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Sub-optimal hand sanitiser usage in a hospital entrance during an influenza pandemic, New Zealand, August 2009

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The hand hygiene behaviours of the public in response to the current H1N1 influenza pandemic 2009 (or other pandemics) have not previously been described. An observational study was undertaken to examine hand hygiene behaviours by people passing a hand sanitiser station in the foyer of a public hospital in New Zealand in August 2009. Of the 2,941 subjects observed, 449 (18.0%, 95% confidence interval: 16.6, 19.6) used the hand sanitiser. This is a far from optimal result in response to the health promotion initiatives in the setting of a pandemic. These findings suggest the need for more effective health promotion of hand hygiene and also provide baseline measurements for future evaluation of hygiene practices.

New Zealand surveillance and research efforts have described various aspects of the influenza A(H1N1)v pandemic in 2009. This work has covered the descriptive epidemiology of the pandemic [1-3], key epidemiological parameters [4], and characteristics of the virus [5]. However, there has been no analysis to date on the behavioural responses of the public to the pandemic in this country – including in the area of hygiene behaviour. Here we describe an observational study to measure hand sanitiser use at the entrance to the Wellington Regional Hospital in New Zealand (the main hospital in the capital city) in August 2009.

Pandemic influenza intervention recommendations from the World Health Organization state that 'handwashing (...) should be routine for all and strongly encouraged in public health messages; such practices should be facilitated by making hand-hygiene facilities available' [6]. There is strong evidence to indicate that good hand hygiene is effective in reducing the spread of infection [7]. Alcohol-based sanitisers (e.g. Sterigel™) are as effective as hand washing (with soap and water) for not visibly soiled hands [8-10]. The convenience of alcohol-based sanitisers increases hand washing compliance and reduces healthcare-associated infection rates [6,7].

Methods

Starting in July 2009 and continuing to the present (mid-September 2009), Wellington Regional Hospital had a hand sanitiser station placed in the middle of the entrance foyer (approximately 8 m from the entrance). This station included two Sterigel™ pump dispensers positioned at a height of 1 m, an A3 laminated sheet recommending respiratory hygiene and a large banner stating 'please CLEANSE your hands when entering and leaving'. The Capital and Coast District Health Board (CCDHB) responsible for this hospital state that their goal in providing the sanitiser station was to create an environment where public and staff would cleanse their hands going into and out of the hospital.

In this study, people were observed entering and leaving the hospital foyer using the main entrance as the reference point. An initial data set was collected over four hours by two observers (one hour per day for four days), one noting the number of people who passed in and out of the hospital entrance and the other counting those who used the hand sanitiser. This allowed an estimation of the proportion of people who used the hand sanitiser.

A further phase of the study involved observation with the collection of additional demographic data (gender and estimated age-group), direction (entering or leaving), and an assessment on whether the person was a member of the public or hospital staff (identified as wearing a uniform or identity tag). We observed 30 min periods in the morning, midday and afternoon of a single day.

Data were analysed using Microsoft Office Excel 2003 and OpenEpi. Inter-observer variation was measured by two observers individually recording hand sanitiser use and demographics over an additional 30 min observation period. Cohen's kappa scores were then calculated.

Results

Data from all observations showed the proportion of people using hand sanitiser in the foyer of Wellington Regional Hospital was 18.0% (95% confidence interval (CI): 16.6%-19.6%) (Table). Use of hand sanitiser on entering the hospital was significant higher than use when leaving (risk ratio (RR) = 4.8, 95% CI: 2.8 to 8.1). It was also significantly higher for adults than for children and teenagers (Table). However, no difference was identified with regards to gender or time of day.

Comparison of the individual data from the two observers showed variation only in the category of people entering or leaving the hospital. The kappa score for this activity was calculated as 0.84,

indicating high levels of chance-corrected agreement between the two observers.

Discussion

Key findings and interpretation

A level of hand sanitiser use of 18% in a hospital entrance and during an influenza pandemic is clearly far from optimal. Unfortunately there is no comparative data, as hand sanitisers are not routinely promoted to the public in New Zealand hospitals in non-pandemic situations. The fact that no signage for the hand sanitiser was visible to people exiting the hospital may explain the even lower usage rate (5%) for those exiting through this doorway. The reason for higher sanitiser use by adults compared to children and teenagers is not obvious but may reflect the fact that the dispenser is psychologically aimed at adults due to the signage and table height and that adults are more aware of the need for infection control.

Study validity and limitations

This observational study showed that it is feasible to systematically observe hand sanitiser use in a hospital setting (indeed, this is the first such study that we know of). The kappa score of 0.84 indicates it is unlikely there was substantive inter-observer variation.

Nevertheless, the single location and restricted time of data collection mean that the results may not be truly representative of hand-sanitising activity in the hospital, or may not hold external validity for other parts of New Zealand. Also, other opportunities to practice hand hygiene in the hospital setting (e.g. hand sanitisers on some of the wards) may have contributed to the lower proportion of people using the sanitiser in the entrance hall when leaving the hospital. Another issue was a possible Hawthorne effect, as we suspect that some people were aware of being observed and this may have increased sanitiser usage. Finally, it was not possible to reliably distinguish staff from members of the public through observation.

Policy implications

Changes to the design and location of the hand sanitiser station would probably increase compliance. Such measures could include: positioning the station closer to the door, targeting signage and visual promotional material to both inflowing and outflowing traffic, ensuring that prompts are multi-lingual and simple, life-size posters depicting 'model behaviour' (e.g. of a nurse using the sanitiser) and, to encourage even higher compliance, having an official hospital worker present overseeing sanitiser use.

Part of the New Zealand Ministry of Health's response to the pandemic was to increase public awareness in the area of good

Table

Hand sanitiser use in a hospital entrance by activity, gender, age-group and time of day, Wellington Regional Hospital foyer, August 2009

Characteristics	Used hand sanitiser	Passed hand sanitiser		Risk Ratio (95% confidence interval)
	Number	Number	%	
All observations (5.75 hours)	449	2,492	18.0 (95% CI: 16.6-19.66)	
Observation period with additional data collection				
Direction of movement*				
Entering the hospital	90	407	20.1	4.8 (2.8-8.1)
Leaving the hospital	15	324	4.6	Reference (1.0)
Total	105	731	14.4	
Gender**				
Male	43	287	15.0	1.1 (0.7-1.5)
Female	55	386	14.2	Reference (1.0)
Age group**				
Child (<12)	0	14	0.0	
Teenager (12-18)	0	12	0.0	
(Child/Teenager Combined)	(0)	(26)	0.0	Undefined
Adult (>18)	98	647	15.1	Undefined (p=0.031)***
Time of day**				
Morning (08:20-08:50h)	23	179	12.8	1.0 (0.6-1.7)
Mid-day (12:50-13:20h)	46	263	17.5	1.4 (0.9-2.1)
Afternoon (15:55-16:25h)	29	231	12.6	Reference (1.0)
Total**	**98**	**673**	**14.6**	

CI: confidence interval
* Total of 1.75 hours of observation with data excluded from those 'milling around' (i.e. those who had no clear direction of movement) and using the hand sanitiser.
** Total of an additional 1.5 hours of observation with data included from those 'milling around' and using the hand sanitiser.
*** Result was statistically significant (p=0.031) using Fisher exact test, 2-tailed.

hand hygiene practices through a televised mass media campaign. As hand hygiene during a pandemic has not, to our knowledge, been measured before, we cannot draw conclusions on the effectiveness of such media campaigns. Our findings could, however, be used as baseline measurements to allow for future campaign evaluation.

Research implications

Further research, be it observational or interventional, could aim to capture staff versus public activity, eliminate possible Hawthorne effects and capture additional data on children and teenagers. The possible occurrence of 'clustering effects' could also be studied: The observers noticed that people were more likely to stop and sanitise if they saw another person using the hand sanitiser. For the design of more effective hygiene promotional material, an interventional study could be undertaken investigating the effect of depicting authority figures role-modelling appropriate hygiene behaviours in hospital settings.

Members of the Wellington Respiratory and Hand Hygiene Study Group included:
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References

1. Jackson G, Thornley S. Burden of novel influenza A virus (H1N1) in Auckland and Counties Manukau DHBs (July 2009): a capture-recapture analysis. N Z Med J. 2009;122(1301):66-69.
2. Baker MG, Wilson N, Huang QS, Paine S, Lopez L, Bandaranayake D, et al. Pandemic influenza A(H1N1)v in New Zealand: the experience from April to August 2009. Euro Surveill. 2009;14(34):pii=19319. Available online: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=19319
3. Centers for Disease Control and Prevention (CDC). Surveillance for the 2009 pandemic influenza A (H1N1) virus and seasonal influenza viruses - New Zealand, 2009. MMWR Morb Mortal Wkly Rep. 2009;58(33):918-21.
4. Nishiura H, Wilson N, Baker M. Estimating the reproduction number of the novel influenza A virus (H1N1) in a Southern Hemisphere setting: preliminary estimate in New Zealand. N Z Med J. 2009;122(1299):73-7.
5. Huang QS, Lopez LD, McCallum L, Adlam B. Influenza surveillance and immunisation in New Zealand, 1997-2006. Influenza Other Respi Viruses. 2008;2(4):139-45.
6. Bell DM, World Health Organization Writing Group. Non-pharmaceutical interventions for pandemic influenza, national and community measures. Emerg Infect Dis. 2006;12(1):88-94.
7. Collignon PJ, Carnie JA. Infection control and pandemic influenza. Med J Aust. 2006;185 (10 Suppl):S54-7.
8. Fendler EJ, Ali Y, Hammond BS, Lyons MK, Kelley MB, Vowell NA. The impact of alcohol hand sanitizer use on infection rates in an extended care facility. Am J Infect Control. 2002;30(4):226-33.
9. Boyce JM. Using alcohol for hand antisepsis: dispelling old myths. Infect Control Hosp Epidemiol. 2000;21(7):438-41.
10. Widmer AF. Replace hand washing with use of a waterless alcohol hand rub? Clin Infect Dis. 2000;31(1):136-43.

Economic consequences to society of pandemic H1N1 influenza 2009 – preliminary results for Sweden

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Experiments using a microsimulation platform show that vaccination against pandemic H1N1 influenza is highly cost-effective. Swedish society may reduce the costs of pandemic by about SEK 2.5 billion (approximately EUR 250 million) if at least 60 per cent of the population is vaccinated, even if costs related to death cases are excluded. The cost reduction primarily results from reduced absenteeism. These results are preliminary and based on comprehensive assumptions about the infectiousness and morbidity of the pandemic, which are uncertain in the current situation.

Introduction

In cooperation with the epidemiological unit at the Swedish National Board of Health and Welfare, researchers at the Swedish Institute for Infectious Disease Control and the Royal Institute of Technology micro-modelled the effects of a possible future scenario of an outbreak of pandemic H1N1 influenza in Sweden, projected for the autumn of 2009. An executable simulation model [1] was used together with registry data from Statistics Sweden (Statistiska centralbyrån, SCB) [2] to link the entire Swedish population together in a large spatially explicit social network. The overall aim of developing the model has been to allow for the simulation of the spread of infection in a population in a realistic manner, and examine the effects of applying different policy strategies. Individuals in the stochastic model go to kindergarten, schools, work, healthcare facilities, and travel to places where they may be exposed to the risk of infection. Since all places have explicit coordinates, the geographical spread can be studied.

Method

The simulations were run with the following assumptions (see detailed description in the Annex at the end of the article): The outbreak of pandemic influenza in Sweden starts on 1 September 2009, and is mild. The infection rate produces an R0-value of approximately 1.4, but here only cases from the first waves of the epidemic (first 180 days) and not from the whole outbreak are reported. Children and adolescents are assumed to be more susceptible and more infectious than adults. For all ages, the following allocation of morbidity holds: 16% are asymptomatically ill (i.e. show no symptoms), 34% are mildly ill, 40% display a typical illness, while 10% have a severe form of illness. The latter category includes patients referred to specialised care at a hospital, which does not necessarily entail hospitalisation. One adult in the household stays home from work for as many days as a child younger than 12 years is sick.

The 90% coverage scenario amounts to mass vaccination, since 10% of the population are assumed to be impossible to vaccinate. Each simulation covered 180 days and began with 50 randomly selected individuals infected on day 0. Each scenario was simulated five (or ten for the most likely scenarios of 50%, 60%, or 70% vaccination coverage) times with different random seeds to obtain

Table

Distribution of the level of immunity. Simulation of pandemic H1N1 influenza in Sweden.

Level of immunity after dose 1	Proportion of vaccinated	Proportion of individuals with 40% immunity after dose 2	Proportion of individuals with 60% immunity after dose 2	Proportion of individuals with 80% immunity after dose 2	Proportion of individuals with 100% immunity after dose 2
100%	15%				100%
80%	20%			40%	60%
60%	25%		40%	40%	20%
40%	20%	10%	40%	35%	15%
30%	15%	40%	35%	25%	0%
10%	5%	40%	35%	25%	0%

robust results and to examine variability. Vaccination started after 30 days (on 1 October). The doses were delivered weekly at a rate that gave all people time to be vaccinated with two doses over 14 weeks. For immunity, the following assumptions were made: Dose 1 gives partial immunity, which is then increased through the second dose (Table 1). For example, an individual who after the first dose gained 40% immunity (i.e. risk of getting the infection reduced by 40%) will after the second dose stand a 10% chance of staying at the same level, a 40% chance of increasing the immunity to 60%, a 35% chance of reaching 80% immunity, and finally a 15% chance of obtaining full immunity (i.e. being no longer susceptible). If a vaccinated individual is infected, the disease will be milder and the infectivity lower than that of an unvaccinated individual.

To compare the societal costs of the six scenarios, the following cost estimations — obtained from health economists at the Swedish Ministry of Health and Social Affairs — were used:

- Cost of one-day absence from work per employee: SEK 2,000 (this includes average daily salary of SEK 1,500 and secondary costs (taxes, overhead) of SEK 500).
- Cost of treatment by a doctor in primary care: SEK 2,000.
- Cost of one-day inpatient care: SEK 8,000.
- Cost of vaccine and administration of vaccination per person: SEK 300.

For all scenarios, the SEK 300 vaccine costs are based on the assumption that the entire population is vaccinated (a total of 18 million doses), split evenly between vaccine cost and vaccine administration. This means that no savings on vaccine administration are attributed to a lower number of vaccinated than 90%. The model presupposes absent workers to take care of sick children, and thus the event of sick children does not produce the SEK 2,000 cost in a family where a parent is already ill. The inpatient care does not include expensive specialist care, but is based on the average cost of one day in inpatient care (SEK 8,119, according to figures from 2007, obtained from: http://sjvdata.skl.se).

Direct costs related to death cases are considered, using the figure of SEK 22 million per deceased (as employed by the Swedish Institute for Transport and Communications Analysis), but the case fatality rate (CFR) is hard to assess. Since the CFR for pandemic H1N1 influenza is still unknown [3], one way to proceed is to use a best estimate. Three scenarios were used for the present analysis, motivated by the early figures from New Zealand: 0.005%, 0.010%, and 0.050%. The first of these is considered the most likely scenario [4]. A similar cost assessment could be made regarding those suffering permanent health damages from the disease, but this is not reported here. Finally, neither deaths resulting from vaccination, nor import infections (i.e. cases of infected individuals travelling to Sweden from abroad) have been included in the model.

Results

For the scenario in which no policy interventions are made, the outbreak reaches its peak in weeks 16-20 in the five simulations run, each with over 100,000 newly infected in that peak week (Figure 1). More than a third of the individuals were infected at home (Figure 2). The neighbourhood is an aggregate of all contacts in geographical and social proximity, outside the home. That schools play a relatively important role in spreading a new infection is in

Figure 1

The number of infected persons per week during five simulations of the scenario without intervention. Estimation of costs of pandemic H1N1 influenza 2009 for Sweden.

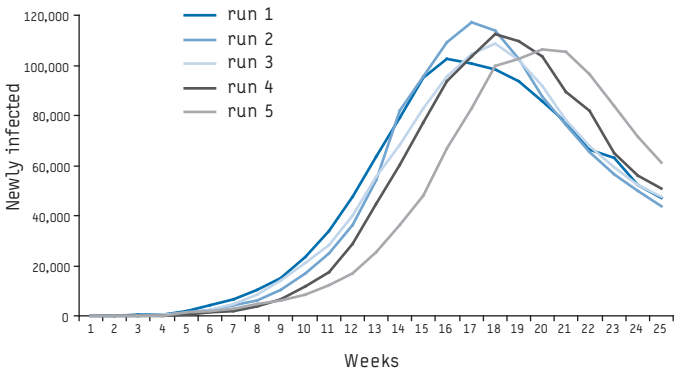
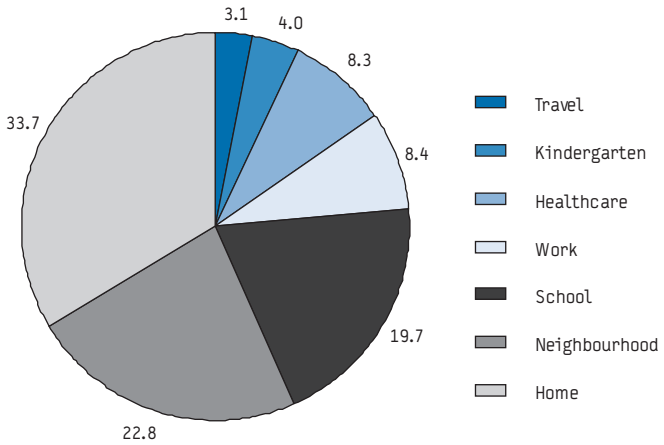


Figure 2

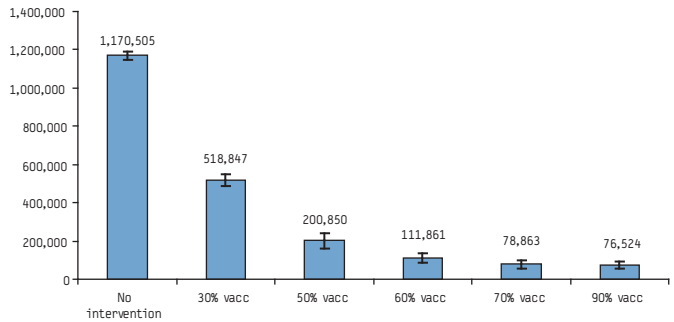
The place distribution of infected individuals, for the scenario without intervention. Estimation of costs of pandemic H1N1 influenza 2009 for Sweden.



Note: Numbers denote percentages, averaged over the five runs of this particular scenario.

Figure 3

The total number of infected individuals (y axis), for all runs. The error bars indicate one standard deviation of uncertainty. Estimation of costs of pandemic H1N1 influenza 2009 for Sweden.



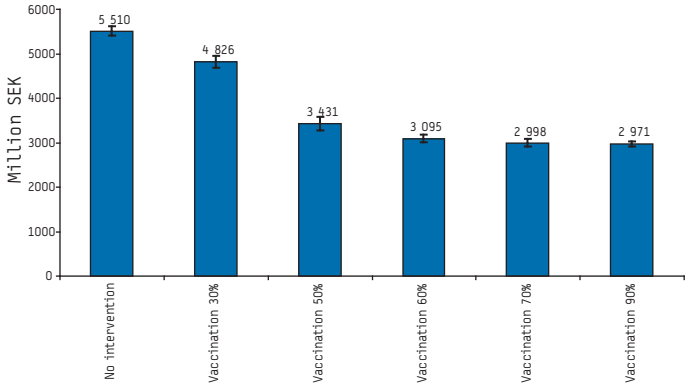
part a result of the assumption of increased infectiousness in the young population.

In Figure 3, the total numbers of infected individuals are presented, for all runs. The age distribution is not presented here, but is largely consistent with reports from actual spread, with an overrepresentation of the youngest and an underrepresentation of the oldest individuals.

The societal costs have been computed for four levels of CFR, including a baseline zero risk scenario depicted in Figure 4 (total costs) and Figure 5 (costs broken down into five categories). The two figures do not include the vaccine cost for the baseline scenario, even though it should be noted that Sweden has already ordered 18 million doses, putting the baseline scenario out of step with the actual fact. This fact notwithstanding, the scenario without interventions proves the most costly, and Figure 5 makes it evident that the mounting costs related to sick leave is the dominating factor. Including costs related to death cases provides even more evidence for the preliminary result that a vaccination level of at least 60% should be recommended (Figure 6). Figure 7 provides a simple sensitivity analysis, where the cost related to the deceased become the major cost as the most plausible CFR (0.005% of infected individuals) is increased by a factor of ten.

FIGURE 4

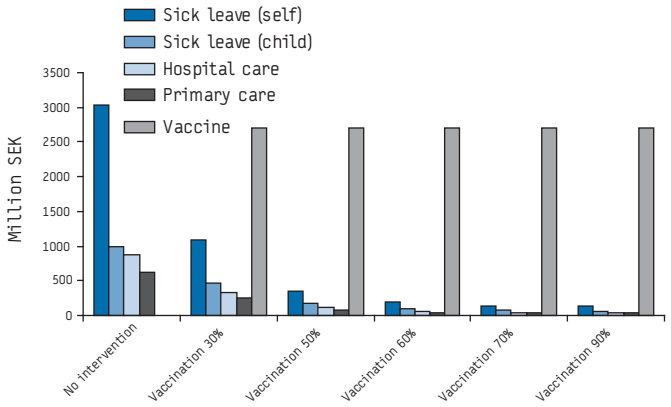
Total costs for the six scenarios, averaged over all runs. Estimation of costs of pandemic H1N1 influenza 2009 for Sweden.



Note: Case fatality rate is here set to zero. The "no intervention" scenario acts as a base line with zero cost for vaccination, while the other five scenarios all have actual cost for vaccination (SEK 2.7 billion). Error bars denote the standard error of the mean.

FIGURE 5

Detailed costs for the six scenarios. Estimation of costs of pandemic H1N1 influenza 2009 for Sweden.



Note: Case fatality rate is set to zero.

Discussion

There are many reasons to be careful when interpreting the results of these simulation experiments, since the assumptions made might not reflect the actual characteristics of the current pandemic. However, as the effects of the pandemic are being assessed,,new assumptions and new sensitivity analyses can relatively easily be made, following the same methodology as described here. And, we believe, that the overall conclusion stands, namely that given an outbreak of pandemic H1N1 influenza of the size contemplated here, vaccinating at least 60% of the Swedish population is recommended, from an economic perspective. When the actual doses arrive in Sweden, they will be distributed among the counties based on county population: the more people, the more doses. In Sweden, vaccination will be voluntary, but for the purpose of these simulation experiments it was assumed, somewhat unrealistically [5], that everyone offered vaccination will accept it. A recent survey, conducted on behalf of the National Board of Health and Welfare, on attitudes towards vaccination in Sweden, found a 72% willingness-to-vaccinate. The survey was conducted between July 27 and August 23, and consisted of 2,000 interviews.

The time to reach the peak of an outbreak in these simulation experiments was more than two weeks longer than what has been reported for the actual outbreaks in the southern hemisphere. This is likely to favour immunisation. Our hypothesis is that the relatively rapid, especially in view of the R0 values reported, peaks in Australia and New Zealand could be explained by the earliest cases going unrecognised, and a constant influx of new cases from abroad. In the model presented here, all cases are recognised, including the earliest asymptomatic cases, pushing back the start date of the epidemic. The fact that cases from abroad were not included can to some extent be justified by the relatively small number of people travelling to Sweden in the early fall.

A recent study [6] suggests that vaccinating school children and their parents leads to a reduction of spread, in large part thanks to herd immunity [7]. The MicroSim model is highly suitable for investigating the efficiency of such policies, since the social network allows for identifying the parents, and a replication study is under way.

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Figure 6

Detailed costs for the six scenarios, including the costs related to the deceased, where the CFR is set to 0.005%. Estimation of costs of pandemic H1N1 influenza 2009 for Sweden.

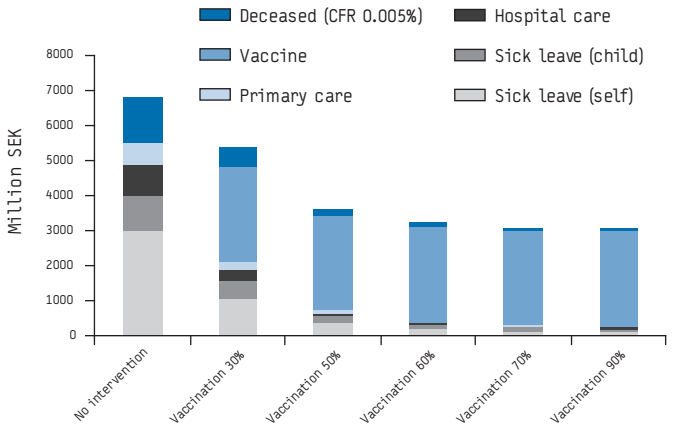
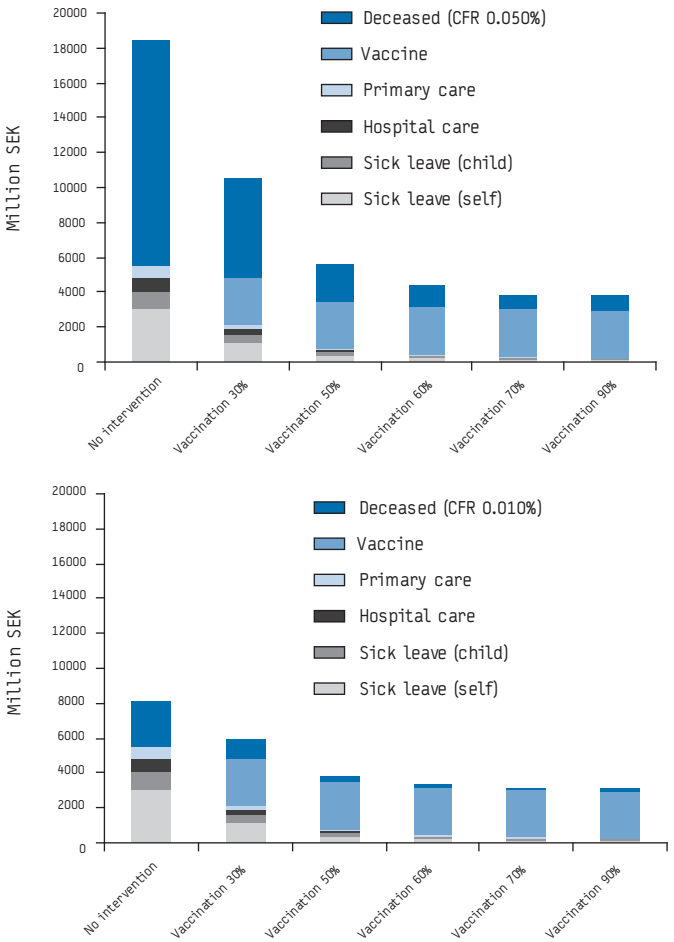


Figure 7

Detailed costs for the six scenarios, including the costs related to the deceased, where the CFR is set to 0.050% (top) and 0.010% (bottom). Estimation of costs of pandemic H1N1 influenza 2009 for Sweden.



References

1. Brouwers L, Camitz M, Cakici B, Mäkilä K, Saretok P. MicroSim: modeling the Swedish population. arXiv:0902.0901. Available from: http://arxiv.org/abs/0902.0901
2. Statistics Sweden (Statistiska centralbyrån, SCB). Homepage on the internet: http://www.scb.se
3. Garske T, Legrand J, Donnelly CA, Ward H, Cauchemez S, Fraser C, et al. Assessing the severity of the novel influenza A/H1N1 pandemic. BMJ. 2009;339:b2840.
4. Baker MG, Wilson N, Huang QS, Paine S, Lopez L, Bandaranayake D, et al. Pandemic influenza A(H1N1)v in New Zealand: the experience from April to August 2009. Euro Surveill. 2009;14(34):pii=19319. Available from: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=19319
5. Chor JS, Ngai KL, Goggins WB, Wong MC, Wong SY, Lee N, et al. Willingness of Hong Kong healthcare workers to accept pre-pandemic influenza vaccination at different WHO alert levels: two questionnaire surveys. BMJ. 2009 Aug 25;339:b3391.
6. Medlock J, Galvani A P. Optimizing influenza vaccine distribution. Science. 2009 Aug 20. [Epub ahead of print].
7. Fine PE. Herd immunity: history, theory, practice. Epidemiol Rev. 1993;15(2):265-302.
8. Carrat F, Luong J, Lao H, Sallé AV, Lajaunie C, Wackernagel H. A 'small-world-like' model for comparing interventions aimed at preventing and controlling influenza pandemics. BMC Med. 2006;4:26.
9. Ferguson NM, Cummings DA, Cauchemez S, Fraser C, Riley S, Meeyai A, et al. Strategies for containing an emerging influenza pandemic in Southeast Asia. Nature. 2005;437(7056):209-14.
10. Moser MR, Bender TR, Margolis HS, Noble GR, Kendal AP, Ritter DG. An outbreak of influenza aboard a commercial airliner. Am J Epidemiol. 1979;110(1):1-6.
11. Swedish Association of Local Authorities and Regions (SALAR). Sjukvårdsdata i focus [Healthcare data in focus]. 2006. Available from: http://sjvdata.skl.se/sif/start/
12. Swedish Social Insurance Agency (Försäkringskassan). Homepage on the internet: http://www.forsakringskassan.se
13. Swedish Institute for Transport and Communications Analysis (Statens Institut för Kommunikationsanalys SIKA). RES 2001. Den nationella reseundersökningen [National Travel Survey]. Stockholm: Birger Gustafsson AB; 2002. Available from: http://www.sika-institute.se/Doclib/Import/100/ss2002_2.pdf

Annex: Assumptions prior to the experiment

1. Introduction of infection

On the first day of simulation, 50 individuals are randomly selected to be the initially infected.

$$R_0 = \frac{-\ln\left(\frac{A}{B}\right)}{1-\frac{A}{B}}$$

2. R0 value

R0 is defined as the average number of individuals a typical person infects under his/her full infectious period, in a fully susceptible population. Here parameter values were used that, on average, cause outbreaks with R0-value 1.4. This value was calculated using the following formula:

B: Total number of susceptible individuals before the outbreak

A: Total number of susceptible individuals after the outbreak

Note that 7,978,105 out of 8,861,388 individuals in Sweden belong to the giant component, that is to say, they are connected to the social contact network. We use this lower value instead of the total population for the "susceptible before" value in the calibrations in order to avoid overestimating the infectiousness.

To reach the required R0-value, we adjusted the amplitude of the epidemic profiles. We used a factor 0.997 as the escape probability to obtain the required R0-value (4,000,080 infections).

3. Infectiousness profiles

We use different infectiousness profiles for different disease severities. Additionally, we assume that children are both more infectious and more susceptible. The infectiousness is the risk of transmission through personal contact, i.e. when an infectious and a susceptible person meet (during a period of eight hours). See Annex Figures 1 through 4 below for the corresponding profile graphs.

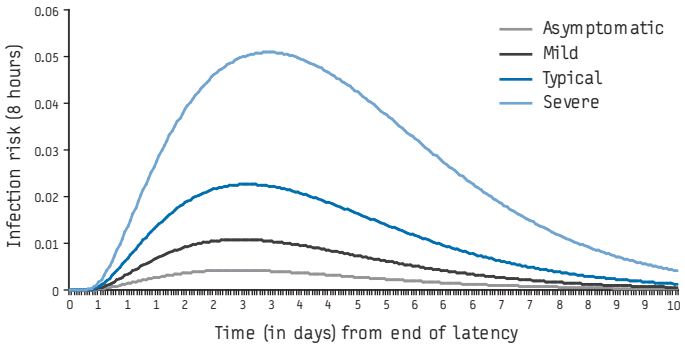
The infectiousness profiles are adapted from Carrat et al. [8], where a static latency period is included. We chose to remove this latency period from the Carrat profiles and instead introduced a varying latency period (12 to 60 hours), generated from a Weibull distribution with scale parameters 1.1 and 2.21 [9,10].

4. Disease profiles

In the experiments, all infected individuals are assigned a certain disease profile with the following proportion: asymptomatic (16%), mild (34%), typical (40%) and serious (10%). The infected individuals display different levels of illness depending on their disease profile (Annex Figure 5).

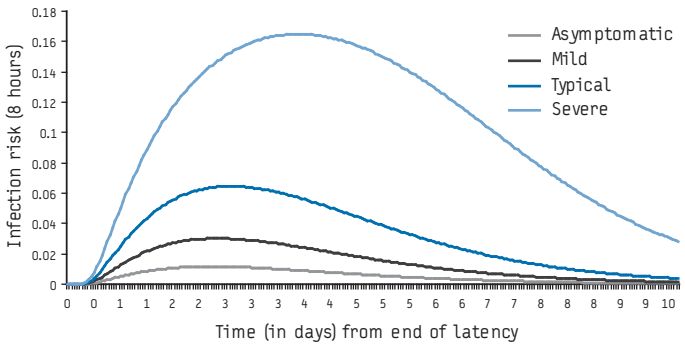
Annex: Figure 1

Infectiousness profiles adult -> adult



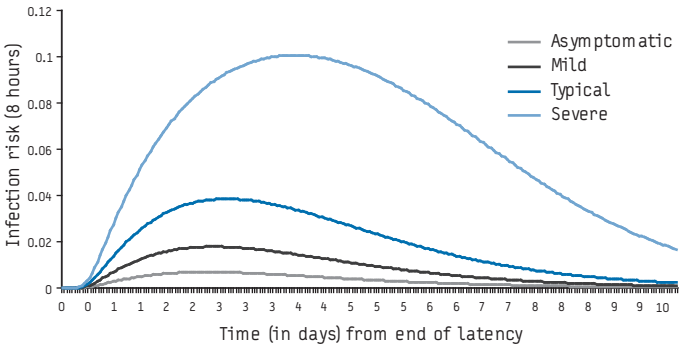
Annex: Figure 2

Infectiousness profiles child -> child



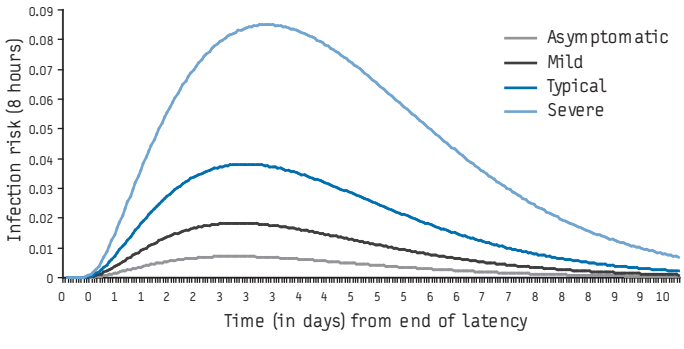
Annex: Figure 3

Infectiousness profiles child -> adult



Annex: Figure 4

Infectiousness profiles adult -> child



The number of deaths was calculated externally, after the simulations, due to the uncertainty of case fatality rates. We multiplied the number of infected individuals by the CFR 0.005% estimated in another study [4].

5. Choice of place according to disease level

Depending on their disease level, the individuals spend their day in different settings (Annex Figure 6). The choice of place is determined randomly. Persons with the same disease level can spend the day in different settings: one stays at home from work, another is at work, and a third person visits the emergency room. Disease level 0 represents all individuals who are not infected, as well as those infected without symptoms.

Settings in the model extracted from register data

By using different SCB (Statistics Sweden) register data [2] individuals have been linked to their workplaces and their residences. Individuals are also linked together in their families.

In the model, each person object contains the family identifier, birth year, gender, coordinates for the family residence (indicated at the level of 100 x 100 meter squares), and workplace identifier. Workplace representations include the workplace identifier, county, and coordinates of the workplace. The workplace identification number is used to connect the person and the workplace. Place objects include a list of members; for residences this list contains the family members and for workplaces it contains employed individuals.

Unit size

We have decided on a maximum number of persons, x, to belong to any one unit. This means that an individual is in close contact with a maximum of x other individuals at his/her workplace, school, nursery centre, etc.

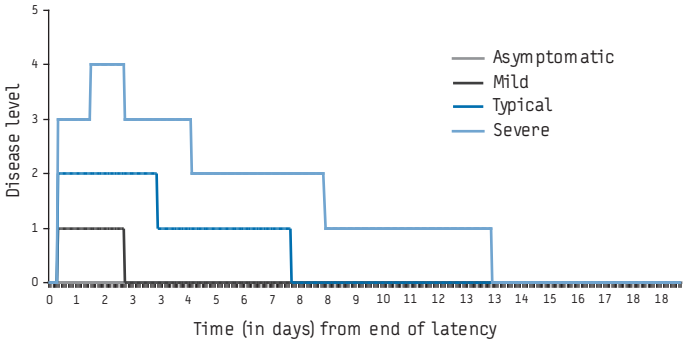
At large places, it is also possible to transmit infection between units.

Since the individuals in the model lack memory, it is possible for them to visit primary care one day, go to work the next day and visit primary care again on the third day. To avoid this issue, we created a place choice rule to limit emergency room visits to one.

The number of visits to emergency rooms and primary treatment are based on information gathered by the Swedish Association of Local Authorities and Regions (SALAR) in 2006 [11]. This database is also the source of the costs for 24 hours of inpatient care, as noted in the paper.

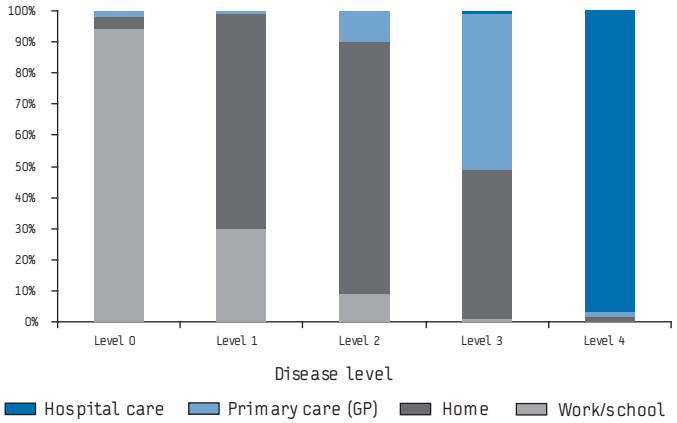
Annex: Figure 5

Disease levels



Annex: Figure 6

Place distribution by disease level



Annex: Table 1

Maximum size of places

Type of place	Maximum size of unit/group
Kindergarten	no unit division
School	25
Office	25
Emergency room	no unit division
Infectious diseases clinic	no unit division

Annex: Table 2

Number of outpatient visits

Visits to general practitioners (excluding antenatal and paediatric care)	25,238,500
All other visits (including day care treatment)	34,131,400
Total:	59,369,900
Per day:	162,657

Annex: Table 3

Number of infected individuals

Scenario	Number of infected	Standard deviation	Number of runs
No interventions	1,170,505	45,345	5
Vaccination coverage 30%	518,847	63,742	5
Vaccination coverage 50%	200,850	40,653	10
Vaccination coverage 60%	111,861	52,219	10
Vaccination coverage 70%	78,863	45,586	10
Vaccination coverage 90%	76,524	37,307	5

In the model, the daily risk of visiting primary care (for an individual with disease level 0) has been determined to be 0.0184 (162,657/8,860,000).

The estimates of daily probability of staying home from work due to illness or for other reasons at disease level 0 are based on data from SCB [2] and the Swedish Social Insurance Agency [12]. The absence, as indicated in the data, varies over time depending on changes in compensation levels and regulations. We use 4%, a relatively low level, for the current model.

6. Ad hoc contacts

In addition to contacts within the social network, we include two additional place types to represent ad hoc contacts: neighbourhood and travel. Neighbourhood infections are used to represent infections in an individual's geographical vicinity, while travel indicates infectious spread between Sweden's 81 regions.

Neighbourhood

Infection transmission in the neighbourhood occurs in two steps for each region:

1) Calculate the total number of new infections for each region:

N = Current number of infected in region

C = Number of contacts (=10, for the current model)

R = Risk of infection: the mean value of the four disease profiles

$$I = N \times C \times R$$

The number of individuals infected in the neighbourhood decreases over time, as described by multiplying the right-hand side of the above equation by the fraction S/T, where S is the number of susceptible individuals and T is the total number of individuals.

2) Choose the individuals to be infected

We pick an infectious person at random from the list of infectious individuals in the region, and search for a susceptible person within a radius of 15km to infect. If no susceptible individuals are found, we increase the radius and try again.

Travel

The daily number of travellers from one region to another has been estimated using statistics about travel [13]. This number is used to calculate the new infections that will occur as a result of infected individuals travelling within the country.

7. Vaccine availability

We assume that 346 boxes of vaccine arrive in Sweden every week. Each box contains eight cases, and each case contains 500 doses. Vaccination can be initiated three days after the boxes' arrival. One to two days are needed to administer 346x8x500 doses of vaccine. After 14 weeks we will have received 19 million doses, which is enough to vaccinate the entire population using two doses for each individual.

8. Total number of infected individuals

The table below presents the total number of infected individuals, averaged over all 180 day runs, for the six scenarios, with their standard deviations (Figure 3 in the article above).

Multidrug-resistant Neisseria gonorrhoeae with reduced cefotaxime susceptibility is increasingly common in men who have sex with men, Amsterdam, the Netherlands

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Antimicrobial resistance is an increasing problem in *Neisseria gonorrhoeae* (NG) treatment. Presently, third-generation parenteral cephalosporins, like ceftriaxone and cefotaxime, are the first option. Resistance to oral, but not to parenteral, third-generation cephalosporins has been reported previously. We analysed the microbial susceptibility (as minimum inhibitory concentration - MIC) of NG cultures obtained from high-risk visitors of the largest Dutch outpatient clinic for sexually transmitted infections (STI) in Amsterdam, the Netherlands. Among 1,596 visitors, we identified 102 patients with at least one NG isolate with reduced susceptibility to cefotaxime (0.125 µg/ml < MIC ≤ 0.5 µg/ml). The percentage of NG isolates with reduced susceptibility to cefotaxime rose from 4.8% in 2006 to 12.1% in 2008 (chi^2 17.5, p<0.001). With multivariate logistic regression, being a man who has sex with men (MSM) was significantly associated with reduced susceptibility to cefotaxime (p<0.001). Compared to susceptible NG isolates, those with decreased susceptiblity to cefotaxime were more often resistant also to penicillin (16.5% vs. 43.3%), tetracycline (21.5% vs. 68.9%) and ciprofloxacin (44.4% vs. 90.0%, all p<0.001). The increased prevalence of NG strains with reduced susceptibility to cefotaxime among MSM may herald resistance to third-generation parenteral cephalosporins. A considerable proportion of these strains show resistance to multiple antibiotics which could limit future NG treatment options.

Introduction

Gonorrhoea is a highly contagious sexually transmitted infection caused by *Neisseria gonorrhoeae* (NG). In the majority of cases NG urogenital infections in males cause symptoms like discharge or urethritis whereas anal and pharyngeal NG infections and urogenital NG infections in females are asymptomatic in a large proportion of cases. Uncomplicated urogenital NG infections can lead to salpingitis in females and epididymitis in males, conditions that are associated with infertility. In some cases localised NG infections can lead to haematogenic dissemination causing severe complications like sepsis, meningitis and endocarditis [1].

Previously we reported a rise in the proportion of fluoroquinolone-resistant *N. gonorrhoeae* (FRNG) isolates among NG isolates obtained from men who have sex with men (MSM) visiting the Amsterdam clinic for sexually transmitted infections (STI) from 0.2% in 2000 to 10.5% in 2003 and among those obtained from men who have sex with women from 0.7% to 3.2%, respectively [2]. A year later, in 2004, a prevalence of FRNG up to 15% was found among heterosexual visitors of STI clinics [3], and in 2008 the proportion of FRNG has risen to 45% among STI clinic visitors throughout the Netherlands [4].

Similar increases in circulating FRNG isolates among STI visitors were documented earlier in the United Kingdom around 2000 [5], and during the 1990s throughout Asia [6]. As soon as the prevalence of antibiotic–resistant strains of a circulating pathogen in a patient population exceeds 5%, both the Centers for Disease Control and Prevention (CDC) and the World Health Organization (WHO) recommend to stop using this antibiotic for treatment of patients infected with this pathogen [1,6]. Therefore since 2004 fluoroquinolones have no longer been recommended as first line treatment for NG in the Netherlands and in many other countries. In the national clinical guidelines issued by the Dutch Dermatological and Venereological Society (Nederlandse Vereniging voor Dermatologie en Venereologie, NVDV) and the Dutch General Practitioners Society (Nederlands Huisartsen Genootschap, NHG), both parenteral third-generation cephalosporins - ceftriaxone and cefotaxime - are the first line treatment option for patients infected with NG [7]. Before fluoroquinolones were abandoned as the first treatment option, penicillin and tetracycline had already been discontinued as preferred treatment option for NG infections due

to unacceptable high prevalence of circulating NG strains resistant to these antibiotics [6].

In collaboration with the Municipal Health Service Public Health Laboratory, we have been closely monitoring the resistance to antibiotics of NG isolates found in the visitors to the STI clinic in Amsterdam. In addition to penicillin (both chromosomal and plasmid mediated resistance), tetracycline and ciprofloxacin, since 2004 we have also monitored the resistance to cefotaxime.

In this article we report an alarming increase in the proportion of multidrug-resistant NG strains with reduced susceptibility to cefotaxime among isolates obtained from visitors frequenting the Amsterdam STI outpatient clinic in 2008 compared to 2006-2007. These NG strains with reduced susceptibility to cefotaxime are found for the larger part among MSM with high-risk behaviour for other STI's. Evolvement of true resistance to third-generation cephalosporins would seriously hamper effective control of NG infections.

Methods

Time frame and study population

The Amsterdam STI outpatient clinic is the largest setting of its kind in the Netherlands, with nearly 28,000 new consultations in 2008 [8]. Upon arrival at the clinic, visitors are prioritised based on a short questionnaire to estimate the risk for having an STI. The prioritising system is described in more detail elsewhere by Heijman et al. [9]. In short, all visitors that are either referred by a healthcare professional, have a sex partner with an STI, had STI-related complaints, or are MSM, are considered high-risk patients and get a full STI check-up including, for the largest part, the collection of swabs for NG cultivation from the pharynx, urethra, cervix and/or rectum depending on the sex technique practiced in the previous six months. Those with negative answers to the questionnaire, are considered low-risk visitors. From these no NG isolates are available since only a nucleic acid amplification test is used to perform NG diagnostics in this group.

All demographic and clinical characteristics used in this study were recorded in an electronic patient database as described earlier [10]. Patients diagnosed with an NG infection (urogenital, anal or pharyngeal) were treated with 500 mg ceftriaxone i.m. according to the national guidelines of the Dutch Dermatological and Venereological Society (NVDV) [7]. In case symptoms persisted one week after treatment, visitors were requested to return to the clinic and additional swabs for NG cultivation were obtained to see if the treatment had been successful ("test for cure"). Moreover, all visitors were screened for *C. trachomatis* infections (including lymphogranuloma venereum in MSM), syphilis, hepatitis B and upon consent HIV, as described elsewhere [10]. All data and samples for this study were collected as part of the routine clinical procedure; therefore no Ethical Committee approval was needed. Care was provided in accordance with the Helsinki Declaration of 1975, as revised in 1983 [11].

All NG isolates with available MIC information collected between October 2006 and December 2008 from high-risk patients were included in the analysis. We examined whether there was a trend in the proportion of NG isolates with reduced susceptibility per quarter. From patients with more than one isolate with a MIC value, the isolate with the highest MIC value was used in the analysis, for all antibiotics tested. In a sub-analysis we examined whether susceptibility to cefotaxim was associated with the anatomical site from which the isolate was originating. Statistical analysis was performed using SPSS version 15.0 (SPSS, Inc., Chicago, IL, US) and Stata version 9.2 (Stata Corporation, College Station, TX, US). We examined the association between reduced susceptibility to cefotaxime and age, sexual orientation, nationality, previous and current STI diagnoses, including HIV status and result of *Treponema pallidum* haemagglutination (TPHA) test. Factors associated with reduced susceptibility in univariate analysis at $p<0.20$, were included in a logistic regression model. Factors that were not significantly associated with the outcome were one by one omitted from that model (level of significance set at $p=0.05$).

N. gonorrhoeae susceptibility testing

All swabs for NG cultivation were swiped on selective feeder plates (GC-LECT; Becton Dickinson, Franklin Lakes, NJ, United States) as described previously [2]. In short, the plates were incubated immediately at 37 ºC in CO2 enriched atmosphere before and after transportation in "candle jars" to the laboratory. After 40-48 hrs the plates were inspected for colony formation. Determination of NG isolates was based on Gram-staining, oxidase-, sugar fermentation-, and aminopeptidase reactions and hybridisation with a DNA probe (Accuprobe, Biomerieux). In cultured NG isolates, the minimum inhibitory concentration (MIC) of penicillin, tetracycline, ciprofloxacin, and cefotaxime was measured using E-tests (AB Biodisk, Solna, Sweden). Moreover, plasmid-mediated penicillin resistance was tested with the help of a beta-lactamase test.

For MIC validation and quality control, the public health laboratory of the Municipal Health Service Amsterdam participated in the European Surveillance of STI (ESSTI) NG isolate panel exchange collaboration programme in 2008. This panel included WHO strains K and L, which both display a reduced susceptibility to third-generation cephalosporins due to a pen A mosaic allele (K) or an A501 mutation in the penA gene (L) [12]. These strains had an MIC for cefotaxime of 0.5 and 0.25 µg/ml, respectively.

Before 2006, ceftriaxone was not available in the Netherlands in acceptable dosages for treating patients with NG infections. Therefore, cefotaxime was the nationally recommended first treatment option and, consequently, the Dutch health authorities (Rijksinstituut voor Volksgezondheid en Milieu, RIVM) provided cefotaxime tests to NG reference laboratories throughout the country for the monitoring of parenteral third-generation cephalosporin susceptibility. From 2006 onwards, the 500 mg ceftriaxone i.m. dosage became available and was then recommended as the first treatment option for NG infections, but the government continued to provide the cefotaxime susceptibility test. Therefore during the study period we treated NG infections with ceftriaxone while testing susceptibility for cefotaxime. Since structural homologies between both molecules are high, we consider cefotaxime susceptibility an appropriate marker for susceptibility to all third-generation cephalosporins. This was confirmed by our finding that genetically well-described WHO reference strains with diminished susceptibility to cefixime and ceftriaxone had also increased MICs for cefotaxime, whereas all other ESSTI control strains were fully susceptible. According to the guidelines and recommendations of the Clinical and Laboratory Standards Institute (CLSI) the following MIC cut-off values were used to define antibiotic susceptibility [13]:

- For cefotaxime: susceptible ≤0.125 µg/ml; 0.125 µg/ml < reduced susceptibility ≤0.5 µg/ml; resistant >0.5 µg/ml.

- For penicillin: susceptible ≤0.06 µg/ml; 0.06 µg/ml < reduced susceptibility < 2.0 µg/ml; resistant ≥2.0 µg/ml.
- For tetracycline: susceptible ≤0.25; 0.25 µg/ml < reduced susceptibility <2.0 µg/ml; resistant ≥2.0 µg/ml.
- For ciprofloxacin: susceptible ≤0.06 µg/ml; 0.06 µg/ml < reduced susceptibility <1.0 µg/ml; resistant ≥1.0 µg/ml.

Results

From October 2006 until December 2008, gonorrhoea was diagnosed in 1,821 high-risk patients (out of the total number of 35,411 high-risk patients who visited the clinic in this period, which gives 5.1% gonorrhoea prevalence in this group) and in 115 low-risk patients (out of 25,304 low-risk patients in total, which gives 0.5% gonorrhoea prevalence in this group). In 225 of the high-risk patients NG isolates were not available, in most cases because the gonorrhoea diagnosis was based on a nucleic acid amplification test or because the gonorrhoea culture did not grow. From the remaining 1,596 patients a total of 1,883 NG isolates obtained from various locations were available for which MIC testing was performed. We compared the demographic and clinical characteristics of the high-risk patients with MIC information versus those without MIC information. There was no significant difference between the two groups regarding concurrent syphilis (i.e. stage 1, 2 or early latent syphilis) or lymphogranuloma venereum infection at the time of consultation, past syphilis infection (i.e. TPHA seropositivity), HIV seropositivity and age distribution. However, the proportion of MSM was significantly higher in the group without MIC information (p<0.001, chi^2 test).

Among the 1,596 patients with MIC data, we identified 102 with at least one NG isolate with reduced susceptibility to cefotaxime (0.125 µg/ml < MIC <0.5 µg/ml, Table 1). No NG isolates resistant to cefotaxime were identified and isolates obtained from the remaining 1,494 patients were all susceptible to cefotaxime (MIC ≤ 0.125 µg/ml). Between October 2006 and December 2008 an important and significant rise in both absolute and relative terms was observed in the number of patients with NG isolates with reduced cefotaxime susceptibility: from 8 (4.8%) in the fourth quarter of 2006 to 23 (12.1%) in the fourth quarter of 2008 (chi^2 for trend = 17.5, p<0.0001, Figure).

Demographic and clinical characteristics of patients with reduced cefotaxime susceptibility

The following patient characteristics were significantly associated with having an NG isolate with reduced susceptibility to cefotaxime (Table 1): age >35 years (p=0.004), MSM (p<0.001), a concurrent lymphogranuloma venereum infection at the time of consultation (p=0.04), positive HIV serology, either as a new diagnosis or known HIV seropositivity (p=0.023), positive TPHA serology (p=0.01, for all comparisons a chi^2 test was used). In a multivariate logistic regression model, only being MSM was significantly associated with reduced susceptibility to cefotaxime (OR=2.9, 95% CI 1.4-5.8, p<0.001, adjusted for age).

Table 1

Demographic and clinical characteristics of 1,596 patients with at least one *Neisseria gonorrhoeae* isolate; STI outpatient clinic, Amsterdam, the Netherlands, 2006-2008

Patient characteristics	cefotaxime MIC ≤ 0.125 µg/ml (n= 1,494)	cefotaxime MIC > 0.125 µg/ml, (n=102)	OR (95%CI)	Overall p value[1]
Age				
≤35 years	847 (56.7%)	43 (42.2%)	1 (ref)	0.004
>35 years	647 (43.3%)	59 (57.8%)	1.8 (1.2-2.7)	
Sexual preference				
Men who have sex with women (exclusively)	317 (21.2%)	9 (8.8%)	1 (ref)	<0.001
Women who have sex with men	192 (12.9%)	3 (2.9%)	0.6 (0.1-2.1)	
Men who have sex with men (and/or women)	985 (65.9%)	90 (88.2%)	3.2 (1.6-6.5)	
HIV serology				
Positive, new diagnosis	39 (2.6%)	7 (6.9%)	3.1 (1.3-7.3)	0.023
Known positive	337 (22.6%)	30 (29.4%)	1.5 (1.0-2.4)	
Negative	952 (63.7%)	55 (53.9%)	1 (ref)	
Not tested	166 (11.1%)	10 (9.8%)	1.0 (0.5-2.1)	
Concurrent infectious syphilis[2]				
No infectious syphilis	1,441 (96.5%)	101 (99%)	1 (ref)	0.165
Infectious syphilis	53 (3.5%)	1 (1.0%)	0.3 (0.04-1.96)	
Syphilis serology[3]				
TPHA-negative	1,145 (76.9%)	67 (65.7%)	1 (ref)	0.011
TPHA-positive	345 (23.2%)	35 (34.3%)	1.7 (1.1-2.7)	
Concurrent lymphogranuloma venereum				
No lymphogranuloma venereum	1,466 (98.1%)	97 (95.1%)	1 (ref)	0.04
Lymphogranuloma venereum	28 (1.9%)	5 (4.9%)	2.7 (1.02-7.1)	

Data are number of patients (% of total).
1p values were calculated with chi^2 test.
2Infectious syphilis infections are stage 1, 2 or early latent stages diagnosed at the date of visit.
3Data on TPHA (Treponema pallidum haemagglutination) test missing for n=4.

NG isolates from MSM

In total 1,231 isolates with MIC information were obtained from 1,075 MSM patients (Table 2). Of these, 1,134 isolates from 985 patients showed good susceptibility to cefotaxime (MIC ≤ 0.125 µg/ml) and 97 isolates from 90 patients showed decreased cefotaxime susceptibility (MIC >0.125 µg/ml). A considerable number of these isolates originated from rectal location (respectively 534 [47.1%] and 48 [49.5%]). Site of infection was not significantly associated with reduced susceptibility (p=0.61).

We analysed the susceptibility to antibiotics other than cefotaxime of all 1,231 NG isolates from 1,075 MSM patients (Table 3). Isolates with decreased susceptibility to cefotaxime showed significantly more often resistance to penicillin (43.3% vs. 16.5%), tetracycline (68.9% vs. 21.5%) and ciprofloxacin (90.0% vs. 44.4%, for all antibiotics separately p<0.001) compared to NG strains susceptible to cefotaxime.

Moreover, multiple resistance to the additionally tested antibiotics (penicillin, tetracycline and ciprofloxacin) was significantly more frequent among isolates with decreased susceptibility to cefotaxime compared to those susceptible (77.5% and 22.9% for at least two of the three additionally tested antibiotics, respectively, and 30.3% and 4.7% for all three additionally tested antibiotics, both comparisons p<0.001).

Discussion

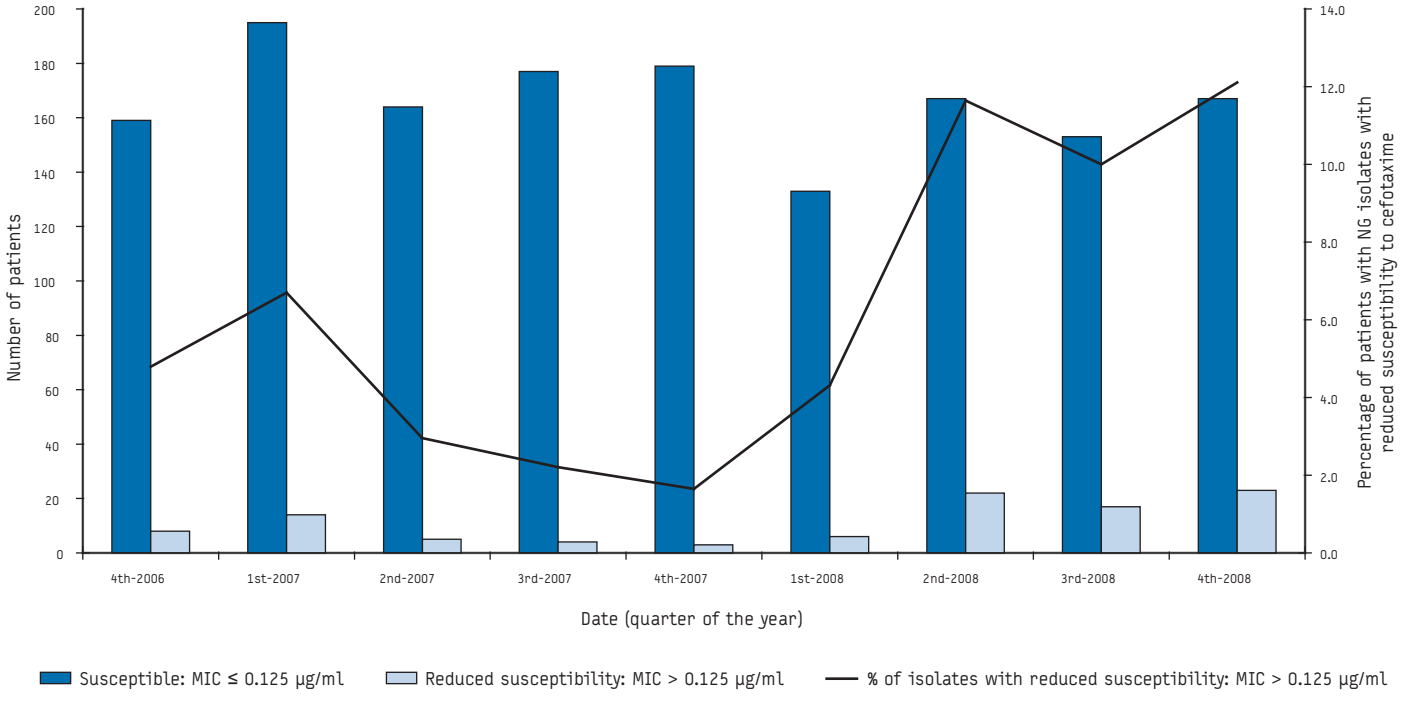
We report on an alarming significant increase of NG isolates with reduced susceptibility for cefotaxime, a parenteral third-generation cephalosporin, among STI clinic visitors in Amsterdam, the Netherlands from 2006 to 2008. Following the resistance to sulfanilamide in the 1940s, penicillin and tetracycline in the 1980s and, lastly, fluorochinolones in the early 1990s, third-generation cephalosporins have nowadays become the first option of treatment in most countries [1,6].

N. gonorrhoeae strains with reduced susceptibility to oral third generation cephalosporins have been described in Japan [14,15], Sweden [16], Australia [17] and Greece [18]. Mosaic patterns of the *penA* gene, encoding penicillin binding protein 2, partly originating from the pharyngeal commensal species *N. cinerea* and *N. perflava* have been reported in such strains [14,16]. In addition, alterations in *mtrR*, resulting in increased expression of efflux pumps, *porB1b*, resulting in altered permeability of the porin *por1B*, and *ponA*, leading to decreased affinity of penicillin binding protein 1 to beta-lactam antibiotics have been reported [16]. In contrast to susceptible strains, these strains cannot always be eradicated using two 200 mg doses of oral cefixime [19]. For this reason, the European Committee on Antimicrobial Susceptibility Testing (EUCAST), recommends to use only a susceptible/resistant breakpoint for all cephalosporins at > 0.12 [20]. According to EUCAST guidelines, all 102 strains with an MIC > 0.125 µg/ml found in our study would have been considered resistant against third-generation cephalosporins.

It is feasible that a decrease in cefotaxime susceptibility among circulating NG strains will necessitate the use of larger doses of third-generation cephalosporins for effective elimination of gonorrhoea in infected individuals, a phenomenon already experienced in the treatment of gonorrhoea patients with penicillin in the past. For uncomplicated gonorrhoea infections, the CDC recommends 125 mg ceftriaxone i.m. [21] and the International

Figure

Numbers and percentages of patients with *Neisseria gonorrhoeae* isolates susceptible and with reduced susceptibility to cefotaxime, by quarter of the year, 2006-2008, STI outpatient clinic, Amsterdam, the Netherlands



Union against sexually transmitted infections (IUSTI)/WHO guidelines a dose of 250 mg ceftriaxone i.m. [22]. Possibly because in the Netherlands we already use higher than recommended doses of 500 mg ceftriaxone i.m., we have not experienced treatment failure in patients treated with ceftriaxone yet (although we do not systematically test all patients treated for gonorrhoea in our clinic to see if the treatment has been successful – "test for cure"). Finally, it is likely that decreasing susceptibility to third-generation cephalosporins will lead to the loss of this class of antimicrobials for the treatment of gonorrhoea [23].

In the present paper, we show that the number of *N. gonorrhoeae* strains with reduced susceptibility to cefotaxime is sharply increasing. The increase is mainly found among MSM patients and is associated with high-risk behaviour as indicated by increased prevalence of other STIs. This differs from the recently published outbreak in Greece, in which 17 patients were infected with NG strains of reduced susceptibility to cephalosporins after casual male-to-female sexual contacts [18].

N. gonorrhoeae with reduced cefotaxime susceptibility found mainly among MSM

A sharp increase in the percentage of NG isolates with decreased susceptibility to cefotaxime has been observed since the last quarter of 2007 and the rising trend was significant for the whole study period. During the last three quarters of 2008 the prevalence of NG isolates with reduced susceptibility to cefotaxime was continuously above 10% which indicates sustained circulation of these strains.

Patients bearing NG strains with reduced cefotaxime susceptibility (MIC >0.125 µg/ml) were significantly more often 35 years old or older, MSM, HIV–positive, and had concurrent STI, compared to those with cefotaxime-susceptible NG strains. Similar characteristics (MSM, >=35 years, multiple concurrent and previously documented STI, especially HIV) were also identified in patients with emerging STIs like lymphogranuloma venereum and sexually acquired hepatitis C [24,25].

The present finding of NG strains with reduced susceptibility to cefotaxime and multiple resistances to other antibiotics circulating in this MSM core group once again underlines the importance of tailored and intensified STI care for high-risk MSM patients focused on multiple concurrent chronic and incident STI infections. This is important for the individual patient but also for the population at large because emerging STIs circulating within a core group can easily spread to the population at large as experienced with ciprofloxacin-resistant NG strains in the Netherlands.

The incidence of NG isolates with reduced susceptibility to cefotaxime was highly associated with MSM patients, also in multivariate analysis. For this reason we focused the second part of our analysis on the characteristics of NG strains collected from MSM patients only. We did not find an association between antibiotic susceptibility to cefotaxime and the various collection sites. Almost half of the NG strains originated from rectal swabs, both among NG strains susceptible to cefotaxime (47.1%) and among those with reduced cefotaxime susceptibility (49.5%) (Table 2). Rectal gonorrhoea infections are an increasing problem among MSM as reported earlier, and should always be considered since many of these infections are asymptomatic [26].

Multidrug-resistance common in NG strains with reduced susceptibility for cefotaxime

Among the NG isolates with reduced susceptibility to cefotaxime obtained from MSM patients, a considerable number was found resistant to multiple antibiotics such as penicillin, tetracycline and ciprofloxacin. This implies that if the trend of reduced susceptibility to cefotaxime progresses towards resistance to all third-generation

Table 2

Collection site of 1,231 isolates obtained from 1,075 men who have sex with men; STI outpatient clinic, Amsterdam, the Netherlands 2006-2008

Site	cefotaxime MIC ≤ 0.125 µg/ml (n=1,134)	cefotaxime MIC > 0.125 µg/ml (n=97)	Overall p value[1]
Urethra	469 (41.4%)	41 (42.3%)	0.61
Rectum	534 (47.1%)	48 (49.5%)	
Pharynx	131 (11.6%)	8 (8.2%)	

Data are the number of isolates (% of total) including 1,134 isolates from 985 patients with a cefotaxime MIC value ≤ 0.125 µg/ml and 97 isolates from 90 patients with a cefotaxime MIC value > 0.125 µg/ml.
1p value based on chi^2 test.

Table 3

Other antibiotic resistance characteristics of 1,231 isolates obtained from 1,075 men who have sex with men; STI outpatient clinic, Amsterdam, the Netherlands, 2006-2008

Antibiotic resistance	cefotaxime MIC ≤ 0.125 µg/ml, (n= 985)	cefotaxime MIC > 0.125 µg/ml, (n=90)	Overall p value[1]
Penicillin resistance[2]	162 (16.5%)	39 (43.3%)	<0.001
Tetracycline resistance[3]	211 (21.5%)	62 (68.9%)	<0.001
Ciprofloxacin resistance[4]	437 (44.4%)	81 (90.0%)	<0.001
Resistance to at least two of the following: penicillin, tetracycline and ciprofloxacin[5]	224 (22.9%)	69 (77.5%)	<0.001
Resistance to all three antibiotics: penicillin, tetracycline and ciprofloxacin[5]	46 (4.7%)	27 (30.3%)	<0.001

Data are the number of isolates (% of total).
1p values are based on chi^2 test.
2For penicillin either chromosomal or plasmid-mediated resistance, missing data on penicillin resistance (excluded from analysis) n=4.
3Missing data on tetracycline resistance (excluded) n=2.
4Missing data on ciprofloxacin resistance (excluded) n=2.
5Missing data (excluded) n=6.

cephalosporins, a switch to previously recommended antibiotics for gonorrhoea will not be an option. It has been suggested that spectinomycin, which is structurally unrelated to third-generation cephalosporins, should be used as the primary therapy for gonorrhoea but this drug is not available everywhere, at least not in the Netherlands [14]. Other treatment options are gentamycin, carbapenems and dual antibiotic therapy [6].

Although we only included high-risk patients in our analysis and excluded the low-risk group we do not think that this could have led to significant bias since the prevalence of NG among low-risk visitors was only 0.5%. Moreover, we compared the selection of patients diagnosed with gonorrhoea with available MIC values to those without MIC data. Being MSM was the only characteristic overrepresented in the group without MIC data. Since the compositions of the two groups were similar, the group with MIC info can be considered representative of the whole.

At present we are working on the molecular typing of the NG isolates with decreased susceptibility to ceftriaxone to investigate if the cefixime-associated mosaic patterns of the *penA* gene are also associated with our findings. The increased prevalence of NG strains with reduced susceptibility to cefotaxime among MSM may herald the evolution towards third-generation cephalosporin-resistant NG strains and this trend needs to be closely monitored. Gonorrhoea can still be treated effectively with third-generation parenteral cephalosporins, however, previous experience has demonstrated that the use of increased doses of antimicrobials only postpones the development of resistance, but does not prevent the eventual demise of the drug.

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References

1. Tapsall J. Antimicrobial resistance in Neisseria gonorrhoeae. Geneva: World Health Organization; 2001. Available from: http://www.who.int/drugresistance/Antimicrobial_resistance_in_Neisseria_gonorrhoeae.pdf
2. Kolader M, Peerbooms PG, Vader PC, van Bergen JE, Fennema JS, de Vries HJ. [The rise in fluoroquinolone-resistant Neisseria gonorrhoeae among people attending the Municipal Health Service's clinic for sexually transmitted diseases in Amsterdam, the Netherlands; cefotaxime now first-choice treatment for uncomplicated gonorrhoea]. Ned Tijdschr Geneeskd. 2004;148(43):2129-32. Dutch
3. Borgen K, van Loo I, Koedijk FD, van de Laar MJ. Increase of gonococcal quinolone resistance in the Netherlands from 2002 – 2004. Euro Surveill. 2005;10(46):pii=2836. Available from: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=2836
4. Koedijk FDH, Vriend HJ, van Veen MG, Op de Coul ELM, van den Broek IVF, van Sighem AI, et al. Sexually transmitted infections, including HIV, in the Netherlands in 2008. Bilthoven, the Netherlands: National Institute for Public Health and the Environment (RIVM); 2009. Available from: http://www.rivm.nl/cib/binaries/090292_SOAHIVjaarrapport_2009_tcm92-60978.pdf
5. Fenton KA, Ison C, Johnson AP, Rudd E, Soltani M, Martin I, et al. Ciprofloxacin resistance in Neisseria gonorrhoeae in England and Wales in 2002. Lancet. 2003;361(9372):1867-9.
6. Workowski KA, Berman SM, Douglas JM, Jr. Emerging antimicrobial resistance in Neisseria gonorrhoeae: urgent need to strengthen prevention strategies. Ann Intern Med. 2008;148(8):606-13.
7. Domain group for Sexually Transmited Infections (NVDV). [Diagnostics and Treatment of Sexually Transmited Infections. Version 2008/2009]. Amsterdam: SOAAIDS the Nederlands; 2009. Dutch. Available from: http://www.soaaids-professionals.nl/medische_richtlijnen/nvdv
8. van Rooijen M. [Annual report 2008. STI outpatient clinic Health Service Amsterdam]. Amsterdam, the Netherlands: GGD Amsterdam; 2009. Dutch. Available from: http://www.gezond.amsterdam.nl/Infectieziekten--hygine/SOA--HIV/Cijfers-soa-en-hiv
9. Heijman TL, Van der Bij AK, de Vries HJ, van Leent EJ, Thiesbrummel HF, Fennema HS. Effectiveness of a risk-based visitor-prioritizing system at a sexually transmitted infection outpatient clinic. Sex Transm Dis. 2007;34(7):508-12.
10. Van der Bij AK, Spaargaren J, Morré SA, Fennema HS, Mindel A, Coutinho RA, et al. Diagnostic and clinical implications of anorectal lymphogranuloma venereum in men who have sex with men: a retrospective case-control study. Clin Infect Dis. 2006;42(2):186-94.
11. World Medical Association Declaration of Helsinki, Ethical Principles for Medical Research Involving Human Subjects. Available from: http://www.wma.net/e/policy/b3.htm.
12. Unemo M, Fasth O, Fredlund H, Limnios A, Tapsall J. Phenotypic and genetic characterization of the 2008 WHO Neisseria gonorrhoeae reference strain panel intended for global quality assurance and quality control of gonococcal antimicrobial resistance surveillance for public health purposes. J Antimicrob Chemother 2009;63(6):1142-51.
13. Clinical and Laboratory Standards Institute (CLSI). Performance Standards for Antimicrobial Susceptibility Testing. 19th Informational Supplement. M100-S19 edition. 2009.
14. Ito M, Deguchi T, Mizutani KS, Yasuda M, Yokoi S, Ito S, et al. Emergence and spread of Neisseria gonorrhoeae clinical isolates harboring mosaic-like structure of penicillin-binding protein 2 in Central Japan. Antimicrob Agents Chemother. 2005;49(1):137-43.
15. Yokoi S, Deguchi T, Ozawa T, Yasuda M, Ito S, Kubota Y, et al. Threat to cefixime treatment for gonorrhea. Emerg Infect Dis. 2007;13(8):1275-7.
16. Lindberg R, Fredlund H, Nicholas R, Unemo M. Neisseria gonorrhoeae isolates with reduced susceptibility to cefixime and ceftriaxone: association with genetic polymorphisms in penA, mtrR, porB1b, and ponA. Antimicrob Agents Chemother. 2007;51(6):2117-22.
17. Whiley DM, Limnios EA, Ray S, Sloots TP, Tapsall JW. Diversity of penA alterations and subtypes in Neisseria gonorrhoeae strains from Sydney, Australia, that are less susceptible to ceftriaxone. Antimicrob Agents Chemother. 2007;51(9):3111-6.
18. Tzelepi E, Daniilidou M, Miriagou V, Siatravani E, Pavlidou E, Flemetakis A. Cluster of multidrug-resistant Neisseria gonorrhoeae with reduced susceptibility to the newer cephalosporins in Northern Greece. J Antimicrob Chemother. 2008;62(3):637-9.
19. Deguchi T, Yasuda M, Yokoi S, Ishida K, Ito M, Ishihara S, et al. Treatment of uncomplicated gonococcal urethritis by double-dosing of 200 mg cefixime at a 6-h interval. J Infect Chemother. 2003;9(1):35-9.
20. European Committee on Antimicrobial Susceptibility Testing (EUCAST). Data from the EUCAST MIC distribution website. Available from: http://www.eucast.org/
21. Centers for Disease Control and Prevention, Workowski KA, Berman SM. Sexually transmitted diseases treatment guidelines, 2006. MMWR Recomm Rep. 2006;55(RR-11):1-94.
22. Bignell CJ, European branch of the International Union against Sexually Transmitted Infection and the European Office of the World Health Organization. European guideline for the management of gonorrhoea. Int J STD AIDS. 2001;12 Suppl 3:27-9.
23. Barry PM, Klausner JD. The use of cephalosporins for gonorrhea: the impending problem of resistance. Expert Opin Pharmacother. 2009;10(4):555-77.
24. de Vries HJ, van der Bij AK, Fennema JS, Smit C, de Wolf F, Prins M, et al. Lymphogranuloma venereum proctitis in men who have sex with men is associated with anal enema use and high-risk behavior. Sex Transm Dis. 2008;35(2):203-8.
25. van de Laar TJ, van der Bij AK, Prins M, Bruisten SM, Brinkman K, Ruys TA, et al. Increase in HCV incidence among men who have sex with men in Amsterdam most likely caused by sexual transmission. J Infect Dis. 2007;196(2):230-8.
26. Kent CK, Chaw JK, Wong W, Liska S, Gibson S, Hubbard G, et al. Prevalence of rectal, urethral, and pharyngeal chlamydia and gonorrhea detected in 2 clinical settings among men who have sex with men: San Francisco, California, 2003. Clin Infect Dis. 2005;41(1):67-74.

Biogeographical origin and varicella risk in the adult immigration population in Catalonia, Spain (2004-2006)

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Immigrants to the European Union may have a higher susceptibility to varicella-zoster virus primo-infection than the indigenous population. There is no evidence as yet that this is caused by genetic or social factors. Therefore, susceptibility could be due to a lesser transmission of the virus in their ecosystems of origin. A multicentre observational study was performed from July 2004 to June 2006 in four primary healthcare centres in Catalonia, Spain, monitoring varicella incidences and comparing standardised incidence rates and standardised rate ratios among different populations classified according to their biogeographical origin (holarctic, Asian paleotropical, African paleotropical or neotropical). Overall, 516 varicella cases were recorded. The standardised incidence rates per 1,000 inhabitants per year were: holarctic: 2.17 (95% confidence interval (CI): 1.95-2.39); autochthonous 2.26 (95% CI: 2.03-2.49); immigrants 3.59 (95% CI: 2.92-4.26); neotropical 4.50 (95% CI: 3.28-5.71); non-holarctic 5.38 (95% CI: 4.27-6.14); Asian paleotropical 7.03 (95% CI: 4.77-9.28); and African paleotropical 7.05 (95% CI: 1.12-23.58). The difference to the autochthonous population was greatest in immigrants of neotropical origin (standardised rate ratio = 2.07 (95% CI: 1.61-2.64) or 4.5 excess cases per 1,000 inhabitants per year) and Asian paleotropical origin (standardised rate ratio = 3.24 (95% CI: 2.47-4.11) or 9.6 excess cases per 1,000 inhabitants per year). Biogeographical origin may therefore account for the vulnerability of certain immigrant populations to varicella, in particular those from Asian paleotropical (Indostan and Southest Asia) and neotropical (South America and the Caribbean) ecosystems. Vaccination of immigrants at high risk (fertile women, healthcare workers) could be recommendable.

Introduction

Varicella is typically a childhood disease (87% of the cases in Spain between 1997 and 2004 were reported in children under the age of 15 years) caused by varicella-zoster virus (VZV)-induced primo-infection [1]. Although the disease is usually self-limiting, some cases can be serious, with 2-6% of them resulting in complications and a hospitalisation rate in the European Union ranging from 1.3 to 4.5 per 100,000 population per year [2]. While the disease is universally distributed, some studies (based on comparison of seroprevalence) point to a greater incidence in cold or temperate northern hemisphere countries – holarctic ecosystem – particularly in winter and spring. There is no explanation to date for the possible lower incidence in Asian, African and South American climates – Asian paleotropical, African paleotropical and neotropical ecosystems, respectively [3]. No hypothesis points to an explanation based on genetics; consequently, it has generally been attributed to a mixture of underreporting and ecological reasons, i.e. VZV would be less transmissible in those ecosystems, although the mechanisms responsible have never been accurately defined [4,5].

However, if these ecological factors do exist, more varicella cases should be seen in the adult immigrant population from tropical-subtropical climates compared to the autochthonous population in Spain who would have a greater degree of immunity from childhood. Furthermore, significant differences should exist between populations of holarctic origin and those of other origin, but not between holarctic immigrants and the autochthonous population.

After centuries of net emigration, Spain has experienced large-scale immigration since the year 2000. Currently, the country has the second highest immigration rate of Western Europe just after Cyprus [6]. According to official statistics, 4,144,000 immigrant residents were recorded in Spain in 2005, of whom over 1,500,000 were from Morocco, Ecuador and Romania [6]. Therefore, Spain represents an excellent platform to carry out such a study since the country received a large number of immigrants over a relatively short period of time, which may allow verifying epidemiologically whether or not there was an increase in the incidence of varicella within the immigrant population. Such an analysis of varicella

incidence should take into account the ecological (biogeographical) and not the geopolitical (according to nationality) origin of the subjects [7-9].

The aim of the present study was to ascertain and compare varicella incidence rates in autochthonous and immigrant populations in Spain according to their biogeographical origin using case registration analysis in primary healthcare centres. It further aimed at discussing the value of the findings with regard to prevention policies to be employed in a country which, in the medium term, is going to receive many more immigrants.

Methods

This was a multicentre, longitudinal study based on new varicella cases in adults (>14 years, i.e. the unvaccinated population, as vaccination was included in the official vaccination calendar in 2004) from four primary healthcare centres (Centre d'Atenció Primaria, CAP) with a substantial immigrant population, registered between July 2004 and June 2006. Two of these centres are located in Santa Coloma de Gramenet, one in the city of Barcelona and one in Sant Adrià del Besòs. All belong to the Catalan Health Institute (Institut Català de la Salut). The 59 general practitioners (GPs) working in these centres were all blinded to the study question.

The study population consisted of all patients assigned to the four primary healthcare centres. According to data from the Primary Health Informatics System, this comprised 103,902 patients in June 2008. This is a local registration under the primary healthcare system and therefore may reflect more complete and reliable data than census or other general records.

All individuals born in the World Health Oganization (WHO) European Region were defined as autochthonous, and those born outside as immigrants. People born in WHO European Region to immigrant parents were considered to be autochthonous. Classification of the study population according to their ecological zone of origin was based on classical bioregion mapping, which divides the emerged land surfaces into seven zones (Figure) [10]:

- the holarctic region (North America, Europe, Maghreb, Near East, Central Asia, Siberia, China, Korea and Japan),
- the African paleotropical region (sub-Sahara Africa except the western half of South Africa),
- the Asian paleotropical (Indian sub-continent and Southeast Asia),
- the neotropical region (Central America, Caribbean islands and South America),
- and three other regions (South Africa, Antarctica and Oceania) that were excluded since no immigrants from these regions were registered during the study period.

For example, the autochthonous population and immigrants from Morocco or China were considered 'holarctic', while immigrants from Ecuador were classified as 'neotropical'.

A varicella case was defined as a patient registered by the GP as varicella infection in the 'conditions and problems' (diagnosis) section of the computerised clinical records. Cases labelled as 'suspected' or 'probable' were excluded. The variables of age, sex and biogeographical origin were collected.

Age-specific and crude incidence rates were calculated using the population of the four healthcare facilities in the study area as the denominator. Rates were stratified by sex and biogeographical origin, and standardised by the direct method, using the population of Catalonia as a reference according to data available from the 2005 Catalan Statistics Institute [11]. Confidence intervals (CI) for the age-specific and age-standardised rates were calculated using the normal distribution in all groups except for the group of African paleotropical immigrants that contained such a small number of cases that the exact approximation method was used.

Age-standardised rate ratios (ASRR) were defined as the incidence rates of varicella in each group divided by the corresponding incidence rate in the reference group. CIs of ASRR were calculated using the median unbiased estimation method; the CIs which did not include the value '1' were considered significant ($p<0.05$) [12]. Subtraction among standardised rate ratios (SRR) represented the excess or deficit of cases per 1,000 inhabitants per year compared to the incidence of the autochthonous population once the differences attributable to population structure had been eliminated.

The EPIDAT 3.0 and R programmes were used for calculating the incidence rates and their respective CIs.

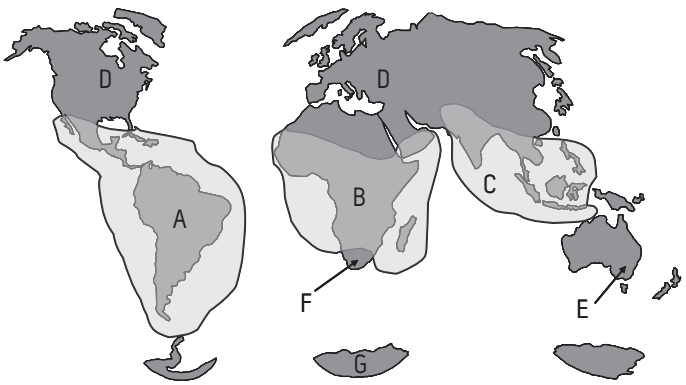
Results

The study population comprised 103,902 individuals with medical records in their healthcare centres. Of these, 14,387 (13.8%) were immigrants. According to the biogeographical origin of those immigrants, 5,470 (5.3%) belonged to the holarctic, 2,806 (2.7%) to the Asian paleotropical, 338 (0.3%) to the African paleotropical, and 5,773 (5.6%) to the neotropical ecosystem.

There were 516 recorded cases of varicella: 296 (57.4%) men and 220 (42.6%) women with a mean age of 28.1 (standard deviation (SD) 10.5) years. The total incidence of the disease in the adult population, standardised according to age and sex, was 2.44 (95% CI: 2.23-2.65) cases per 1,000 inhabitants per year. According to biogeographical origin, the incidence rates standardised per 1,000 persons per year were, in ascending order: holarctic 2.17 (95% CI: 1.95-2.39), autochthonous 2.25 (95% CI:

Figure

Biogeographical regions of the world



A: Latin America (neotropical); B Africa (African paleotropical); C: Tropical Asia (Asian paleotropical); D: Holoarctic; E: Oceania; F: South Africa; G: Antarctica.

2.02-2.47), all immigrants 3.59 (95% CI: 2.92-4.26), neotropical immigrants 4.50 (95% CI: 3.28-5.91), non-holarctic immigrants 5.38 (95% CI: 4.27-6.14), Asian paleotropical immigrants 7.03 (95% CI: 4.77-9.28) and African paleotropical immigrants 7.05 (95% CI: 1.12-23.58).

The crude rates obtained during the study period and those standardised for age are presented in Tables 1-3. Crude and age-standardised rate ratios according to biogeographical origin are shown in Table 4. The resulting excess of varicella cases per 1,000 inhabitants is displayed in Table 5.

Table 1

Crude, age-specific and age-standardised incidence rates of varicella, by geographical origin (autochthonous/immigrants) and sex in the urban health area of Barcelonès nord-Maresme, Catalonia, Spain, 2004-2006

Sex and age group	Autochtonous			Immigrants			Total		
	Number of cases	Study population	Rate (95% CI)	Number of cases	Study population	Rate (95% CI)	Number of cases	Study population	Rate (95% CI)
Male									
15-34 years	147	12,768	5.75 (4.82-6.68)	73	4,642	7.86 (6.6-9.66)	220	17,410	6.32 (5.48-7.15)
35+ years	62	27,887	1.11 (0.83-1.37)	14	3,546	1.97 (0.94-3.01)	76	31,433	1.19 (0.92-1.46)
Total / crude rate	209	40,655	2.57 (2.23-2.93)	87	8,188	5.32 (4.25-6.55)	296	48,843	3.00 (2.65-3.34)
Age-standardised rate			2.66 (1.33-3.04)			3.94 (3.03-4.86)			2.91 (2.58-3.24)
Female									
15-34 years	134	14,422	4.64 (3.86-5.43)	40	3,637	5.50 (3.79-7.20)	174	18,059	4.82 (4.10-5.54)
35+ years	36	34,438	0.52 (0.35-0.68)	10	2,562	1.95 (0.74-3.16)	46	37,000	0.62 (0.44-0.80)
Total / crude rate	170	48,860	1.73 (1.48-2.01)	50	6,199	4.04 (2.99-5.31)	220	55,059	1,99 (1.73-2.26)
Age-standardised rate			1.90 (1.61-2.18)			3.14 (2.15-4.12)			2.02 (1.75-2.29)
Total									
15-34 years	281	27,190	5.16 (4.56-5.77)	113	8,279	6.82 (5.65-8.33)	394	35,469	5.55 (5.01-6.11)
35+ years	98	62,325	0.78 (0.62-0.93)	24	6,108	1.96 (1.18-2.75)	122	68,433	0.89 (073-1.1)
Total / crude rate	379	89,515	2.11 (1.90-2.34)	137	14,387	4.76 (3.99-5.63)	516	103,902	2.48 (2.26-270)
Age-standardised rate			2.26 (2.03-2.49)			3.59 (2.92-4.26)			2.45 (2.24-2.67)

Rates are per 1,000 inhabitants per year. The age-standardised rates were calculated using the age distribution of the 2005 Catalan population census and confidence intervals (CI) rates of 95%.

Table 2

Crude, age-specific and age-standardised incidence rates of varicella by biogeographical origin (holarctic/non-holarctic) and sex in the urban health area of Barcelonès nord-Maresme, Catalonia, Spain, 2004-2006

Sex and age groups	Holarctic			Non-holarctic			Total		
	Number of cases	Study population	Rate (95% CI)	Number of cases	Study population	Rate (95% CI)	Number of cases	Study population	Rate (95% CI)
Male									
15-34 years	152	13,950	5.45 (4.58-6.31)	68	3,079	11.04 (8.42-13.66)	220	17,029	6.46 (5.63-7.37)
35+ years	62	29,391	1.05 (0.79-1.32)	14	1,865	3.75 (1.78-5.72)	76	31,256	1.21 (0.96-1.52)
Total / crude rate	214	43,341	2.47 (2.15-2.82)	82	4,944	8.29 (6.59-10.29)	296	48,285	3.07 (2.72-3.43)
Age-standardised rate			2.52 (2.19-2.86)			6.19 (4.61-7.77)			2.97 (2.63-3.31)
Female									
15-34 years	139	15,300	4.54 (3.78-5.30)	35	2,504	6.99 (4.67-9.31)	174	17,804	4.88 (4.17-5.67)
35+ years	37	35,534	0.52 (0.36-0.69)	9	1,469	3.65 (1.06-5.06)	46	37,003	0.62 (0.46-0.88)
Total / crude rate	176	50,834	1.73 (1.48-4.00)	44	3,973	6.54 (4.03-7.43)	220	54,807	2.00 (1.75-2.29)
Age-standardised rate			1.86 (1.59-2.14)			4.37 (2.83-5.92)			2.05 (1.28-2.32)
Total									
15-34 years	291	29,250	4.97 (4.41-5.54)	103	5,583	9.22 (7.44-11.00)	394	34,833	5.65 (5.10-6.23)
35+ years	99	64,925	0.76 (0.61-0.91)	23	3,334	3.45 (2.04-4.86)	122	68,259	0.89 (0.74-1.07)
Total / crude rate	390	94,175	2.07 (1.87-2.28)	126	8,917	7.07 (5.88-8.41)	516	103,902	2.50 (2.28-2.70)
Age-standardised rate			2.17 (1.95-2.39)			5.38 (4.27-6.14)			2.49 (2.27-2.70)

Rates are per 1,000 inhabitants per year. Age-standardised rates were calculated using the age distribution of the 2005 Catalan population census and confidence intervals (CI) rates of 95%.

In summary, significant differences in the varicella incidence rates were found between the autochthonous and immigrant populations. These differences were accentuated when the group of holarctic populations was compared with the group of non-holarctic populations, and when the holarctic population was compared to the neotropical and, particularly, Asian paleotropical populations. In contrast, no statistically significant differences were observed when the autochthonous and holarctic populations were compared.

Discussion and conclusions

The possible epidemiological changes in the number of varicella infections due to the influx of immigrants had already been noted

Table 3

Crude, age-specific and age-standardised incidence rates of varicella by biogeographical origin and sex in the urban health area of Barcelonès nord-Maresme, Catalonia, Spain, 2004-2006

Sex and age groups	Holarctic			Asian paleotropical			African paleotropical			Neotropical		
	Number of cases	Study population	Rate (95% CI)	Number of cases	Study population	Rate (95% CI)	Number of cases	Study population	Rate (95% CI) [1]	Number of cases	Study population	Rate (95% CI)
Male												
15-34 years	152	13,950	5.45 (4.58-6.31)	27	1,418	9.52 (6.28-13.58)	1	156	3.21 (0.8-17.86)	40	1,505	13.29 (9.49-18.09)
35+ years	62	29,391	1.05 (0.79-1.32)	9	922	4.88 (2.23-9.25)	0	90	0.00	5	853	2.93 (0.95-6.84)
Total / crude rate	214	43,341	2.47 (2.15-2.82)	36	2,340	7.69 (5.39-10.65)	1	246	2.04 (0.05-11.33)	45	2,358	9.53 (6.96-12.77)
Age-standardised rate			2.52 (2.19-2.86)			6.43 (3.99-8.87)			1.07 (0.003-1.47)			6.40 (4.20-8.59)
Female												
15-34 years	139	15,300	4.54 (3.78-5.30)	11	253	21.74 (10.85-38.89)	0	77	0.00	24	2,174	6.52 (3.53-8.21)
35+ years	37	35,534	0.52 (0.36-0.69)	2	213	4.69 (0.57-16.96)	2	15	66.66 (8.08-240.82)	5	1,241	2.02 (0.65-4.70)
Total / crude rate	176	50,834	1.73 (1.48-4.00)	13	466	13.95 (7.43-23.85)	2	92	10.87 (1.31.-39.26)	29	3,415	4.24 (2.84-6.10)
Age-standardised rate			1.86 (1.59-2.14)			10.40 (4.30-16.5)			44.36 (5.37-160.22)			3.19 (1.80-4.57)
Total												
15-34 years	291	29,250	4.97 (4.41-5.54)	38	1,671	11.37 (8.04-15.60)	1	233	1.14 (0.06-11.95)	64	3,679	8.7 (6.70-9.81)
35+ years	99	64,925	0.76 (0.61-0.91)	11	1,135	4.84 (2.42-8.67)	2	105	9.52 (1.15-34.40)	10	2,094	2.39 (1.14-4.39)
Total / crude rate	390	94,175	2.07 (1.87-2.28)	49	2,806	8.73 (6.46-11.54)	3	338	4.44 (0.91-12.97)	74	5,773	6.41 (5.04-8.05)
Age-standardised rate			2.17 (1.95-2.39)			7.03 (4.77-9.28)			7.05 (1.12-23.58)			4.50 (3.28-5.71)

Rates are per 1,000 inhabitants per year. Age-standardised rates were calculated using the age distribution of the 2005 Catalan population census and confidence intervals (CI) rates of 95 %.
1 Because of the small number of cases for the African paleotropical group, 95% CIs were calculated using exact methods.

Table 4

Crude rate ratio (CRR) and age-standardised rate ratio (ASRR) for varicella by geographical and biogeographical origin and sex in the urban health area of Barcelonès nord-Maresme, Catalonia, Spain, 2004-2006

Sex	Male		Female		Total	
Geographical and biogeographical origin	CRR (95% CI)	ASRR (95% CI)	CRR (95% CI)	ASRR (95% CI)	CRR (95% CI)	ASRR (95% CI)
Autochtonous	1 (reference)	1 (reference)	1 (reference)	1 (reference)	1 (reference)	1 (reference)
Immigrants	2.09 (1.61-2.67)	1.48 (1.14-1.87)	2.33 (1.69-3.18)	1.65 (1.19-2.24)	2.27 (1.86-2.75)	1.60 (1.25-1.96)
Holarctic	1(reference)	1 (reference)	1 (reference)	1 (reference)	1 (reference)	1 (reference)
Asian paleotropical	3.12 (2.16-4.39)	2.55 (1.79-3.60)	8.15 (4.40-13.75)	5.57 (3.21-9.51)	4.22 (3.10-5.63)	3.24 (2.47-4.11)
African paleotropical	0.94 (0.04-4.10)	0.42 (0.01-3.89)	6.76 (1.05-21.02)	23.76 (5.94-69.2)	2.25 (0.54-5.88)	3.25 (0.8-5.84)
Neotropical	3.87 (2.77-5.29)	2.53 (1.83-3.44)	2.45 (1.63-3.59)	1.71 (1.26-2.03)	3.10 (2.40-3.95)	2.07 (1.61-2.64)
Non-holarctic [1]	3.36 (2.59-4.32)	2.45 (1.88-3.11)	3.20 (2.28-4.42)	2.34 (1.68-3.24)	3.41 (2.78-4.16)	2.48 (2.03-3.02)

Rate ratio is statistically significant ($p<0.05$) if non-holarctic group is not included.
1 Non-holarctic represent Asian paleotropical, African paleotropical and neotropical grouped together.

in the mid-twentieth century. Causes of the changes have been assumed to be: the presence of adult immigrants susceptible to the disease, and the introduction of new strains of VZV [13,14]. Those studies were based on seroprevalence – generally on samples from blood banks – and on genetic analyses of the isolated viruses. Subsequently, a substantial series of articles was published that warned of epidemic outbreaks in immigrant communities and their immunological vulnerability. Articles cited in PubMed between 1997 and 2007 defined as risk communities immigrants from the Indian subcontinent [15-17], South America [18,19], Africa [20] or a combination of two of them [21]. Only one study (in Oceania, a non-holarctic ecosystem) did not report differences between immigrants and autochthonous individuals [22]. Although the evidence provided in these studies is based solely on the greater seronegativity in immigrants, they clearly suggest that this vulnerability is due to factors related to the transmissibility of VZV in their respective ecosystems of origin. No hypothesis regarding genetic or cultural causes was raised.

The present study is consistent with the approaches above in that it confirms – from a purely epidemiological point of view – higher incidence rates in individuals from ecological environments that are very different from the European or holarctic region. Furthermore, the fact that no differences were found between the autochthonous European population and immigrants of other holarctic biogeographical origin (i.e. people from the Maghreb, Near East, central Asia, China and North America) is not only in line with the ecological hypothesis but an argument against a role of genetic factors. One of the limitations of this study is the lack of a comparative analysis of varicella incidence rates in children and further research in that direction should be encouraged. One would expect the differences between the groups to disappear when comparing rates in children of autochthonous families and children of immigrant families born in Europe.

Two further limitations of the study should be noted: firstly, the possible bias due to different social situations in the study groups (e.g. housing conditions or the proportion of women of childbearing age) that could facilitate the spread of the infection in a given group, and secondly, the strictly epidemiological approach of the study which did not take into account seroprevalence. Also, immigrants from certain areas, particularly Africa, could be under-registred in Spain and therefore cause a bias. Nevertheless, the local demographic records used are considered trustworthy and, thus, any bias was likely small.

Table 5

Excess of cases per 1,000 inhabitants by biogeographical origin with respect to the autochthonous population, Catalonia, Spain, 2004-2006

Biogeographical origin	Excess of cases per 1,000 inhabitants
Autochthonous	0 (reference)
African paleotropical	-1.25
Holarctic	0.16
All immigrants	2.68*
Neotropical	4.5*
Non-holarctic	6.26*
Asian paleotropical	9.56*

*p< 0.05

The observed differences were most marked in male individuals under the age of 35 years. However, this may be a coincidental observation and due to the limited number of cases in people over 35 years of age. Nevertheless, the differences between the sexes could be due to possible inequalities in the access to healthcare for female immigrants who may be discriminated against. Other epidemiology-based studies also raised this possibility [23,24].

Similarly, the African paleotropical population was not strongly represented which makes it difficult to draw definitive conclusions. The incidence CI in this group compared with the autochthonous and holarctic populations was not significant for the men, but significant for the women. Only one earlier study has described an African paleotropical population with a seroprevalence of antibodies against VZV that was significantly lower than that in the European population [18], but these were immigrants from East Africa (refugees from Somalia) who are very rare in Spain and therefore do not allow a comparison with our study. From our data, we can only conclude that significant differences do not appear to exist between the sub-Saharan African population from West Africa, the largest in Spain and the local autochthonous population.

The vulnerability to varicella in immigrants from the holarctic ecosystem (i.e. China or Turkey) appeared to follow a similar age pattern as in the autochthonous population. Both groups had fewer cases in adults than in children. This probably reflects a higher VZV transmission in childhood and the development of permanent immunity after infection. In contrast, immigrants from the neotropical and, particularly, Asian paleotropical ecosystems show an epidemiological pattern typical of an adult population with greater susceptibility to the disease [25].

All varicella cases were identified according to the diagnosis made by 59 GPs. Considering their experience, this purely clinical ascertainment, albeit subjective, is likely to be reliable. There is no reason to suppose that the diagnosis should be more or less accurate for immigrants than for autochthonous groups.

Preventive measures for these populations with lower immunity should be considered. Although determining the cost-effectiveness of a vaccination against VZV was not the aim of this study, it should be emphasised that some institutions do recommend (United States Army) [26] or consider it (Catalan Autonomous Government) [27]. Studies that supported the inclusion of the chickenpox vaccine in the childhood vaccination schedule defined an incidence threshold for cost-effectiveness at much lower rates than those found in vulnerable immigrant communities [28]. Selective vaccination of the young adult population from South America or the Indian subcontinent without a history of chickenpox in childhood would very likely be cost-effective as far as direct and particularly indirect expenses (loss of productivity) are concerned [29]. Within these populations, two high-risk groups can be defined: women of childbearing age, in order to protect them against varicella infection during pregnancy and to ensure the transplacental diffusion of maternal antibodies to the foetus [30,31], and immigrant healthcare personnel, who are at a high risk of acquiring the infection [32]. An additional argument in favour of selective vaccination is the possibility that immigrants might introduce holarctic strains of VZV to their countries of origin when visiting their families. Such transcontinental spreads caused the measles epidemic in Ecuador in 2008 [33] and, probably, the outbreak of German measles in Brazil in 2007 [34].

Those high-risk target populations could be vaccinated without the need for a serological study [35]. In countries with limited resources such as Spain (where patients must purchase a vaccine at prices ranging from EUR 45 to 60), a possible strategy aimed at increasing social acceptance would be to test healthy immigrant women for varicella antibodies (usually accounting for less than EUR 15) in primary care screening programmes, which usually include the routine test for German measles [36].

Varicella vaccination would surely be indicated for adult immigrants living together with child index cases, since epidemic outbreaks with a high attack rate within families have been described [37]. Thereby, cases of chickenpox in pregnant women and neonates could be avoided, the two groups in whom severe cases occur most frequently. This would require an improved coordination of GPs and epidemiologists, a faster response to an increase in the number of cases, and easier access to vaccination without costs for the individuals [38].

In conclusion, certain immigrant populations, particularly from neotropical and Asian paleotropical biogeographic origin, present a higher incidence rate of varicella than autochthonous inhabitants of Spain and other holarctic populations. The reasons for this elevated vulnerability probably depend on ecological factors that limit transmissibility of the virus in their ecosystems of origin. Thus, the implementation of preventive measures using biogeographical origin as a criterion could be effective. Analysis of the influence of different biogeographical origins on the epidemiology of infectious microorganisms should be completed with further studies.

References

1. Instituto de Salud Carlos III. National Epidemiology Centre. Epidemiological Surveillance Service. Situación de la varicela en España. [Varicella situation in Spain]. National Epidemiology Centre. 2006. [Spanish]. Available from: http://www.isciii.es/htdocs/centros/epidemiologia/pdf/InformevaricelaCNE.pdf
2. Bonnani P, Breuer J, Gershon A, Gershon M, Hryniewicz W, Papaevangelou V, et al. Varicella vaccination in Europe – taking the practical approach. BMC Med. 2009;7:26.
3. Lee BW. Review of varicella zoster seroepidemiology in India and Southeast Asia. Trop Med Int Health. 1998;3(11):886-90.
4. Weller TH. Varicella: historical perspective and clinical overview. J Infect Dis. 1996;174 Suppl 3:S306-9.
5. Kjersem H, Jepsen S. Varicella among immigrants from the tropics, a health problem. Scand J Soc Med. 1990;18(3):171-4.
6. National Institute for Statistics. Statistical exploitation of the municipal census until 1 January 2006. [Explotación estadística de los datos del padrón a 01 de enero de 2006]. National Institute for Statistics. 1 March 2007. [Spanish]. Available from: http://www.ine.es/prensa/np447.pdf
7. Ramette A, Tiedje JM. Biogeography: an emerging cornerstone for understanding prokaryotic diversity, ecology, and evolution. Microb Ecol. 2007;53(2):197-207.
8. Green JL, Bohannan BJ, Whitaker RJ. Microbial biogeography: from taxonomy to traits. Science. 2008;320(5879):1039-43.
9. Horner-Devine MC, Carney KM, Bohannan BJ. An ecological perspective on bacterial biodiversity. Proc Biol Sci. 2004;271(1535):113-22.
10. Brown JH, Gupta VK, Li BL, Milne BT, Restrepo C, West GB. The fractal nature of nature: power laws, ecological complexity and biodiversity. Philos Trans R Soc Lond B Biol Sci. 2002;357(1421):619-26.
11. Statistical Institute of Catalonia. 2005 Census population data. Statistical Institute of Catalonia. [Spanish]. [Accessed 15 September 2009]. Available from: http://www.idescat.cat/territ/BasicTerr?TC=8&V3=863&V4=435&ALLINFO=TRUE&PARENT=1&VO=3&V1=0&CTX=B&VN=3&VOK=Confirmar
12. Rothman KJ, Greenland S. Modern Epidemiology, Second edition. Philadelphia: Lippincot- Raven Publishers. U.S.A.;1998:353-5.
13. Hawrami K, Hart IJ, Pereira F, Argent S, Bannister B, Bovill B, et al. Molecular epidemiology of varicella-zoster virus in East London, England, between 1971 to 1995. J Clin Microbiol. 1997;35(11):2807-9.
14. Ross LF, Lantos JD. Immunisation against chickenpox. BMJ. 1995;310(6971):873.
15. Gabutti G, Fedele A, Aprile V, Guido M, Lopalco P. Immigration flows and new epidemiological evidence in Southern Italy. Vaccine. 2003;21(5-6):399-400.
16. Uduman SA, Tahira AM, Al-Wash R, Usmani MA, Bener A. Varicella susceptibility among children and healthy adults in the United Arab Emirates. East Mediterr Health J. 2001;7(4-5):604-8.
17. Kubeyinje EP, Belagavi CS, Desai S. Ethnic differences in the severity of varicella in adults in northern Saudi Arabia. East Afr Med J. 1997;74(11):727-8.
18. Barnett ED, Christiansen D, Figueira M. Seroprevalence of measles, rubella and varicella in refugees. Clin Infect Dis. 2002;35(4):403-8.
19. Goldberg JM, Ziel HK, Burchette R. Evaluation of varicella immune status in an obstetric population in relation to place of birth. Am J Perinatol. 2002;19(7):387-94.
20. Nysse LJ, Pinsky NA, Bratberg JP, Babar-Weber AY, Samuel TT, Krych EH, et al. Seroprevalence of antibody to varicella among Somali refugees. Mayo Clin Proc. 2007;82(2):175-80.
21. Avrahami-Heller Y, Cohen D, Orr N, Slepon R, Ashkenazi I, Danon YL. Immunity to varicella zoster virus in young israelí adults. Isr Med Assoc J. 2000;2(3):196-9.
22. O'Grady KA, Merianos A, Patel M, Gilbert L. High seroprevalence of antibodies to varicella zoster in adult women in a tropical climate. Trop Med Int Health. 2000;5(10):732-6.
23. Valerio L, Milozzi J, Figueredo A, Reina MD, Martínez-Cuevas O, Pérez-Quílez O. [Prevalence of diabetes mellitus in young Asian Indian immigrants in Santa Coloma de Gramenet (Spain)]. Med Clin (Barc). 2006;126(2):53-6. [Spanish].
24. Faura T, García F, Isla P, López C, Robau M, Moreno A, et al. Health problems and social vulnerability in immigrants admitted for an infectious disease: a case-control study. Rev Clin Esp. 2007;207(5):234-9.
25. Liyanage NP, Fernando S, Malavige GN, Mallikahewa R, Sivayogan S, Jiffry MT, et al. Seroprevalence of varicella zoster infections in Colombo district, Sri Lanka. Indian J Med Sci. 2007;61(3):128-34.
26. Struewing JP, Hyams KC, Tueller JE, Gray GC. The risk of measles, mumps and varicella among young adults: a survey of US Navy and Marine Corps recruits. AM J Public Health. 1993;83(12):1717-20.
27. Plans P, Costa J, Espuñes J, Plasència A, Salleras L. Prevalence of varicella-zoster antibodies in pregnant women in Catalonia (Spain). Rationale for varicella vaccination of women of childbearing age. BJOG. 2007;114(9):1122-7.
28. Lenne X, Díez-Domingo J, Gil A, Ridao M, Lluch JA, Dervaux B. Economic evaluation of varicella vaccination in Spain: results from a dynamic model. Vaccine. 2006;24(47-48):6980-9.
29. Díez-Domingo J, Gil A, San-Martín M, González A, Esteban J, Baldó JM, et al. Seroprevalence of varicella among children and adolescents in Valencia, Spain. Reliability of the parent's reported history and the medical file for identification of potential candidates for vaccination. Hum Vaccin. 2005;1(5):204-6.
30. Pinot de Moira A, Edmunds WJ, Breuer J. The cost-effectiveness of antenatal varicella screening with post-partum vaccination of susceptibles. Vaccine. 2006;24(9):1298-307.
31. Smith WJ, Jackson LA, Watts DH, Koepsell TD. Prevention of chickenpox in reproductive-age women: cost-effectiveness of routine prenatal screening with post-partum vaccination of susceptibles. Obstet Gynecol. 1998;92(4 Pt 1):535-45.
32. Hastie IR. Varicella-zoster virus affecting immigrant nurses. Lancet. 1980;2(8186):154-5.
33. Ecuador Ministry of Public Health. Informe de investigación de la epidemia de sarampión. [Measles Outbreak Investigation Report]. Available from: www.msp.gov.ec/index.php?option=com_content&task=view&id=223&Itemid=1
34. Pan American Health Organization. Elimination of rubella and of congenital rubella syndrome. Progress report. Pan American Health Organization. 2007. [Spanish]. Available from: http://www.paho.org/Spanish/AD/FCH/IM/InformeProgreso_RubeolSRC_2007.pdf
35. Wagenpfeil S, Neiss A, Banz K, Wutzler P. Empirical data on the varicella situation in Germany for vaccination decisions. Clin Microbiol Infect. 2004;10(5):425-30.
36. Bernaola E, Jiménez F, Baca M. Criterios de inclusión de vacunas en el calendario de la Asociación Española de Pediatría. [Criteria for bacines inclusion in the Schedule of the Spanish Association of Pediatrics]. An Pediatr. 2008;68(1):58-62.
37. Macartney K, McIntyre P. Vaccines for post-exposure prophylaxis against varicella (chickenpox) in children and adults. Cochrane Database Syst Rev. 2008;(3):CD0019833.
38. Centers for Disease Control and Prevention (CDC). Public Health Response to Varicella Outbreaks--United States, 2003-2004. MMRW Morb Mortal Wkly Rep. 2006;55(36):993-5.