transmissions routs of HIV-1 infections in Norway by year of diagnosis.

Transmission route	**2010**	**2010**	**2011**	**2012**	**2013**	**2014**	**Total**	**%**
Heterosexual	2229	157	155	142	124	130	**2937**	52,2
- HIV-1 infected while living in Norway	*691*	*57*	*46*	*46*	*31*	*47*	*918*	-
-HIV-1 infected before imigrating to Norway	*1538*	*100*	*109*	*96*	*93*	*83*	*2019*	-
Homosexual	1369	85	97	76	98	107	**1832**	32,6
Intravenious drug abuse	564	11	10	11	8	7	**611**	10,9
Blood and blood products	47					1	**48**	0,9
From mother to child	63	1	4	7	1	3	**79**	1,3
Unknow/other	99	4	2	6	3	1	**115**	2,1
Total	**4371**	**258**	**268**	**242**	**234**	**249**	**5622**	**100,0**

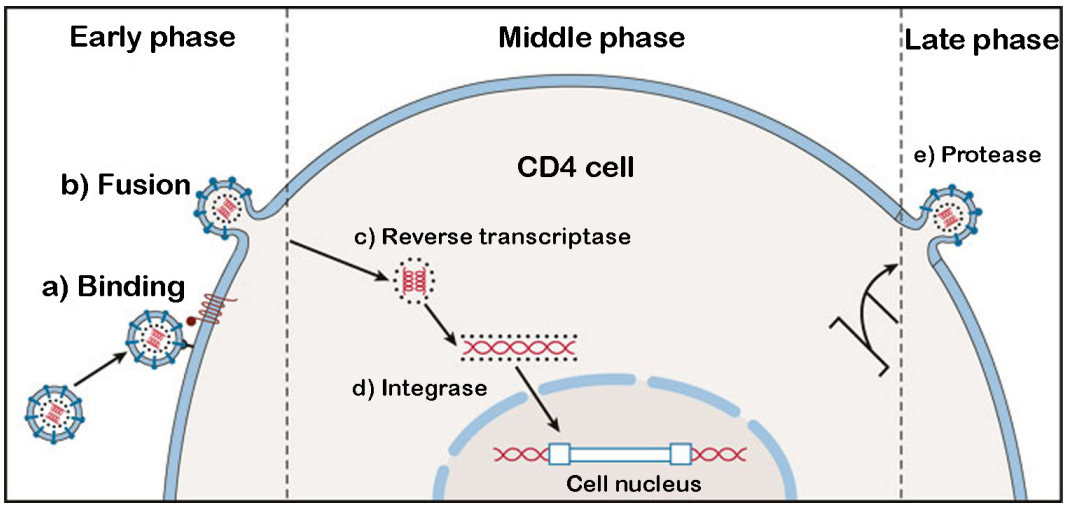

Figure 1. HIV-1 life cycle and attack points for antiviral drugs (4).

viral gp120 and the chemokine receptor CCR5, fusion inhibitors prevent fusion between the viral gp41 and the cell membrane, nucleoside and non-nucleoside reverse transcriptase inhibitors are directed against the reverse transcriptase and inhibits transcription of RNA into DNA, integrase inhibitors prevent integration of pro-viral DNA into the host cell DNA, and protease inhibitors prevent cutting of poly-proteins (Figure 1).

The antiretroviral therapy is based on the principle that during prolonged treatment of HIV-1, combinations of at least two drugs with different attack points must be used. Mono-therapy may favour the development of resistant viruses, while combination therapy targeting e.g. both reverse transcriptase and protease keeps the replication so low that the risk of developing resistance decreases. Recommended treatment therefore consists of a combination of at least three different drugs from at least two different classes.

HIV-1 binds first to the CD4 molecule on the cell surface and thereafter to coreceptor CCR5 (or CXCR4). The fusion with the cell membrane is mediated by gp41. The viral RNA is transcribed to viral DNA by reverse transcriptase in the cytoplasm and is integrated into the cell nucleus by integrase. New virus particles bud off from the cell membrane and the protease cleaves the major poly-proteins to functional proteins. Early phase: a) blocking of CCR5, b) blocking of fusion with the cell membrane. Interphase: c) Nucleoside and non-nukleoside reverse transcriptase inhibitors, d) integrase inhibitor. Late phase: e) Protease inhibitors.

The treatment does not eliminate the virus, but can effectively reduce the production of new virus particles so that for most patients, HIV-1 RNA levels in plasma remain stable below the limit of detection. The effect of treatment is monitored by increase in CD4 counts and decrease in HIV-1 RNA copy numbers in plasma. Detectable levels of HIV-1 RNA in plasma may indicate the development of resistant virus. There is a considerable genetic variation in the HIV-1 genome, not only from patient to patient, but also within the individual patient. This genetic variation is mainly due to the fact that the reverse transcriptase does not correct errors (mutations) that occur during DNA synthesis. Mutation rate is estimated to be approximately one substitution per viral genome per replication cycle. The variation is amplified by the fact that HIV-1 has a high replication rate, up to 10^{10} viral particles produced each day in an untreated patient. Different variants will soon be able to be selected upon changes in the environment. At suboptimal treatment, resistant viruses are selected, resulting in therapy failure.

With today's antiretroviral treatment, effective control of viral replication and full suppression of plasma viral load are achieved for most patients with chronic HIV-1 infection. However, antiretroviral drug-resistant virus strains are emerging, and HIV-1 resistance testing has become an important component of the clinical management of patients with HIV-1 infection (5-8). There is some transmission of drug-resistant virus, but in most cases, resistance develops as a result of persistent viral replication during antiretroviral treatment, often due to suboptimal drug levels. Still, the dynamics of drug resistance development is not yet fully understood.

There are two main methods for determining the resistance of HIV-1, phenotypic and genotypic. Phenotypic susceptibility tests measure viral replication in cell culture in the presence of serial dilutions of the drugs in question, but these methods are slow and compli-

cated, and are not used as routine tests. In Norway, as in most other countries, only genotypic assays are used, and all HIV-1 resistance testing is currently performed at the National reference laboratory for HIV at Oslo University Hospital Ullevål.

The genotypic assays involve amplification of the relevant part of the HIV-1 genome with RT-PCR, followed by nucleotide sequencing of the PCR product. The routine assays include sequencing of the genes coding for the protease and reverse transcriptase, the viral enzymes targeted by the main classes of antiretroviral drugs. The integrase gene can also be investigated on request, but the analysis is only performed in samples from patients currently or previously treated with integrase inhibitors. The resulting amino acid sequence is subsequently interpreted through identification of amino acid alterations that have been found to be associated with reduced drug susceptibility. More than 200 amino acid sequence positions of relevance for resistance have been identified. There are numerous genotypic interpretation systems available that take accumulated clinical data into account, and they are updated regularly. In addition, all samples showing genotypic resistance in Norway are individually interpreted by an experienced HIV clinician and microbiologist in collaboration, and the interpretation often includes treatment suggestions. In order to make such recommendations, it is important that all information about previous and current antiretroviral treatment is communicated to the laboratory. A special referral form designed for this purpose is available at www.oslo-universitetssykehus.no (Avdeling for mikrobiologi, henvisningsrutiner).

A new class of antiretroviral drugs called CCR5 antagonists work by blocking the binding of HIV-1 to CCR5 chemokine-receptors on the surface of the target cells. Most HIV strains depend on binding to CCR5 as a co-receptor for viral entry. However, some HIV strains use another chemokine receptor (CXCR4) as co-receptor, rendering CCR5 antagonists ineffective. Co-receptor usage is correlated with the amino acid sequence of the V3 loop of the HIV protein gp120. If viruses with CXCR4 tropism are detected by sequencing of the V3 loop, the patient should not be treated with CCR5 antagonists. Genotypic tropism testing can be performed on request at the HIV reference laboratory at Ullevål.

The most important rationale for performing resistance testing in clinical practice, is virological failure. Resistance testing is also recommended in pregnancy, or from the source after a needle stick injury. Furthermore, it is recommended that all patients with a newly diagnosed HIV-1 infection are tested for resistance mutations for surveillance purposes. It is not commonly recommended to perform resistance testing prior to initiation of treatment.

HIV-1 drug resistance testing requires plasma samples for analysis, and in general a viral load of at least 500 copies/mL is required for genotypic resistance. However, samples with lower viral loads may sometimes be successfully sequenced, while some samples with higher viral loads may not, mainly due to variation in the quantification assay or individual sequence variations. Clinicians are encouraged to contact the laboratory if they have samples with low viral loads where resistance testing is of particular importance.

One major limitation of genotypic resistance testing is its inability to detect variants of HIV-1 that represent only small fractions of the patients total virus population. For a mutation to be detected, it must account for 20–30% of the virus population in the sample. Therefore low-level mutations with possible clinical consequences cannot be ruled out. The presence of antiretroviral drugs acts as selection pressure, rendering HIV-1 variants containing resistance mutations a relative growth advantage. This positive selection of resistant virus depends on the presence of the specific drug. When the medication is stopped or altered, the growth advantage of the mutant virus ceases, and wild type virus or other variants will usually reappear and dominate. Therefore, when testing for drug resistance mutations in a patient with virological failure, it is important that the sample is collected while the patient is still receiving the failing regimen.

Surveillance of HIV-1 drug resistance

There are variations in the observed rate of transmission of drug resistant HIV-1 in countries where antiretroviral treatment is available. The variation in prevalence is due to several factors, e.g. occupational testing bias, different treatment regimes at the population level, differences in risk behaviour and access to medicines among risk groups, different definitions of resistance, and different time periods between exposure and sampling. Different results from different countries illustrate the importance of national monitoring systems and standardised methods for surveillance monitoring. WHO recommends a set of Surveillance Drug Resistance Mutations (SDRM) that should be monitored in transmitted HIV-1 resistance surveillance. The list of SDRMs is updated regularly (Appendx A1), and used in the analysis tools provided by databases that can be used for genotypic interpretation of HIV-1 drug resistance, such as the Stanford HIV Drug Resistance Database http://hivdb.stanford.edu/hiv/ and the Los Alamos National Laboratory HIV Drug Resistance Database http://hiv -web.lanl.gov. The monitoring of primary HIV-1 resistance in Norway is conducted according to WHOs SDRM-list of 2009 and analysed by using the Calibrated Population Resistance (CPR) tool at Stanford HIV Drug Resistance Database, (http://hivdb.stanford.edu).

Surveillance findings in Norway in 2006–2014

Resistance surveillance was carried out in less than half of the newly diagnosed HIV-1 cases during the first years of implementation, but in the last year there has been an increase in the percentage of cases where samples are received for testing. The annual percentage of sequences analysed for primary HIV-1 drug resistance from newly diagnosed cases of HIV-1 in Norway since 2006 is shown in figure 2.

SDRM detected in monitoring of primary HIV-1 resistance is presented in figure 3 as percentage of the sequences with detected SDRM in total. There may be several SDRM per sequence.

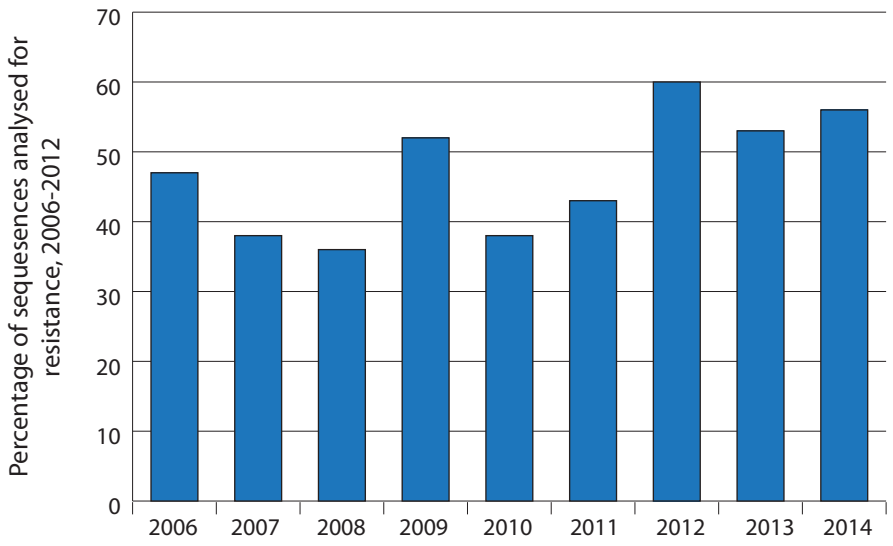

Figure 2. Percentage of newly diagnosed cases of HIV-1 infection where samples were sent for resistance testing (2006–2014).

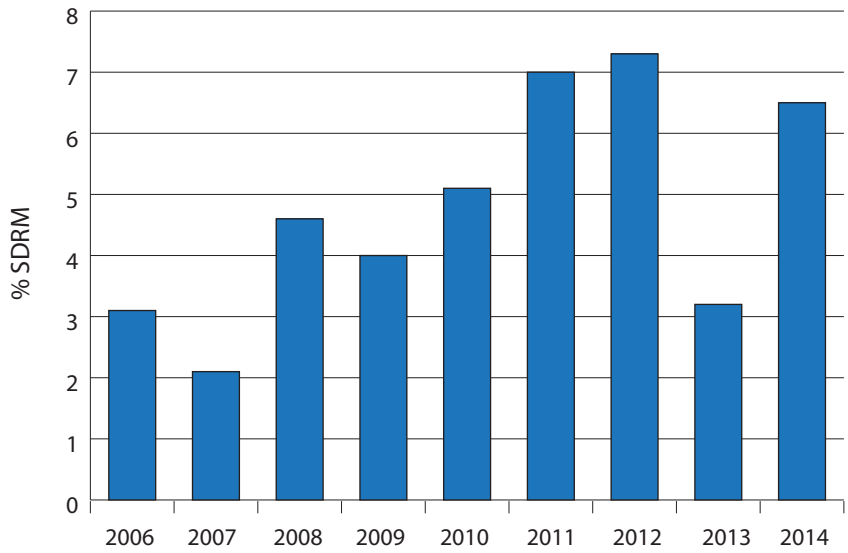

Figure 3. Percentage of analysed sequences with Surveillance Drug Resistance Mutations, (SDRMs) in 2006–2014.

Findings of clinical significance in Norway in 2013–2014

The analysed sample are from patients who had their HIV-1 infection confirmed in Norway and anonymously reported to MSIS during the respective year. A number of these patients are immigrants who were infected before arrival to Norway. Many of these patients were also diagnosed before arrival to Norway, and some have also received treatment in their home countries. Thus, it should be noted that the numbers above showing the frequence of resistance mutations in samples from all patients with newly diagnosed HIV-1 infection in Norway, do not reflect the risk for being infected in Norway with a drug resistant strain of HIV. Among the patients infected in Norway, the corresponding numbers are even lower.

Furthermore, in order to facilitate comparisons of the surveillance data, WHOs standard list of SDRM was used for the monitoring of primary HIV-1 resistance in Norway (appendix A1). The WHO list is designed for surveillance purposes, and does not give information on individual drugs, nor does it take into account the genetic barrier of a drug, and the presence of mutations from this list does not imply resistance of clinical significance. Therefore, the numbers above does not necessarily translate into the number of newly diagnosed patients with clinical drug resistance. They represent surveillance data, and should not be used for recommendations and clinical practice.

In 2013 and 2014, SDRMs from the WHO list were detected in 3% and 6% of the analysed sequences, respectively. The SDRMs detected in the Norwegian material is shown in table 2 and 3. All four mutations detected in the 2013 samples were of clinical significance, causing high level resistance to efavirenz and nevirapine, which are often used in first line regimens. However, only 6 of the 9 samples from 2014 had drug mutation patterns that would be interpreted as clinically relevant drug resistance, and most of these patients were infected abroad. In conlusion, the risk for being infected with drug resistant HIV in Norway is still very low.

Table 2. Total sequences (n=125) with SDRMs in 2013

SequenceID	NRTI SDRMs	NNRTI SDRMs	PI SDRMs
1	None	K103N	None
2	None	V106M	None
3	D67N, K70R, M184V, T215F, K219E	K103N	None
4	T215S	K103N	None

Table 3. Total sequences (n=139) with SDRMs in 2014

SequenceID	NRTI SDRMs	NNRTI SDRMs	PI SDRMs
1	**D67N, K219Q**	**None**	**M46L**
2	None	None	**M46L**
3	**M184V, T215F**	**K103N, Y181C**	None
4	D67G, M184V	G190A	None
5	**T215D**	**L100I, K103N**	**V32I, I47V, F53L**
6	**M184V**	None	None
7	None	**K103N**	None
8	None	**G190E**	None
9	None	None	**F53Y**

Conclusions

In recent years, a large number of drugs have been developed to control HIV replication. This has dramatically improved both the patients' quality of life and their life expectancy. However, the treatment is very demanding, with a lifelong therapy and risk of serious side effects. Furthermore, if the drug regimen is not properly followed by the patient, there is a considerable risk of development of drug resistant viruses. The patient's health could deteriorate and there is a risk of spreading of resistant virus into the community. Resistance mutations was detected in between 2,1% and 7,3% of the sequences from the newly diagnosed HIV patients in 2006–2014. Surveillance of HIV resistance is important to be able to make decisions on implementing preventive measures to control dissemination of resistant HIV strains.

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Hepatitis B virus

Hepatitis B is a potentially life-threatening liver infection caused by the hepatitis B virus (HBV). It is a DNA virus (about 3 kb) within the *Hepadnaviridae family* that is converted to a highly stable mini-chromosome upon infection in liver cells. Despite the tiny size of its genome HBV is one of the most successful human pathogens. It is a major global health problem and the most serious type of viral hepatitis. It can cause chronic liver disease and puts people at risk of death from cirrhosis of the liver and liver cancer.

Worldwide, an estimated two billion people have been infected with HBV and more than 240 million have chronic liver infections (1). About 600 000 people die every year due to the acute or chronic consequences of hepatitis B. HBV is transmitted between people by direct blood-to-blood contact or contact with semen and vaginal fluid of an infected person. HBV can cause both acute and chronic disease. The likelihood that a HBV-infection becomes chronic depends upon the age at which a person becomes infected. Young children are the most likely to develop chronic infections. Ninety % of infants infected during the first year of life and 30–50% of children infected between one to four years of age develop chronic infections. Twenty-five % of adults who became chronically infected during childhood die from hepatitis B-related liver cancer or cirrhosis, whereas 90% of healthy HBV- infected adults will recover and get completely rid of the virus within six months.

Norway is generally a low prevalence country (0.5%) (2). The immigrant populations from highly endemic countries have an impact on overall prevalence, as the majority of cases infected with chronic HBV-infections (95%) are immigrants from middle- and high endemic regions infected before they entered Norway. However, further transmission of HBV from the immigrant population is quite limited. Although the mode of transmission is unknown in the majority of cases, it is assumed that almost all have been infected at birth or early in childhood. In recent years around 700 new cases of CHB are notified yearly in Norway (3), and the majority of these cases were among immigrants from Somalia, Afghanistan, Vietnam, Thailand and Eritrea. Among CHB with Norwegian ethnicity 50% are transmitted through sex or drug use, while in the remaining cases the transmission route is unknown.

Development of resistance

The ultimate goal of hepatitis B treatment is to prevent cirrhosis, hepatic failure, and hepatocellular carcinoma (HCC) (4). The nucleos(t)ide analogues (NAs) used in treatment for CHB suppress viral replication by inhibiting the viral polymerase, whereas interferon therapy works by enhancing the host immune response. The clinical benefit is dependent on the ability to maintain sustained suppression of HBV replication and to induce remission of liver disease. Despite recent advances in treatment of CHB using NAs, these approved treatments seldom eradicate the virus with the risk of viral resistance during long-term treatment. There are 8 primary mutations associated with drug resistance and cross-resistance occurs between several of these drugs (Table 1).

Lamivudine, adefovir, emtricitabine, tenofovir, entekavir and telbivudine are antivirals used for treating chronic hepatitis B with various barrier to resistance development. Currently, entecavir or tenofovir disoproxil are recommended as first-line monotherapy, given their antiviral potency and favorable resistance profile. The rates of resistance at 5 years in NA naive patients are <1.5% and 0% for entecavir and tenofovir disoproxil, respectively (4). Treatment response should be regularly monitored by quantification of the virus in blood. Resistance should be identified when there is a viral breakthrough (i.e. increase in viral load) as early as possible before biochemical breakthrough (increased ALT), and ideally identification of the pattern of resistance mutations should be used to adapt therapeutic strategies. Clinical and virological studies have demonstrated the benefit of an early treatment adaptation, as soon as viral load increases.

Table 1: Nucleos(t)ide analogue cross-resistance data for resistant HBV variants

	Cross-resistance data for resistant HBV variants				
HBV-variants (mutations)	Lamivudine	Telbivudine	Entecavir	Adefovir	Tenofovir
Wild type	S	S	S	S	S
M204I	R	R	I	S	S
L180M + M204V	R	R	I	R	I
A181T/V eller N236T	R	R	S	R	R
L180M + M204V/I ± I169T ± M250V	R	R	R	S	S
L180M + M204V/I ± T184G ± S202I/G	R	R	R	S	S

S= sensitive, R= resistance, I = intermediate

Surveillance of HBV drug resistance

Materials & Methods

The NIPH is a national reference laboratory for hepatitis B receiving samples from microbiological laboratories in Norway for confirmation or characterization by alternative or supplementary analysis including antiviral resistance testing. Sequencing of the polymerase gene that covers the mutations that give resistance to the NAs is frequently used for resistance determination. It is the current method of choice at NIPH, although the resistance population must reach 20–30% before it is detectable by this method.

Surveillance of HBV resistance is based on a selection of patients with chronic infection that has been tested for drug resistance in relation to treatment og patients that are genotyped for HBV. The latter patient group was selected because sequence information on antiviral resistance was available as part of a HBV-genotyping (S-gene) analysis previously requested.

Drug resistance surveillance data

In 2013-14 no drug resistance was found (0/321) among patients that were genotyped as part of their patient managment and no information had been given on antiviral treatment (Table 2). HBV-variants with resistance towards NAs were found in 3 of 26 patients tested for HBV-resistance in the same periode.

Conclusion

A recent multi-centre survey in 18 European countries (including Norway) among NA-experienced CHB patients showed that drug resistance was observed in half of the cases genotypically tested for drug resistance (5). Lamivudin monotherapy was still the most frequently used drug and hence associated with the majority of cases of drug resistance development in Europe. Lamivudin-associated drug resistance mutations confer cross-resistance to entecavir, and were also frequently present in patients with entecavir-therapy failure according to this study. Similar data is also observed in Norway among the very few cases of drug resistence tested. However, patients in Norway are given primarily first-line therapy (entecavir and tenofovir disoproxil) that seems to effectively suppress virus replication and limit drug resistance. Since 2010 there is a clear increase in the use of first-line drugs in Norway, whereas the less potent drugs (i.e. lamivudine, adefovir and telbivudine) commonly associated with drug resistance are decreasingly used. Development of drug resistance during treatment of HBV infection thus seems to be a minor problem in Norway for the time being, but very few samples are referred to antiviral susceptibility testing. The presented data supports the use of resistance testing in cases of therapy failure, particularly in patients previously exposed to less potent drugs as lamivudine, adefovir and telbivudine.

Table 2. Surveillance of drug resistance among patients on treatment and among patients where HBV-genotyping has been requested in 2011–14.

HBV–variants resistant to NAs	Among treated patients				Among HBV-genotyped patients			
Year	*2011*	*2012*	*2013*	*2014*	*2011*	*2012*	*2013*	*2014*
Total analysed	14	3	9	17	131	156	185	136
Wild type	11	2	8	15	130	156	185	136
M204I	1a	0	1a	1c	0	0	0	0
L180M + M204V	1b	1a		1c	1d	0	0	
A181T/V eller N236T	1a	0	0	0	0	0	0	0
L180M + M204V/I ± I169T ± M250V	0	0	0	0	0	0	0	0
L180M + M204V/I ± T184G ± S202I/G	0	0	0	0	0	0	0	0

a=entecavir, b=tenofovir, c=lamivudine, d=treatment unknown

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Antiviral drug resistance of human herpes viruses

Effective antiviral treatment exists in four of the eight human herpes viruses, namely Herpes simplex virus (HSV) 1 and 2, Varicella zoster virus (VZV) and Cytomegalovirus (CMV). Aciclovir or its prodrug valaciclovir are the most commonly used drugs for treatment of HSV1-2 and VZV infections, other options are cidofovir and foscarnet that have to be parenterally administered.

The majority of patients treated for CMV infections are given ganciclovir (GCV) or its prodrug valganciclovir (VGCV), and the consumption of these drugs has been increasing steadily. This is due to the fact that several diseases are treated aggressively with immunosuppressive drugs or biologicals. Furthermore, valganciclovir prophylaxis is now commonly used during the first 6-12 months after solid organ transplantation in patients at risk of serious CMV-disease. In the case of ganciclovir resistance, foscarnet (FOS) or cidofovir (CDV) can be given as anti-CMV treatment.

Cytomegalovirus

The Department of Microbiology at Oslo University Hospital-Rikshospitalet, is a national reference laboratory for CMV and the only laboratory performing CMV genotypic resistance testing.

Mutations in the *UL97* gene lead to GCV resistance only, whereas mutations in the *UL54* gene (CMV-DNA-polymerase) may result in resistance to GCV, CDV or FOS. The appearance of UL54 mutations during GCV therapy in the absence of UL97 resistance mutations is uncommon.

Based on parallel phenotypic and genotypic resistance testing, the UL97 GCV-resistance mutations are categorized as moderate, low or insignificant when the increase in GCV ED50 is 5-15x, 2-5x or <2 fold respectively (Lorain & Chou). In blood specimens from 23 patients in 2013 and 21 patients in 2014 tested for genotypic GVC-resistance, 8 patients in 2013 and 7 patients in 2014 were found to harbor CMV-UL97 resistance mutations. In 10 patients only one resistance mutation was detected, whereas in 5 patients two resistance mutations were observed. No UL54 resistance mutations were recorded.

All UL97 resistance mutations detected belonged to the moderate resistance group, the reason may be that they were seen in patients developing treatment failure on longstanding GCV treatment and not as part of a screening program. For treatment of CMV infections that are moderately resistant to GCV an alternative drug i.e. FOS or CDV is recommended. Low grade GCV-resistance is handled by increased GCV dosage.

UL97 mutations detected x no of strains	Fold increase in GCV ED50 (1)
M460V x 2	5-15x
M460I x 1	5-15x
H520Q x 6	5-15x
A549V x2	5-15x
L595S x2	5-15x
L595W x1	5-15x
L595F x3	5-15x
C603W x 3	5-15x

Table 1. GCV-resistance mutations recorded in 2013-2014 and the number of detected mutations are also given.

Herpes simplex virus

Herpes simplex virus 1-2 and Varicella zoster virus infections resistant to acyclovir seem to be a minor problem. Specimens for genotypic aciclovir resistance tests are sent to the Public Health Agency of Sweden (Folkhälsomyndigheten). As seen in Table 2, specimens from only 12 patients were sent for analysis during the last six years and among these, 3 were found to be acyclovir resistant (Lottie Schloss, personal communication).

Conclusion

GCV-resistant CMV-infection is now quite common and is mainly seen in solid organ or hematopoietic stem cell transplant recipients on long term GCV treatment. The recognition of adequate dosing of GCV for reducing the incidence of GCV-resistance has been important. Despite high prevalence of herpes infections and high consumption of ACV, resistant HSV-strains are rarely seen.

References

Lurain NS Chou S. Antiviral drug resistance of human cytomegalovirus. Clin Microbiol Rev 23: 689-712

Table 2. Number of HSV positive specimens analyzed for genotypic acyclovir resistance.

Year	Number of specimens	Number of resistant strains
2009-2010	0	0
2011	4	1 (HSV1)
2012	3	0 (two unsuccessful)
2013	2	1 (HSV1)
2014	3	1 (HSV2)

Abbreviations

ACV	Aciclovir
AIDS	acquired immunodeficiency syndrome, caused by HIV
ART	antiretroviral therapy
CMV	Cytomegalovirus
CHB	chronic hepatitis B infection
DDA	direct acting antiviral drugs
DDD	defines daily dose
ESAR	European Society of Antiviral Resistance
GCV	Ganciclovir
HBV	Hepatitis B virus
HCV	Hepatitis C virus
HIV	Human immunodeficiency virus
HSV	Herpes Simplex virus
MSIS	Meldingssystem for smittsomme sykdommer
NA	nucleoside/nucleotide analogues
NAI	neuraminidase inhibitor
NIPH	Norwegian Institute of Public Health
NNRTI	nonnucleoside RTinhibitor
NRTI	nucleoside RTinhibitor
PCR	polymerase chain reaction
PI	proteaseinhibitor
PFA	Foscavir
RT	reverse transcriptase
RTI	reverse transcriptase inhibitor
SDRM	Surveillance Drug Resistance Mutations
SPREAD	Strategy to Control Spread of HIV Drug Resistance
VZV	Varicella Zoster virus

Appendix A1. List of Surveillance Drug Resistance Mutations,SDRM, recommended by WHO.

HIV-1 RT and Protease Mutations For Drug Resistance Surveillance

NRTI		NNRTI		PI	
Position	Mutation	Position	Mutation	Position	Mutation
M41	L	L100	I	L23	I
K65	R	K101	E, P	L24	I
D67	N, G, E	K103	N,S	D30	N
T69	D, Ins	V106	M, A	V32	I
K70	R, E	V179	F	M46	I
L74	V, I	Y181	C, I, V	LI47	V, A
V75	M, T, A, S	Y188	L, H, C	G48	V, M
F77	L	G190	A, S, E	I50	V, L
Y115	F	P225	H	F53	L, Y
F116	Y	M230	L	I54	V, L, M, A, T, S
Q151	M			G73	S, T, C, A
M184	V, I			L76	V
L210	W			V82	A, T, F, S, C, M,L
T215	Y, F, I, S, C, D, V, E			N83	D
K219	Q, E, N, R			I84	V, A, C
				I85	V
				N88	D, S
				L90	M

The following considerations were used to develop this list of drug resistance mutations*

the mutations should cause or contribute to drug resistance, defined as being present on three or more of five expert lists of drug resistance mutations **.
the mutations should not occur in untreated persons (i.e. they should be nonpolymorphic, and should not occur at highly polymorphic positions.),
the mutation list should be applicable to all group M subtypes, and
the mutation list should be simple, unambiguous, and parsimonious, excluding mutations resulting exceedingly rarely from drug pressure.

*** HIV-1 pretease and reverse transcriptase mutations for drug resistance surveillance,** AIDS 2007, 21:215-223 Shafer R et al.

Drug Resistance Mutations for Surveillance of Transmitted HIV-1 Drug-Resistance: 2009 Update, PLoS One 2009;4:e4724. Bennett DE et al.

**ANRS drug resistance interpretation algorithm (2008.07),HIVdb drug resistance interpretation algorithm (4.3.7), IAS-USA Mutations Associated With Drug Resistance (March/April 2008), Los Alamos National Laboratories HIV Sequence database (2007), or Rega Institute Drug Resistance Interpretation Algorithm (7.1.1).

The prevalence of all protease and RT mutations according to subtype and treatment can be found at http://hivdb.stanford.edu/cgi-bin/MutPrevBySubtypeRx.cgi.

Apendix A2. Surveillance Drug Resistance Mutations, SDRMs present in sequences analysed in Norway 2006-2014.

		2006 n=129	2007 n=95	2008 n=108	2009 n=149	2010 n=95	2011 n=115	2012 N=140	2013 n=125	2014 n=139
NRTI	M184V	1								1
	M41L	1		1			1	1		
	V75A		1							
	T215D/E/I						2	2		
	M41L+ T215D			1	1					
	M41L+T215D+ L210W					1				
NNRTI	G190E									1
	K103N	1			3		3	1	1	1
	K101E			1		1				
	V106M			1					1	
	Y188C			1						
	Y188L							1		
NRTI and NNRTI	K219N+Y181C		1							
	Y181C+G190A						1			
	M184I+K219N, K103N+Y188H					1				
	M184V+K103N+ V106M					1				
	M184V+T125Y+ K103N							1		
	M184V, T215F, K103N, Y181C									1
	D67G, K70R, M184V, T125I, K219E, V106M, Y181C							1		
	D67G M184V, G190A									1
	K65R, M184I V106M, Y181C, G190A							1		
	K70E, M185V K103N							1		
	D67N, K70R, M184V, T215F, K219E K103N								1	
	T215S + K103N								1	
PI	M461L	1								1
	L23I				1					
	F53Y									1
	G73S					1				
	I85V*						1			
NRTI and PI	M41L, D67N, K70R, M184V, T215F, K219Q + I54V,V82A,L90M				1					
	M41L, T215D M46L							1		
	D67N, K219Q, M46L									1
NRTI, NNRTI and PI	T215D, L100I, K103N, V32I, I47V, F53L									1

Appendix B1. Methods for detection of antiviral resistance.

Resistance indicates the virus's ability to multiply in the presence of antiviral agents. Antiviral drugs are targeted against essential steps in the viral life cycle. It may be the viruses' own enzymes such as polymerase or protease, or viral mechanism to penetrate into or out of the host cell. Viruses can develop resistance to these drugs by the occurrence of one or more mutations in genes encoding for the antiviral target protein. The consequence is that the production of new virus particles is no longer inhibited by a drug at a concentration that would normally inhibit the virus.

There are two approaches for the detection of viral resistance, phenotypic testing of the infectious virus in the presence of an antiviral drug, or genotypic testing where mutations associated with antiviral resistance are detected using molecular biology techniques. The genotype describes the composition of nucleotides in the genome, while the phenotype is the functional expression of one or more genotypes in the virus population.

Phenotypic resistance testing is a direct measure of resistance where the virus's ability to replicate in the presence of various concentrations of antiviral drugs is analysed. The virus must first be isolated from the patient in question and then cultured in presence of serial dilutions of the drug. The resistance is analysed for one drug at a time, and the results must be compared with the results from a virus strain sensitive to the analysed drug. Phenotypic methods determine the drug concentration required to inhibit in vitro virus replication in the cell culture by 50%. The concentration is named "inhibitory concentration 50%" (IC50).

One problem with this method is to define the concentration that provides clinically relevant resistance (clinical cut-off level). The method is considered to be the gold standard, but is technically complex, labour- and time-consuming (depending on how fast the virus grows in cell culture), is costly and takes place in a cell culture laboratory (for HIV a biosafety level 3 laboratory is required). Therefore, this is usually not the preferred routine method.

Genotypic resistance testing is an indirect measure of the resistance by which nucleotide mutations, which correlate with resistance to one or more drugs, are detected. Genotypic methods require that the genetic cause of virus resistance has been identified. For interpretation of the genotype a map of known resistance mutations is used. For some medications, a complex interaction between several mutations is causing the resistance. It is common to use a sequencing-based method for the genotypic resistance testing, in which the gene involved in the antiviral activity is sequenced. The method is suitable for routine diagnostics. It requires sophisticated and expensive equipment and interpretation can be complicated, but viral culture is not necessary. It is less expensive in use and faster to perform than phenotypic resistance testing (takes 2–3 days).

Appendix B2. Methods for detection of resistance in influenzavirus.

Influenza resistance tests in Norway are currently performed at the National Influenza Centre at NIPH. The methods used in the laboratory to determine whether the virus isolates can be classified as sensitive or resistant to a drug, are either phenotypic or genotypic. By the phenotypic methods, one can determine the concentration of an antiviral agent that inhibits the virus.

Methods for resistance testing against neuraminidase inhibitors are commonly measuring decrease in neuraminidase enzyme activity with increasing concentration of the pharmaceutically active substance. One may thus determine the IC50, that is the drug concentration which gives 50% inhibition of the viral neuraminidase activity. NAI susceptibility is measured by enzyme inhibition assay. The MUNANA assay determines the sensitivity of influenza viruses to the NA inhibitor compounds, using the substrate MUNANA. MUNANA is a fluorescent substrate 20-(4-methylumbelliferyl)-a-D-N-acetylneuraminic acid (MUN or MUNANA). Cleavage of MUNANA by neuraminidase releases the methylumbelliferone which then fluoresces. The amount of fluorescence therefore directly correlates to the amount of enzyme activity. Any isolate suspected of showing reduced susceptibility in the NA inhibition assay is further characterised by sequencing the NA gene before resistance may be confirmed.

The genotypic methods detect mutations which already are known and have been shown to occur in resistant viruses by analysing gene sequences in specific target areas of the viral genome. Appendix 1 list substitutions in influenza neuraminidase associated with resistance or reduced susceptibility to neuraminidase inhibitors. Genotypic methods require that the genetic cause of virus resistance have been identified. The correlation between the finding of virus mutations and their impact on resistance should be evaluated in studies of the virus. Genotypic methods are used for susceptibility testing for both neuraminidase inhibitors and M2 blockers.

Pyrosequencing is a molecular technique that can be used for the detection and quantitation of neuraminidase inhibitor resistance mutations. This rapid technique can be used both on virus cultures and directly on clinical material which means that it can be used for individual management of severe cases. It has the advantage over alternative methods such as conventional Sanger sequencing which are more time consuming or lack the sensitivity to detect mutations in mixed virus populations.

Pyrosequencing is a real-time DNA sequencing technique which, via a cascade of enzymatic reactions, detects pyrophosphate (PPi) released during DNA synthesis as visible light. The light released is quantitative and enables the rapid generation of sequence information. This is a rapid technique suitable for high throughput surveillance or drug resistance screening, as demonstrated during the emergence of the oseltamivir resistant seasonal influenza A (H1N1) H274Y viruses in Europe in 2007–08 (14).

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