Everybody hands-on to avoid ESKAPE: effect of sustained hand hygiene compliance on healthcare-associated infections and multidrug resistance in a paediatric hospital

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Abstract

Purpose. Hand hygiene is the most important strategy for preventing healthcare-associated infections (HCAIs); however, the impact of hand hygiene in middle-income countries has been poorly described. In this work, we describe the impact of the programme ‘Let’s Go for 100’ on hand hygiene adherence, HCAIs rates and multidrug-resistant (MDR) bacteria, including the molecular typing of methicillin-resistant Staphylococcus aureus (MRSA) strains.

Methodology. A multimodal, hospital-wide hand hygiene programme was implemented from 2013. ‘Let’s Go for 100’ involved all healthcare workers and encompassed education, awareness, visual reminders, feedback and innovative strategies. Monthly hand hygiene monitoring and active HCAI surveillance were performed in every ward. Molecular typing of MRSA was analysed by pulsed-field gel electrophoresis (PFGE).

Results/Key findings. Hand hygiene adherence increased from 34.9 % during the baseline period to 80.6 % in the last 3 months of this study. The HCAI rate decreased from 7.54 to 6.46/1000 patient-days (P=0.004). The central line-associated bloodstream infection (CLABSIs) rate fell from 4.84 to 3.66/1000 central line-days (P=0.05). Negative correlations between hand hygiene and HCAIs rates were identified. The attack rate of MDR-ESKAPE group bloodstream infections decreased from 0.54 to 0.20/100 discharges (P=0.024). MRSA pulsotypes that were prevalent during the baseline period were no longer detected after the 5th quarter, although new strains were identified.

Conclusions. A multimodal hand hygiene programme in a paediatric hospital in a middle-income country was effective in improving adherence and reducing HCAIs, CLABSIs and MDR-ESKAPE bloodstream infections. Sustaining hand hygiene adherence at a level of >60 % for one year limited MRSA clonal transmission.

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Keywords: MRSA; multidrug resistance; hand hygiene; adherence; HCAIs; PFGE.

Abbreviations: CAUTIs, catheter-associated urinary tract infections; CC, clonal complexes; CDC, Centers for Disease Control and Prevention; CLABSIs, central line-associated bloodstream infection; CS, cardiovascular surgery; DHE, Department of Hospital Epidemiology; EM, emergencies; EN, endocrinology; ESKAPE, Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa, and Enterobacter species; GA, gastroenterology; GS, general surgery; HCAIs, healthcare-associated infections; HIMFG, The Children’s Hospital of Mexico Federico Gómez; ICU, intensive care units; IM, internal medicine; IT, Intermediate Therapy; MDR, multidrug-resistant; MRSA, Methicillin-resistant Staphylococcus aureus; ND, nursing department; NICU, neonatal intensive care unit; NS, neurosurgery; PFGE, pulsed-field gel electrophoresis; PICU, pediatric intensive care unit; P, pulsotypes; qt, quarter or quarterly; ST, surgical therapy; UPGMA, unweighted pair group method with arithmetic mean; VAP, ventilator-associated pneumonia; VRE, vancomycin-resistant E. faecium; WHO, World Health Organization.

Two supplementary figures are available with the online version of this article.
INTRODUCTION
Healthcare-associated infections (HCAIs) are a significant cause of mortality worldwide [1, 2], affecting 4 to 20 out of every 100 patients admitted to acute care hospitals. Annual costs for the treatment of these infections have been estimated at €7 billion in Europe and US$ 6.8 billion in the USA [3]. Hand hygiene is the single most important measure for preventing HCAIs [4–9]. However, compliance is low at many centres in both high- and low-income countries. Implementation of a multimodal strategy from the World Health Organization (WHO) has successfully improved hand hygiene adherence in many settings [10–13]. Nevertheless, data on the long-term maintenance of hand hygiene programmes and their impact on HCAIs and multidrug-resistant (MDR) microorganisms in middle-income countries are scarce, especially in paediatric hospitals. Bacteria from the ESKAPE group (Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa and Enterobacter spp.) are common causes of life-threatening HCAIs. Most of these microorganisms are MDR, which is one of the greatest challenges in clinical practice [14, 15]. Hand hygiene is the most important measure for preventing nosocomial infections and has proven to be effective in reducing the attack rate of methicillin-resistant Staphylococcus aureus (MRSA). Some authors have described the effects of infection control measures in reducing MDR bacteria from the ESKAPE group [16–18]. However, the direct impact of hand hygiene on the incidence of these pathogens has rarely been analysed. Furthermore, the correlation between pulstotypes, as indicated by pulsed-field gel electrophoresis (PFGE)-molecular typing of MRSA strains and hand hygiene adherence, has not been addressed. The Children’s Hospital of Mexico Federico Gómez (HIMFG), a public teaching and referral paediatric hospital, has implemented the programme ‘Let’s Go for 100’ (in Spanish, 100 is spelled CIEN, which is the acronym for Infection Control by Integration and Innovative Strategies) based on a multimodal hand hygiene strategy, with the goal of involving 100% of the staff and periodically adding innovative strategies. The aim of this work is to describe the impact of the programme on hand hygiene adherence, HCAIs rates and MDR organisms, including PFGE-molecular typing of MRSA strains during the programme.

METHODS
Setting
This study was performed in a 349-bed tertiary care paediatric teaching hospital that treats more than 7000 patients yearly, mostly serving a low-income population without medical insurance. The hospital has three intensive care units (neonatal, surgical and medical), as well as medical and surgical wards. The main causes of hospitalization are malignant tumours (35%), birth defects (21%), gastrointestinal pathologies (6.6%) and respiratory diseases (6.2%). In September 2013, the ‘Let’s Go for 100’ programme began hospital-wide, and the period between January and August 2013 was considered the baseline period.

Hand hygiene promotion during the baseline period
Monthly training for paediatric residents, on hand hygiene and blood sampling, were provided and presentations of HCAIs rates to hospital leaders were given on a routine basis. Hand hygiene posters were placed in bathrooms but not in clinical areas. Alcohol-based hand rubs were located on nursing trolleys and in every patient unit at the point of care.

‘Let’s Go for 100’ programme
Hands, the number 100, the infinity symbol and a hug were included in the programme logo (Fig. S1, available in the online version of this article). The goal was to accomplish complete and permanent hand hygiene by integrating all hospital staff (Fig. 1). Briefly, this programme consisted of the following components: 1) Medical and administrative leaders, including the head of the Workers’ Union, were convinced to support the program. 2) In every patient unit, alcohol-based hand rubs were placed at the point of care. 3) Periodic education programmes were individualized for each group of healthcare workers (attending physicians, nurses, residents, students and ancillary staff) that highlighted the mortality and costs associated with HCAIs, as well as the efficacy of hand hygiene, including scientific evidence and local data. Incidental education during hospital visits was also performed on a regular basis. 4) Innovative strategies were developed ‘motu proprio’ by different groups. 5) Monthly feedback on HCAIs rates and hand hygiene adherence was provided by group and for the whole hospital. 6) Attractive graphic reminders were located in every ward and changed every 4 months.

Hand hygiene monitoring
Monthly hand hygiene monitoring was carried out covertly in every ward by nursing students according to the hand hygiene tools following a WHO technical reference manual [19]. Hand washing or rubbing the hands with an alcohol-based preparation were both considered to be hand hygiene actions.

Surveillance for HCAIs
Trained nurses from the Department of Hospital Epidemiology (DHE) carried out active surveillance in every ward and reviewed microbiology laboratory results daily from Monday to Friday. The aim was to detect HCAIs and to educate healthcare workers and patients’ relatives. Two infectious disease physicians and one specialized nurse from the DHE reviewed every suspicious case. Centers for Disease Control and Prevention (CDC) definitions [20] were used for the diagnosis of central line-associated bloodstream infections (CLABSIs), ventilator-associated pneumonia (VAP) and catheter-associated urinary tract infections (CAUTIs). For other infections, the definitions were obtained from Mexican regulations. Pathogens were regarded as MDR if they were non-susceptible to ≥1 agent.
in ≥3 antimicrobial categories according to Magiorakos et al. [21].

**Resistance profile and molecular characterization of MRSA strains**

The Kirby–Bauer method was employed to identify MRSA phenotypes according to CLSI-2017. PFGE assays were performed according to previously described protocols [22]. In this study, one MRSA isolate per healthcare-associated bloodstream infection case was included.

**Statistical analysis**

For the statistical analysis, we used Stata software (version 10.1/SE, Stata Corporation, College Station, TX, USA). The Cochran Armitage and Cuzick non-parametric tests were used to analyse trends in hand hygiene adherence and rates of HCAIs, CLABSIs, VAP and CAUTIs; and MDR-ESKAPE...
attack rates for all microorganisms, both together and separately. We used robust regression analysis to determine the relationships between hand hygiene adherence and these rates. \( P < 0.05 \) was considered statistically significant. PFGE pulsortypes were analysed using NTSYS-pc software (version 2.0 Applied Biostatistics, Inc., NY, USA) with the Jaccard similarity coefficient and cluster analysis using the unweighted pair group method with arithmetic mean (UPGMA). The clonality of the MRSA strains was evaluated using the Tenover criteria [23].

**RESULTS**

Between January 2013 and October 2016, 27,975 patients were discharged from the hospital, yielding a total of 266,524 patient-days, 111,642 central line-days, 30,218 ventilator-days and 26,327 urinary catheter-days, as well as 18,660 clean and clean-contaminated surgeries (Table 1). During this period, 30,281 hand hygiene opportunities were registered through monthly observation audits. Nurses, residents and students comprised 75.14% of the observations (Table 1). A total of 1025 education sessions were provided to 17,677 health care workers, and 1254 posters, including 18 banners, were displayed in different areas of the hospital.

**Hand hygiene adherence**

The baseline hand hygiene adherence during the first 8 months was 34.9% (SD 3.52), and this exhibited a statistically significant (\( P = 0.0001 \)) increasing trend throughout the study period, reaching 80.6% (SD 6.3) during the last 3 months of follow-up (Fig. 2). The increase in hand hygiene was statistically significant for hand washing and alcohol-based products \( (z=2.78 \text{ and } P=0.005) \) but not for washing hands \( (z=0.32 \text{ and } P=0.745) \) (data not shown). Hand hygiene adherence increased across all healthcare groups throughout the intervention (Fig. S2).

**HCAIs and hand hygiene**

A total of 1916 HCAIs in 266,524 patient-days were detected during the study period. The rate of HCAIs decreased from 7.54/1000 patient-days (SD 1.82) to 6.46/1000 patient-days (SD 0.32) \( (P=0.004; \) Fig. 2). CLABSIs rates also decreased, from 4.84/1000 central line-days (SD 2.25) at baseline to 3.66/1000 central line-days (SD 0.99) at the end of the programme \( (P=0.05) \) (data not shown). The CAUTIs, VAP and surgical site infection rates did not significantly decrease following implementation of the programme \( (P>0.5) \). Negative correlations between hand hygiene adherence and the rates of HCAIs \( (z=0.07 \text{ to } -0.01, P=0.008) \) and CLABSIs \( (z=-3.02, 95 \% \text{ C.I. } -5.62 \text{ to } -0.41, P=0.02) \) were identified; however, there were no significant associations with other types of infection \( (P>0.5) \).

**MDR organism attack rate and hand hygiene adherence**

MDR microorganisms belonging to the ESKAPE group and MDR \( E. \) coli were isolated from blood culture samples from 4.9% of all HCAIs events (attack rate 0.34/100 discharges), yielding a total of 94 cases \( (E. \) faecalis: 4, \( S. \) aureus: 21, \( K. \) pneumoniae: 41, \( A. \) baumannii: 4, \( P. \) aeruginosa: 7, \( Entero- \) bacter spp.: 2, and \( E. \) coli: 15). The attack rate for MDR-ESKAPE group bloodstream infections significantly decreased, from 0.54/100 discharges (SD 0.34) to 0.20/100 discharges (SD 0.082) at the end of the study period \( (z=-2.25, P=0.024) \) (Fig. 3). When the analysis was performed for each pathogen separately, there were significant decreases in the attack rates for vancomycin-resistant \( E. \) faecium (VRE) and MRSA \( (z=-2.01, P=0.04 \text{ and } z=-1.89, P=0.05, \) respectively), but this pattern was not observed for Gram-negative pathogens. However, there were no cases of MDR \( K. \) pneumoniae, \( Enterobacter \) spp. or \( P. \) aeruginosa infection during the months when hand hygiene adherence was >80%. We observed a negative correlation between hand hygiene adherence and attack rate, not only for MRSA \( (\text{coef. } -17.10, 95 \% \text{ C.I. } -30.67 \text{ to } -3.53, P=0.019) \) and VRE \( (\text{coef. } -54.87, 95 \% \text{ C.I. } -73.28 \text{ to } -36.46, P=0.001) \) but also for \( Enterobacter \) spp. \( (\text{coef. } -33.04, P=0.002, 95 \% \text{ C.I. } -51.14 \text{ to } -14.94) \) and the MDR-ESKAPE group overall \( (-7.76, 95 \% \text{ C.I. } -15.08 \text{ to } 0.37, P=0.059) \) (Fig. 3).

**Genetic relationships among MRSA clinical strains**

A total of 17 DNA pulsotypes that grouped into three clusters (I–III) were identified, revealing patterns involving 11–18 DNA fragments ranging in size from 48.5 to 388 Kb (Fig. 4). Briefly, 33.33% \( (7/21) \) of the pulsotypes grouped in cluster I (pulsotypes 5, 10, 13, 16, 14, 17 and 15), 9.52% \( (2/21) \) grouped in cluster II (pulsotypes 3 and 12) and 57.14% \( (12/21) \) grouped in cluster III (pulsotypes 1, 2, 4, 6, 7, 8, 9 and 11). In cluster I, 42.85% \( (3/7) \) of the MRSA clinical strains with ≥80% genetic similarity were grouped into two sub-clusters (5 and 6). In cluster II, no genetic relationship between the two MRSA clinical strains was identified. In cluster III, 91.66% \( (11/12) \) of the strains with ≥80% genetic similarity were grouped into six sub-clusters (9, 11, 12, 13, 14 and 15) (Fig. 4).

**Association between MRSA pulsotypes and hand hygiene adherence**

A clonal relationship among MRSA clinical strains was observed in pulsotypes 6 (subgroup 12) and 7 (subgroup 9) in cluster III during this study. In addition, the other MRSA strains were diverse and located in the other subgroups. A reduction in the number of MRSA strains was observed during the first year after implementing the multimodal hand hygiene programme; however, pulsotypes 6 and 7 were still identified during this 12-month period. Interestingly, the most prevalent pulsotypes, 6 and 7, were no longer detected after one year with sustained hand hygiene adherence above 60%, but new, unrelated MRSA pulsotypes (P8 to P17) were identified (Figs 4 and 5).

**Discussion**

In this study, we describe the implementation and maintenance of a successful hand hygiene programme in a paediatric teaching hospital in a middle-income country and its impact on HCAIs and MDR bacterial infections. The
Table 1. Hand hygiene opportunities and patient data during the study period

<table>
<thead>
<tr>
<th>Patient-days</th>
<th>Baseline 1st qt</th>
<th>Baseline 2nd qt</th>
<th>1st qt</th>
<th>2nd qt</th>
<th>3rd qt</th>
<th>4th qt</th>
<th>5th qt</th>
<th>6th qt</th>
<th>7th qt</th>
<th>8th qt</th>
<th>9th qt</th>
<th>Last 3 months</th>
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<td></td>
<td>15 680</td>
<td>22 085</td>
<td>21 686</td>
<td>20 492</td>
<td>22 608</td>
<td>24 381</td>
<td>22 961</td>
<td>24 335</td>
<td>24 857</td>
<td>24 260</td>
<td>24 787</td>
<td>18 392</td>
<td>266524</td>
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<tr>
<td>Central line-days</td>
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<td>10 078</td>
<td>9084</td>
<td>10 271</td>
<td>9569</td>
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<td>9628</td>
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<td>111642</td>
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<td>2696</td>
<td>2736</td>
<td>2585</td>
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<td>2535</td>
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<td>2920</td>
<td>2081</td>
<td>1845</td>
<td>30 218</td>
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<td>Clean and clean-contaminated surgeries</td>
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<td>1469</td>
<td>1739</td>
<td>1702</td>
<td>1510</td>
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<td>1374</td>
<td>1873</td>
<td>1121</td>
<td>18 660</td>
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<td>Urinary catheter-days</td>
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<td>2185</td>
<td>2133</td>
<td>2114</td>
<td>2259</td>
<td>1877</td>
<td>2081</td>
<td>2477</td>
<td>2300</td>
<td>2435</td>
<td>2273</td>
<td>26 327</td>
<td>26 327</td>
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<td>Total Hand hygiene opportunities</td>
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<td>2942</td>
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<td>2717</td>
<td>3382</td>
<td>3849</td>
<td>4693</td>
<td>18 521</td>
<td>30 281</td>
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<td>Hand hygiene opportunities by profession</td>
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<td></td>
<td></td>
<td></td>
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<td></td>
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</tr>
<tr>
<td>Nurses</td>
<td>254 (44.33)</td>
<td>152 (22.29)</td>
<td>220 (22.94)</td>
<td>478 (22.63)</td>
<td>1268 (32.19)</td>
<td>1135 (38.58)</td>
<td>891 (40.97)</td>
<td>875 (32.2)</td>
<td>1142 (35.23)</td>
<td>1357 (38.58)</td>
<td>1511 (32.2)</td>
<td>639 (25.98)</td>
<td>9902</td>
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<td>Medical residents</td>
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<td>215</td>
<td>134 (13.97)</td>
<td>292 (13.83)</td>
<td>880 (22.7)</td>
<td>611 (30.77)</td>
<td>430 (19.77)</td>
<td>578 (21.27)</td>
<td>955 (18.35)</td>
<td>739 (19.2)</td>
<td>975 (20.78)</td>
<td>652 (16.66)</td>
<td>606</td>
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<td>69</td>
<td>127 (13.24)</td>
<td>208 (9.85)</td>
<td>521 (3.44)</td>
<td>302 (30.27)</td>
<td>293 (13.47)</td>
<td>355 (13.07)</td>
<td>461 (14.22)</td>
<td>756 (19.64)</td>
<td>652 (16.66)</td>
<td>344 (9.23)</td>
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<tr>
<td>Patient caregivers</td>
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<td>91</td>
<td>83 (8.65)</td>
<td>195 (9.23)</td>
<td>492 (12.49)</td>
<td>68 (2.31)</td>
<td>77 (3.54)</td>
<td>342 (12.59)</td>
<td>254 (7.83)</td>
<td>393 (10.21)</td>
<td>569 (15.27)</td>
<td>227 (6.56)</td>
<td>2839</td>
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<tr>
<td>Physicians</td>
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<td>73</td>
<td>33 (3.44)</td>
<td>90 (4.26)</td>
<td>212 (5.47)</td>
<td>394 (13.39)</td>
<td>104 (4.78)</td>
<td>150 (5.52)</td>
<td>138 (4.26)</td>
<td>274 (7.12)</td>
<td>493 (13.98)</td>
<td>21 (6.56)</td>
<td>2216</td>
</tr>
<tr>
<td>RadioLOGY technicians and respiratory therapists</td>
<td>29 (7.33)</td>
<td>78</td>
<td>43 (4.48)</td>
<td>180 (8.52)</td>
<td>316 (8.15)</td>
<td>93 (3.16)</td>
<td>105 (4.83)</td>
<td>226 (8.32)</td>
<td>313 (9.65)</td>
<td>155 (4.03)</td>
<td>308 (8.66)</td>
<td>280 (7.02)</td>
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<td>Others</td>
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<td>66 (1.7)</td>
<td>283 (9.62)</td>
<td>245 (11.26)</td>
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<td>126 (3.89)</td>
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<td>0</td>
<td>24 (2.50)</td>
<td>99 (4.99)</td>
<td>76 (1.96)</td>
<td>34 (1.16)</td>
<td>25 (1.15)</td>
<td>91 (3.35)</td>
<td>87 (2.68)</td>
<td>145 (3.77)</td>
<td>79 (2.17)</td>
<td>130 (3.77)</td>
<td>780</td>
</tr>
<tr>
<td>Medical students</td>
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<td>0</td>
<td>7 (0.73)</td>
<td>52 (2.46)</td>
<td>66 (1.7)</td>
<td>22 (0.75)</td>
<td>5 (0.23)</td>
<td>50 (1.84)</td>
<td>126 (3.89)</td>
<td>15 (0.39)</td>
<td>53 (1.32)</td>
<td>442 (1.46)</td>
<td>187</td>
</tr>
</tbody>
</table>

All hand hygiene data are presented as n (%). qt=quarter.
success of multimodal programmes has been described previously in other low- to middle-income countries, including México [11, 24–28], although data in the paediatric setting remain scarce [5, 11, 29, 30]. A quasi-experimental study carried out in 43 hospitals found an increase in compliance from 49.8% (34.2–65.5) to 79.4% (73.3–84.4) in paediatric wards [11]. However, these increases are often not sustained. Mazi et al. [31] showed that although a multimodal approach improved the hand hygiene compliance rate, the results were not sustainable in certain critical care areas that lacked team leaders. In our study, maintenance of hand hygiene adherence was accomplished through the periodic introduction of innovative strategies, continuous education and the engagement of leaders. HCAIs rates decreased in our hospital after the implementation of the programme, showing a negative correlation with hand hygiene adherence. Several studies in both high- and low-income countries, including some studies in paediatric and neonatal settings [5, 30, 32], have reported a decrease in HCAIs as a result of improved hand hygiene compliance [4, 7, 33].

Our data show a decrease in CLABSI rates and a correlation with hand hygiene adherence. No other hospital-wide interventions associated with central line insertion and care were introduced during this period to explain this decline. Few studies have addressed the impact of hand hygiene alone on CLABSI rates. Recently, Johnson and co-workers implemented a multimodal action plan that resulted in a marked improvement in hand hygiene compliance (from 58 to 98%) and a decrease in CLABSI rates from 4.08 to 0.42 per 1000 device-days [34]. In a prospective cohort study performed in six intensive care units (ICU) in Colombia, CLABSI rates decreased rapidly after the introduction of alcohol-based hand sanitizer dispensers (−12.7% per year; \( P<0.001 \)) and remained stable thereafter in the absence of other strategies specifically targeting CLABSI prevention [32]. With respect to other device-associated infections,
VAP and CAUTI rates were not modified by the hand hygiene programme, which is consistent with previously reported results [32, 35–37], suggesting that a package of care is needed to improve these rates [36].

Remarkably, we observed a significant decrease in the attack rate of MDR-ESKAPE group bloodstream infections, as well as a negative correlation with hand hygiene adherence, after implementation of the programme. Although we did not measure adherence to contact precautions or antibiotic prescriptions in the hospital during the study period, there were no changes in the associated policies. Therefore, we assume that the reduction in MDR-ESKAPE infection rates was mainly attributable to improvement in hand hygiene. Correlations between hand hygiene adherence and MRSA incidence have been widely reported elsewhere, and MRSA attack rates have been suggested as markers of hand hygiene adherence [4, 6, 8, 37–39]. Multi-component interventions including hand hygiene, as well as other measures (antimicrobial stewardship, screening, contact precautions, selective decolonization and environmental cleaning) have been shown to be useful in controlling MDR Gram-negative bacteria [16, 40].

To investigate other MDR bacteria, Zarpellon et al. [16] carried out a strategy that included hand hygiene promotion, the isolation of patients colonized or infected by MDR bacteria with enforced contact precautions, and terminal cleaning and disinfection of rooms. Although the authors did not aim to measure the attack rate of every MDR bacterial strain, they maintained a low frequency of KPC-producing Enterobacteriaceae and demonstrated a decrease in HCAIs from 5.35% (range: 4.58–6.12%) to 3.62% (range: 3.0–4.24%) over a 10-year period. Regarding the relative contributions of different interventions to the control of MDR micro-organism transmission, Barnes et al. [18] developed a model of patient-to-patient transmission via the hands of healthcare workers and incompletely cleaned rooms in an ICU for 1 year. Nurses and physicians were modelled and demonstrated distinct hand hygiene compliance levels upon entry and exit from patient rooms. For all micro-organisms (MRSA, VRE and A. baumannii), increases in hand hygiene compliance outperformed equivalent increases in the thoroughness of terminal cleaning. Similarly, Pelat et al. [17] developed a transmission model to quantify the effectiveness of interventions (hand hygiene, cohorting and antibiotic use reduction) aimed at reducing the spread of
extended-spectrum β-lactamase-producing Enterobacteriaceae in an ICU. These data suggested that hand hygiene is the most effective intervention for the control of these pathogens, surpassing cohorting and antibiotic use reduction. However, hand hygiene per se has not been proven to lower the attack rate of Gram-negative rods. The transmission of these pathogens by other routes, such as intravenous solutions and equipment, could contribute to their hospital dissemination and explain the fact that the attack rate of Gram-negative rods was not lowered when analysed separately.

According to our PFGE analysis, a similarity of >80% among nosocomial MRSA strains in the baseline period was observed during the implementation of 'Let's Go for 100'. However, after compliance with hand hygiene improved to >60% for one year, the previous clusters were replaced by new MRSA strains, which were not related. These results indicate the impact of hand hygiene on reducing the dissemination of MRSA clones, which is associated with the appearance of new MRSA strains and probably reflects introduction by patients or healthcare workers. Similar to this finding, Lawes and collaborators reported in a national
study that the introduction of three measures (antibiotic control, a hand hygiene campaign and a reduction in the length of hospital stays) resulted in the gradual replacement of hospital MRSA clonal complexes (CC22 and CC30) with community clonal complexes, which were more diverse than the former complexes. Additionally, the authors found that the introduction of a national hand hygiene campaign together with a decrease in the length of hospital stays resulted in a reduction in the prevalence density of CC22 ($C_0$ = $0.143$, 95 % C.I. $C_0$ = $0.231$ to $0.055$, $P$ = $0.006$) [41].

On the other hand, Huang et al. reported that a decrease in the density of MRSA HCAIs, and community clones replaced the endemic clone when measures such as active screening, culturing, isolation, hand hygiene and decolonization were applied in a neonatal ICU [42]. In the present study, we found that the P6 and P7 pulsotypes were replaced by pulsotypes 10 to 17, which were associated with hospital MRSA. To our knowledge, this is the first study to analyse the effects of sustained hand hygiene adherence per se on MRSA diversity.

However, this study had several limitations. As a wide-reaching programme in which all staff received education regarding the impact of HCAIs on patients, it is possible that this information also facilitated improvements in other aspects of the daily work of personnel, such as cleaning and disinfection, resulting in decreased levels of HCAIs; however, the cleaning process was not assessed during the study. Another limitation is that we did not measure adherence to contact precautions or antibiotic prescriptions in the hospital during the study period; nevertheless, we were able to confirm that there were no changes in these policies during the programme. Finally, cases of C. difficile infection were very rare, and we did not include these in the study due to changes in definition and laboratory resources; therefore, the contribution of the programme to C. difficile incidence was not addressed in this study. In conclusion, we demonstrated that a successful hand hygiene programme involving almost every hospital staff group was sustainable and had a significant impact on HCAIs= rates and MDR-ESKAPE microorganisms, primarily MRSA and VRE. We also showed for the first time that sustained hand hygiene adherence greater than 60 % for one year could stop the spread of MRSA clones.

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Conflicts of interest
The authors declare that this experimental and clinical study was conducted in the absence of any commercial or financial relationships that could be construed as potential conflicts of interest.

Ethical statement
This study included data that are routinely collected and reported by the infection control committee and as part of the active surveillance of HCAIs, MDR and MRSA, which are considered quality improvement tools. The investigation and ethics committee approved this study under numbers HIM/2014/048 and HIM/2017/134.

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