Table 1
Viruses detected by polymerase chain reaction in hospitalized children in the flu epidemic weeks.

	Number of identified virus (%)						
	2008–09	2009	2010–11	2011–12	2012–13	2013–14	2014–15
Months	D–F	S–D	D–F	D–F	D–F	D–F	D–F
Patients	229	341	314	248	206	117	157
RSV	121	102	99	121	87	68	89
Rhinovirus	64	118	63	56	61	27	44
Human bocavirus	24	41	45	33	16	7	7
Adenovirus	17	28	43	16	17	8	19
Parainfluenza (1, 2, 3, 4)	15	40	7	7	7	4	3
Human metapneumovirus	8	0	6	8	1	1	6
Influenza (A, B, C)	28	38	24	14	14	7	10
Enterovirus	9	7	2	5	7	1	4
Coronavirus	2	6	1	12	2	0	5
Negative	34	69	98	51	49	18	29

RSV: respiratory syncytial virus, D: December, F: February, S: September.
The number of total virus is superior to patients because the presence of co-infections.

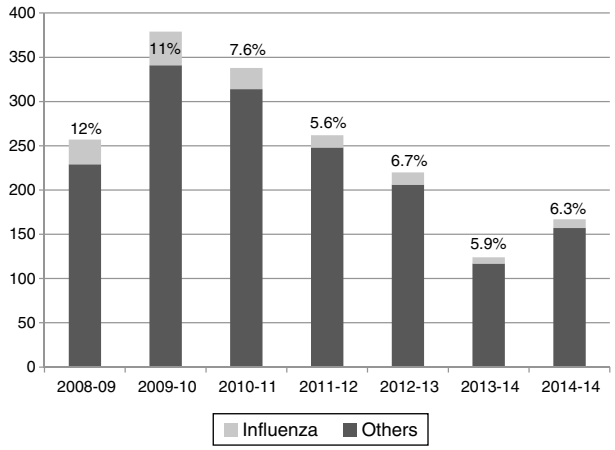

Fig. 1. Influenza cases during the 12 weeks of highest incidence of flu, in hospitalized children.

the further development of new anti-influenza drugs might be the best way for control.[5] Based on our personal experience, we recommend making an effort to improve diagnosis in children with suspected influenza, performing molecular techniques or a rapid diagnostic test with high sensitivity,[6] especially in children with risk factors and children requiring hospitalization. This may help to guide treatment with oseltamivir to patients who really need it.

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Evaluation of combined use of the MALDI-TOF and GenomEra MRSA/SA assay for the direct detection of methicillin resistance in *Staphylococcus aureus* from positive blood culture bottles

Evaluación del uso combinado de ensayo de MALDI-TOF y Genomera MRSA/SA para la detección directa de la resistencia a la meticilina en Staphylococcus aureus *de botellas de hemocultivo positivo*

Staphylococcus aureus bacteremia has been associated with high mortality rates, prolonged hospitalization and increased economic cost. Delay in the initiation of appropriate antimicrobial therapy is known to be an important determinant in clinical outcomes. Therefore, rapid identification of Staphylococcal species and susceptibility profiles in patients with bacteremia assist in the early optimization of therapy that would have a positive clinical impact.[1–2]

The aim of this study is to evaluate the *GenomEra MRSA/SA assay* in combination with MALDI-TOF MS for rapid detection of MRSA and MSSA in positive blood culture bottles.

The *GenomEra MRSA/SA Diagnose* (Abacus Diagnostica, Oy, Finland) is a fully automated closed tube PCR assay that simultaneously detects *S. aureus* specific DNA and a sequence within the *mecA* gene encoding for methicillin resistance with time-resolved fluorescence labels.[3] The sequence of the SA marker has not yet been published, but according to the manufacturer, it

is a new, highly conserved nucleic acid target on the *S. aureus* genome.

Over a period of 16 months, from January 2013 to April 2014 the *GenomEra MRSA/SA assay* was performed to positive blood culture bottles where *S. aureus* ($n = 112$) had been previously identified by MALDI-TOF MS.

Two automatic systems were used in this study: the *BacT/Alert system* (bioMérieux, Durham, NC) and the *Bactec systems* (Becton Dickinson, Sparks, MD). When a blood culture was flagged positive, and a Gram stain confirmed the presence of Gram-positive cocci in clusters, direct identification was done by MALDI-TOF mass spectrometry.[4–5] All positive blood cultures were processed in parallel by conventional microbiological methods. The susceptibility studies were done using the susceptibility cards of VITEK 2 AST-P588 (bioMérieux).

For the analysis of blood culture samples, the *GenomEra MRSA/SA assay* was performed following the manufacturer's instructions.[6] Invalid kit results and mechanical errors were discarded for further analysis.

During the study period, the average turnaround time to identification of MRSA/SA was less than 3 h. A total of 112 blood culture bottles containing *S. aureus* were randomly selected and analyzed. Thirty out of these 112 were methicillin resistant and 82 out of the 112 samples were methicillin susceptible. The GenomEra assay correctly detected all 82 MSSA isolates, and 29 out of the 30 MRSA isolates. In the kit-negative specimen, the *S. aureus* specific DNA was detected but the *mecA* gene was not detected and the system reported the assay as MSSA. In this sample a pure culture of *S. aureus* was confirmed, the susceptibility studies were done using VITEK 2 AST-P588 susceptibility cards (bioMérieux). The strain was identified as MRSA by the VITEK 2 system, although oxacillin MIC was low, 0.5 μg/mL. In order to confirm the diagnosis of MRSA, detection of the *mecA* gene by PCR and the agglutination of PBP2a assay were done resulting both tests negative. In view of these results further investigations were carried out and a specific PCR verified the presence of the *mecC* gene.[7–8]

The sensitivity and specificity of the GenomEra MRSA/SA assay were 96.67% and 100%, respectively (95% confidence intervals, 80.95–99.83% and 94.42–99.89%, respectively). The accuracy of our testing methods using the MALDI-TOF and GenomEra MRSA/SA system was 99.1% with a negative predictive value and positive predictive value of 98.8% and 100%, respectively (95% confidence intervals, 92.54–99.94% and 85.44–99.69%, respectively).

Some authors have reported the use of different commercial PCR assays in positive blood cultures contained Gram-positive cocci in clusters.[6,9] An important drawback of this approach might be the high cost of the test. The inclusion of MALDI TOF-MS identification to select blood cultures containing *S. aureus* would allow optimization of the test, and reduce of costs.

Methicillin resistance is mainly due to the presence of the *mecA* gene, which encodes a modified penicillin-binding protein (PBP2a) that has low affinity for b-lactams. However, it has been recently reported in different European countries isolates of MRSA carrying the *mecC* gene causing human infections.[7,8] This *mecC* gene is not detected by the commercial PCR assays most frequently used to detect rapidly MRSA in blood cultures.[7] In those cases where a *mecC* gene is present we would obtain a false negative as a result (MSSA instead of MRSA). Although nowadays the rates of human infection caused by MRSA carrying *mecC* gene are low, this is a matter of concern when this system is used.

In overall, compared to the conventional methods, significant improvements are achieved in the speed and accuracy of *S. aureus* methicillin resistance detection in blood culture samples using the GenomEra MRSA/SA assay in combination with MALDI-TOF identification. This time reduction in the diagnosis of MRSA might have a significant clinical impact in patients with bacteremia.

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Conflict of interest

None of the authors declare conflicts.

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