

Surveillance reports

CHARACTERISATION OF SWABBING FOR VIROLOGICAL ANALYSIS IN THE SPANISH INFLUENZA SENTINEL SURVEILLANCE SYSTEM DURING FOUR INFLUENZA SEASONS IN THE PERIOD 2002-2006

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This study sought to characterise the swabbing pattern in the Spanish Influenza Sentinel Surveillance System (SISS) and ascertain to what extent the system meets the guidelines currently being drafted by The European Influenza Surveillance Scheme (EISS). Data on seasons 2002/2003 to 2005/2006 were drawn from SISS. The study analysed collection and dispatch of swab specimens for virological analysis by reference to variables relating to patient sex, age group, vaccination status, specimen collection period, period of influenza activity, time of swabbing and epidemiological season. SISS adapts to EISS recommendations with respect to the specimen collection period and period of influenza activity, but there is a tendency to collect fewer specimens than recommended as the age of patients increases, and in the case of elderly patients (65 years and older), frequency of collection is clearly insufficient. Furthermore, sentinel physicians collect a higher percentage of specimens in cases where patients have received the influenza vaccine.

Introduction

SISS forms part of EISS, which covers 30 European countries [1]. Influenza sentinel systems are based on sentinel physicians reporting clinical cases due to influenza-like illness (ILI) and/or acute respiratory infection (ARI), and integrate clinical and virological data collected in the same population.

Via the sentinel systems based in its member countries, EISS ensures the timely collection of epidemiological and virological data and the weekly dissemination of such data during influenza seasons. All physicians belonging to the respective sentinel networks report diagnosed cases of influenza as per a case definition, obtain nasal or nasopharyngeal swabs, or nasopharyngeal aspirates from patients, and then send these specimens to the national reference laboratories for confirmation of diagnosis and characterisation of the influenza viruses in circulation.

EISS is drafting recommendations, which are still under discussion (Tamara Meerhoff, personal communication), to standardise the sentinel swabbing routine used in the networks. The recommendations, which EU Member States would have to adapt, refer to the population in which specimens are to be obtained; the periods in which such specimens must be collected; and the manner of collection and dispatch. This study sought to:

- ▶ Characterise the swabbing pattern in SISS;
- ▶ Ascertain to what extent the system meets the guidelines currently being drafted by the EISS; and
- ▶ Propose any necessary corrections, where applicable.

Methods

Data on four seasons from 2002/2003 to 2005/2006 were drawn from SISS. We included the number of influenza cases reported to this system each season by the sentinel networks that sent individualised data to the central unit (2002/2003: Aragon, Balearic Islands, Canary Islands, Castile & León, Valencian Region and the Basque Country; 2003/2004 and 2004/2005: the above-mentioned plus Castile-La Mancha, Extremadura, Navarre and La Rioja; 2005/2006: each above-mentioned Region plus Catalonia and Ceuta).

In Spain, 16 Autonomous Regions (AR) (Comunidades Autónomas) had influenza sentinel networks in place during the 2005/2006 season, accounting for approximately 90% of the population nationwide, with a total of 413 general practitioners (GPs), 125 paediatricians and 15 support laboratories. The population covered by the system in the 2005/2006 season numbered 771,133, giving an overall coverage of 1.78% of the total population of Spain's 16 AR. Similarly, all the networks complied with a series of requirements as to the minimum population covered (>1%) and representativeness in terms of age, sex and degree of urbanisation.

Clinical information was obtained from network sentinel GPs and paediatricians, who participated on a voluntary basis and submitted individualised reports of all medical visits attributable to influenza syndromes detected in their reference populations in accordance with a case definition as per the International Classification of Health Problems in Primary Care (ICHP-2-D) for "influenza-like illness" (context of influenza epidemic, plus four of the following criteria: onset within 12 hours of cough, fever, chills, prostration and weakness, myalgia or general pain, rhinitis, pharyngitis, contact with a case; or six of those criteria) within the surveillance periods identified as the winter seasons (usually, from week 40 of one year to week 20 of the next). For virological influenza surveillance, sentinel physicians obtained nasal or nasopharyngeal swabs or nasopharyngeal aspirates from a subset of patients, which were then sent to network-affiliated laboratories for determination of influenza virus.

The dossier collected on each case includes epidemiological and clinical data, with virological data incorporated later. These individualised data, together with the population coverage achieved, are available at the central unit within a period of 24 to 48 hours after the end of each week. This allows for swift dissemination of the information on the evolution of influenza activity in Spain,

through periodic reports that are systematically updated on the Internet (see <http://vgripe.isciii.es/gripe/inicio.do>).

The study analysed the collection and dispatch of swab specimens for virological analysis by reference to variables relating to: patient sex, age group and vaccination status; and specimen collection period, period of influenza activity, time of swabbing and epidemiological season. We defined the “influenza activity period” as corresponding to the epidemiological weeks of each season in which influenza incidence exceeded the baseline activity threshold, and the “influenza activity-free period” as the remaining weeks, which tend to coincide with the start and end of the surveillance seasons.

When it came to characterising the time of swabbing in the course of the epidemiological week, we defined “time of swabbing” as the difference between the date of dispatch of the specimen to the laboratory and the middle day of the relevant reporting week (Wednesday, as the epidemiological weeks runs from Sunday to Saturday), in the four seasons analysed.

Firstly, we described the relative frequency of swabbing vis-à-vis the remaining variables reported (calculation of percentages of dispatch of specimens and their variability, including test for trend and deviation from linearity). In a second step, a multivariate logistic regression model was used to estimate the adjusted effects (odds ratio, OR) of the same variables on the performance of a swab specimen. All data analyses were performed using the SPSS v14.0 and Stata v8.0 computer software programmes.

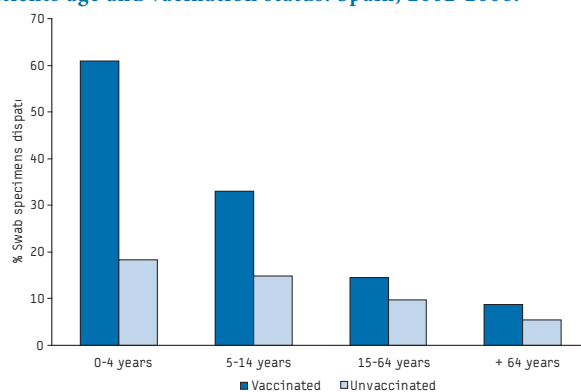
Results

Univariate analysis of the first part of this study showed that during the last four influenza seasons (2002-2006), sentinel network physicians obtained 4,005 swab specimens for dispatch to system-affiliated laboratories, which represented a swab percentage of 11.53% vis-à-vis cases reported with influenza syndrome. When the distribution of this percentage was analysed by reference to patient-related epidemiological variables, significant variations were

observed in terms of sex, age and vaccination status (Table 1), with a higher relative frequency of specimens being dispatched for males versus females and for vaccinated versus unvaccinated patients. There was a gradual decline in swabbing with patients' age. Analysis of this showed a significant trend ($X^2(1\text{ gl})=345.62$; $p<0.001$), which did not deviate from linearity ($X^2(2\text{ gl})=2.57$; $p=0.277$).

Figure 1 shows the percentage of specimens collected from vaccinated and unvaccinated patients according to patients' age. For each age group, there was a significantly higher ($p<0.001$) percentage of swab specimens collected and dispatched among vaccinated versus unvaccinated patients. Differences were more pronounced in patients aged under 15 years old, with triple or double the percentage of swabs collected and dispatched to laboratories for vaccinated versus unvaccinated patients among the 1-4 and 5-14 age groups respectively.

FIGURE 1
Percentage of collected and dispatched swab specimens with respect to number of cases reported with influenza, by patients age and vaccination status. Spain, 2002-2006.



When the characteristics of swabbing for virological analysis were studied in reference to epidemic period, we observed that, with respect to total reported cases, the percentage of specimens collected was lower in periods of influenza activity, though in absolute numbers, the number of specimens dispatched in such periods was logically higher (Table 2). Furthermore, there were also significant variations when the indicator was analysed by season, with a high percentage of swabbing in the last season, 2005/2006. Indeed, the swabbing percentage was almost double that of the previous seasons (Table 2).

When time of swabbing for virological analysis was characterised, we observed, firstly, that the date of specimen dispatch to the laboratory was reported in 3,581 cases, accounting for 90.3% of all swab specimens taken. In 98% of such cases, the patients' swab specimens were obtained by the physicians in the three days prior or subsequent to the middle day of the epidemiological week in which the case was reported, whereas specimens collected at longer time intervals accounted for 2% of the total volume of swabs performed (data not shown).

The multivariate analysis undertaken in the second part of this study assessed the independent effects exerted by each of the patient-related and time-of-swabbing variables on the collection and

TABLE 1

Swab specimens collected in the Spanish Influenza Sentinel Surveillance System, by sex, patient age and vaccination status, in the period 2002-2006

	No. of swab specimens dispatched	% Swab specimens dispatched*	P value**
Sex			
Male	2,152	12.2	0.02
Female	1,831	11.1	
Age group			
0-4 years	498	18.8	<0.001
5-14 years	1184	15.0	
15-64 years	2123	10.1	
65 years and older	199	6.5	
Vaccination status			
Vaccinated	383	13.2	<0.001
Unvaccinated	3,546	11.6	

*Percentage of collected and dispatched swab specimens with respect to number of cases reported with influenza syndrome.
**Chi-squared test was used.

TABLE 2

Table 2. Collection and dispatch of swab specimens, by epidemic period and study season, Spain, 2002-2006

	No. of swab specimens dispatched	% Swab specimens dispatched*	P value**
Epidemic period			
No influenza activity	1,245	19.7	<0.001
Influenza activity	2,760	12.1	
Influenza season			
2002-2003	611	11.2	<0.001
2003-2004	776	10.7	
2004-2005	1,231	8.5	
2005-2006	1,387	18.6	

*Percentage of collected and dispatched swab specimens with respect to number of cases reported with influenza syndrome.

**Chi-squared test was used.

dispatch of swab specimens. Table 3 shows the adjusted OR of the above variables along with their 95% confidence intervals, obtained with logistic regression analysis in a model that included collection and dispatch of swab specimens as the dependent variable, and the remaining variables analysed as the independent variable. All the relationships observed in the univariate analyses remained in evidence after the multivariate analysis. Independently, the collection of swab specimens was linked to age (proving significantly lower in the oldest age groups), sex (higher in males than females) and vaccination record (higher among patients who reported being previously vaccinated). Similarly, the percentage of collection of clinical specimens was lower in periods of peak influenza activity, and significantly higher in the last season, 2005/2006, than in the previous three.

Discussion

A recent EISS analysis on swab forms used by the 30 countries reporting to it reveals that 17 of them, including Spain, show appreciable improvement in terms of the information collected in the most recent season compared to the previous one, and meet the EISS requirements (Tamara Meerhoff, personal communication). Nevertheless, although there are clear recommendations about swab specimen collection procedures in the Spanish system, there are no strict rules as to the number of swab specimens and/or specific population groups in which such specimens are to be collected, in view of the fact that this largely depends on the capacity of the support laboratories and specimen-dispatch logistics.

The number of swab specimens collected and dispatched for virological confirmation in the first two seasons (2002/2003 and 2003/2004) was clearly lower than that in the last two seasons (2004/2005 and 2005/2006). We feel that this could be due to the fact that in the 2003/2004 season, SSISS was enlarged by the addition of four more regional networks that did not consolidate their operations until the 2004/2005 season, the first in which a substantial increase was observed in swab specimens collected by sentinel physicians. Furthermore, in the 2004/2005 season, Spain underwent influenza activity of greater intensity than it had in the preceding seven seasons, with elevated incidence rates across all age groups during the upward phase of the epidemic wave [2]. This situation may have influenced sentinel physicians' readiness to collect a greater number of swab specimens from patients seeking medical attention during the following season, something that may

in turn account for the higher swabbing percentage observed in the last season, 2005/2006, compared to previous seasons.

EISS' draft guidelines, which are still under discussion, indicate that swabbing must be performed during all phases of the epidemic, although it should be boosted at the start and end of the season to ensure that the surveillance system fulfils an early alert function and enables detection of possible strains in circulation. In Spain, SSISS adapts to this recommendation inasmuch as physicians collect respiratory swab specimens from patients throughout the season, although the percentage of swabbing is relatively higher in periods of reduced influenza activity.

In addition, EISS' draft guidelines recommend that the percentage of the collection of specimens should be at least 10% across all age groups. In Spain, as in other European countries [3], a lower number of swab specimens is collected in the 0-4 and 65-and-over age groups. However, while the Spanish system falls short of the necessary percentage of the collection and dispatch of swab specimens in the group of patients aged 65 years and older (6.5%), it exceeds the recommended minimum in patients under the age of 15 years. Indeed, as can be seen from the results, though the percentage collection of swab specimens for all cases reported in the four seasons (11.4%) surpasses the EISS requirement, there is nevertheless a tendency to collect fewer specimens as the age of patients increases, and in the case of elderly patients frequency of collection is clearly insufficient. Accordingly, we would like to encourage specimen collection by GPs in patients aged 65 years and older; to meet EISS guidelines (which require collection of specimens to be at least 10% in all age groups), one third more specimens would have had to be collected among the 65 and older age group (up to 300 specimens in the four seasons studied).

Moreover, history of influenza vaccination in the current season has also been observed to influence swab specimen collection, in the sense that sentinel physicians collect a higher percentage of specimens in cases where patients have received the influenza vaccine. Our study reveals that the younger the patient, the greater the influence of vaccination status on the collection of swab specimens. This is particularly so in the under-15-year group, where the proportion of specimens collected by sentinel physicians among vaccinated children is double (5-14 years) or triple (0-4 years) that among unvaccinated children. This is a major selection bias that could interfere when it comes to assessing the effectiveness of the influenza vaccine. Spain has one of the highest influenza vaccination coverages in the world [4], which in the 2005/2006 season was 70% among the 65 years and older segment of the population [5]. General coverages in Spain decline sharply among persons aged 64 years or under and are estimated to be 6% among children [6]. These figures heighten the importance of the influence of vaccination status on the collection of swab specimens in SSISS, since it is in age groups with lower influenza vaccination coverages that physicians take a far higher proportion of specimens from vaccinated versus unvaccinated patients.

SSISS sentinel physicians have been shown to be representative of the population for age and geographic distribution, including rural and urban distribution [7]. However, the influence exerted by those factors mentioned above may translate as selection bias when it comes to swabbing the monitored population, the basis of virological information that is essential for surveillance. The system's capacity for reliable detection of influenza viruses circulating in the Spanish population could be limited if a certain

degree of representativeness in the collection of specimens and an adequate number of specimens cannot be ensured. The existence of other factors pertaining to the availability of means for collection and transport of specimens is doubtless also a determinant in the selection of cases for virological confirmation, but a lack of knowledge of such factors renders it impossible to judge the role that they play. As a result, the specimen collection protocol of the Spanish System stresses that respiratory specimens for viral isolation, or viral detection of nucleic acids or antigens, be collected in the first four days of the disease, as this is the period of maximum viral excretion and laboratory results depend on good timeliness of swabbing [8]. However, a lack of data on the date of symptom onset among cases (a variable registered by the system but not available in our analysis) prevented us from assessing its determinant role in the selection of specimens. The only fact established was that in 98% of cases specimens were dispatched for analysis in the week when they were collected.

The above problems of representativeness could be resolved by requiring systematic random procedures in the system for collecting swab specimens from patients. However, it is not clear how feasible such procedures would be for a population-based surveillance system with so many reporting physicians and so many limitations in terms of laboratory and preservation resources and dispatch of specimens. For the present, our criterion, akin to the guidelines proposed by EISS, is to continue to insist that a sufficient number of appropriate specimens be obtained at all ages during periods of least activity; likewise, it is essential that the importance of collecting swab specimens, regardless of patients' vaccination status and/or age, be recalled at the beginning of each influenza season. Both recommendations will contribute to ensure early detection of the circulation of influenza virus in the European continent, one of the fundamental goals of any influenza surveillance system.

* This group was formed by the Sentinel General Practitioner Networks of Andalusia, Aragon, Asturias, Balearic Isles, Canary Islands, Cantabria, Castile-La Mancha, Castile & León, Catalonia, Valencian Region, Extremadura, Madrid, Navarre, Basque Country, La Rioja and Ceuta, in collaboration with the following laboratories that participated in virological surveillance: Influenza Centre (WHO), National Microbiology Centre, Majadahonda-Madrid; Influenza Centre (WHO), Valladolid Faculty of Medicine; Influenza Centre (WHO), Barcelona Clinical Hospital; Virgen de las Nieves Hospital, Granada; Miguel Servet Hospital, Zaragoza; Nuestra Señora de

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