## 1 Impact of COVID-19 on the degree of compliance with hand hygiene: a repeated Cross-

## 2 sectional study

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#### 17 ABSTRACT

18 Hand hygiene (HH) is the paramount measure used to prevent healthcare associated infections. A 19 repeated cross-sectional study was undertaken with direct observation of the degree of 20 compliance on HH of healthcare personnel during the SARS-CoV-2 pandemic. Between, 2018-21 2019, 9,083 HH opportunities were considered, and 5,821 in 2020-2022. Chi squared tests were 22 used to identify associations. The crude and adjusted odds ratios were used along with a logistic regression model for statistical analyses. Compliance on HH increased significantly (p < 0.001) 23 24 from 54.5% (95% CI: 53.5, 55.5) to 70.1% (95% CI: 68.9, 71.2) during the COVID-19 25 pandemic. This increase was observed in four of the five key moments of HH established by the 26 World Health Organization (WHO) (p<0.05), except at moment 4. The factors that were 27 significantly and independently associated with compliance were the time period considered, type of healthcare-personnel, attendance at training sessions, knowledge of HH and WHO 28 29 guidelines, and availability of hand disinfectant alcoholic solution in pocket format. Highest HH 30 compliance occurred during the COVID-19 pandemic, reflecting a positive change in healthcarepersonnel's behavior regarding HH recommendations. 31 32

Keywords: Healthcare associated infections; Hand hygiene; Degree of compliance; Health
 personnel; SARS-CoV-2.

35

#### 36 INTRODUCTION

37 Healthcare-acquired infections (HAIs) are a worldwide problem that directly affect hospitalised 38 patients and are, in turn, a complication which can directly affects patient safety. Such infections 39 represent a challenge for public health due to potential adverse clinical events and prolongation 40 of hospital stavs with a consequent increase in healthcare costs [1, 2]. According to data 41 provided by the Study of the Prevalence of Nosocomial Infections in Spain (EPINE in its 42 Spanish abbreviation), the prevalence of HAIs in Spain in 2022 was between 5 and 10%, and in turn, the prevalence of HAIs in our centre (the General University Hospital of Alicante or 43 44 HGUA) in the same year was 8.2% [3]. The most important measure to prevent the transmission of microorganisms and reduce HAIs is hand hygiene (HH) [4] and its proper compliance [5–7] 45 according to the '5 moments' for the application of HH as recommended by the World Health 46 47 Organization (WHO). These are (1) before touching a patient, (2) before performing an aseptic task, (3) after the risk of exposure to body fluids, (4) after patient contact, and (5) after contact 48 49 with the patient's environment [8].

50 In the context of the COVID pandemic caused by the severe acute respiratory syndrome 51 coronavirus 2 (SARS-CoV-2) which is transmitted through close contact and respiratory droplets 52 or aerosols [9], HH, along with the use of personal protective equipment (PPE) and proper 53 respiratory hygiene [9–13], became an important part of the measures adopted to prevent the 54 transmission of the virus. These measures proved to be highly important due to constant 55 exposure of healthcare staff to infected patients and contaminated surfaces, and consequently 56 posed a risk of both acquiring and transmitting the infection [12]. Thus, compliance with HH 57 recommendations and its monitoring were key measures prevent HAIs and, reduce the 58 transmission of COVID-19 infection [4, 14].

Numerous studies have surveyed the HH compliance in different hospital areas and generally,
compliance levels seldom exceeded 50% [5, 7]. In this current study, HH compliance was
monitored through direct observation (the gold standard method) [15], and results were
communicated healthcare staffto better understand how compliance changed with time, and the
factors that may contribute to poor HH practice [11,16–17].
After the start of the COVID-19 pandemic, during the first quarter of 2021, Gras-Valentí *et al.*[18] recorded close to 90% HH compliance in an emergency department. In addition, Wong *et* 

*al.* [19] reported from a study in two paediatric hospital units during the pandemic that full HH

67 compliance by staff was possible. Thus, evaluation of the current situation, post COVID-19,

68 represents an opportunity to determine the impact of the pandemic on HH compliance on HH

and, make healthcare-personnel more aware of its importance in the quality of patient care and

70 hence promote better adherence to WHO guidelines.

## 71 METHODS

#### 72 *Population/measures*

73 This was a repeated cross-sectional study in a tertiary-level hospital. The data on the degree of HH compliance, through direct observation, from 2005 to 2022 were obtained through the 74 75 Epidemiological Surveillance program established by the Center's Preventive Medicine Service. 76 Observations were carried out of one health professional at a time; without prior notice for 1 77 hour during working hours at a single fixed moment in time. No interventions were made, or 78 follow-up over time of the observations. Observers explained the reason for their presence and 79 requested verbal authorisation of the staff to carry out the observation, and completed an 80 anonymous form designed to evaluate HH compliance [17, 19], in terms of the measures 81 performed (positive), and those not performed (negative), as well as recording potential

explanatory variables (sex, age, type of healthcare-personnel, area of care, WHO activity code,
knowledge of the HH leaflet, training session attendance, and availability of pocket-sized
hydroalcoholic hand sanitizer PSAS-solution).. HH actions that did not correspond to a 'WHO
moment' were not recorded. The method of choice for hand hygiene at the center was mainly
hydroalcoholic solution, some occasions was substituted with soap and water with subsequent
application of hydroalcoholic gel.

#### 88 Statistical analysis

89 During the first phase of the study, compliance with recommendations on HH with confidence 90 intervals (95% CI) was calculated as the ratio between the number of HH actions carried out and 91 the number of WHO HH opportunities: Compliance (%) = (actions carried out/opportunities)  $\times$ 92 100. To study the evolution over time, a trend analysis based on the degree of compliance was carried out using estimates for 18 periods (from 2005 to 2022) in the different hospital areas. A 93 94 permutation test for join point regression (JoinPoint®) was used to detect significant percentage 95 changes of the period in the prevalence of degree of complianceThe overall statistical significance level was p=0.05, allowing a maximum of 7 joining points and 8 line segments. 96 97 I year 2018–19 was taken as the reference period prior to the COVID-19 pandemic and from 98 June 2020 to end of April 2022 was considered the pandemic period. To study the impact of the 99 pandemic, the degree of HH compliance was compared before and during the pandemic; the 100 odds ratio (OR) and its 95% CI were calculated for each of the subgroups. Associations between 101 HH compliance with the study period and possible explanatory variables were investigated using 102 Chi-squared tests. The magnitude of any observed associations was expressed as OR 95% CI. 103 Finally, a multivariate analysis was performed with the variables that showed a statistically 104 significant association in order to estimate the adjusted OR with 95% CIs, using a logistic 105 regression model. The level of statistical significance in all tests was p < 0.05 and SPSS software 106 (version 25.0; IBM Corp., Armonk, NY) was used for the analyses. The study was approved by 107 the Drug Research Ethics Committee at the Department of Health (PI2021/181). 108 **RESULTS** 109 A total of 9,083 activities in which HH had been indicated were observed in the HGUA in the 110 pre-pandemic period (2018 to 2019), compared with 5,821 during the pandemic. 111 Table 1 shows the trend in the prevalence of degree of compliance from 2005 to 2022. The 112 lowest compliance was in 2005 with 31.0% (95% CI: 29.6%-32.5%) in contrast with 66.5% in 2020 (95% CI: 64.0%-69.0%); 73% in 2021 (95% CI: 71.5%-74.5%) – the highest compliance 113 114 in the 18 years studied, and 65.3% in 2022 (95%CI:62.3%-68.2%), (Figure 1). Joinpoint 115 regression analysis showed significant inflection points between the periods from 2005 to 2013, 116 from 2013 to 2014, from 2014 to 2015, from 2016 to 2019 and from 2019 to 2021. Non-117 significant inflection points were detected between the period from 2015 to 2016, and from 2021 118 to 2022. HH compliance increased significantly (p < 0.001) to 70.1% (95%CI: 68.9-71.2) during the 119 120 COVID-19 pandemic compared to 54.5% (95%CI: 53.5-55.5) in the prior reference period

121 (Table 2). As shown in Table I, compliance during the pandemic was higher in healthcare-

122 personnel aged under 35 years at 71.4% (1,471), in 70.5% (3433) of women, 73.4% (224) of

123 physicians, and 74.3% (1758) of staff carrying pocket-sized alcohol hand sanitizers. There was a

124 significant increase in the degree of HH compliance at each of the WHO- moments during the

pandemic period (p < 0.05), with the exception of moment 4. Compliance with the latter was

- 126 66.0% (95% CI: 63.8-68.1 in the period prior to the pandemic and no significant differences were
- 127 observed between the first and second periods (p = 0.774). Compliance with WHO moment 2
- 128 was lowest in both periods (43.2% and 55.4% respectively). (Table 2). The highest degrees of

- 130 (72.7%; 95%CI: 68.6-71.9) in the 2020–2022 period (Table 2).
- 131 As shown in Table 3, the factors that were significantly and independently associated with HH
- 132 compliance were the time period (before or during the COVID-19 pandemic), with an ORa of
- 133 2.0 (95%CI: 1.8-2.1), knowledge of the HH leaflet (ORa=1.8; 95%CI: 1.3-2.5), training session
- 134 attendance (ORa=1.6; 95%CI: 1.4-1.8), and availability of PSAS (ORa=1.4; 95%CI: 1.3-1.5), as
- 135 well as medical care areas (ORa=1.2; 95%CI: 1.1-1.3) and critical care units (ORa=1.4;
- 136 95%CI:1.3-1.5), and attention to the WHO moments 1,3,4 and 5.

## 137 **DISCUSSION**

- 138 In recent years, many efforts have been made to monitor the on HH practice in different
- 139 healthcare areas around the world, and several have showed a high level of non-compliance with
- 140 the recommendations [5, 8, 12, 17]. Infection prevention and control measures, especially HH,
- 141 have gained vital importance worldwide in the current context of the COVID-19 pandemic.
- 142 Indeed, several studies, including a meta-analysis by Ying et al. [20], have shown, among others,
- 143 a significant increase in HH practice at the beginning of the pandemic (years 2020–2021) [18,
- 144 19]. Nonetheless, relatively little research has continuously monitored over time to determine if
- 145 compliance was maintained throughout, or varied during the pandemic. Thus, this current work
- 146 provides pertinent data that show a significant improvement in HH compliance which was
- 147 sustained during the COVID-19 pandemic. In fact, the highest levels of compliance recorded in
- 148 recent years (70.1%) were reached during the pandemic, with figures similar to those published
- 149 by Qian Zhou et al [21]. In contrast, other studies reported a lack of increase [22,23], or even a
- 150 decline [24,25], in HH compliance over the period. These results differ from our own
- 151 observations, perhaps due to differences in the methodology used to document this metric.

Indeed, to our knowledge, all of the previous studies monitored the HH compliance through an electronic system, which may have led to certain differences in the observed trend. Current evidence supports such systems as a complement to direct observation for monitoring HH, but the latter continues to be the gold standard method.

156 In addition, we found that the WHO moment was independently and significantly associated 157 with the degree of compliance on HH, as evidenced by the observed improvement in practice in 158 the 2020–2022 period for all, but 'moment' 4 which nonetheless, did not reflect a significant 159 increase in compliance during the pandemic compared with the period beforehand, which stayed 160 constant at around 65%. This finding might suggest that performing HH 'after' direct contact 161 with patients is a deep-rooted behaviour among healthcare-personnel. Moreover, although there was an improvement in compliance for all WHO moments, there was a higher degree of 162 conformance for moments 3, 4, and 5 which all occur 'after' contact or exposure to patients, their 163 164 fluids, or environment. This view is supported by the lower compliance with moments 1 and 2, 165 which occur 'before' the contact or care procedure [8]. Specifically, moment 2 had the lowest 166 degree of compliance in both periods, even though it increased by almost 15 percentage points 167 during the pandemic period. This finding may be of concern considering that the purpose of HH 168 at moments 1 and 2 is to prevent the transmission of infectious agents to patients, while at 169 moments 3, 4, and 5, its primary purpose is prevention of the risk of transmission to healthcare-170 personnel and contamination of the care area [26].

These results coincide with previously published studies [7, 16, 17, 21, 27] and could possibly reflect a behaviour guided by healthcare-personnel being concerned for their self-protection after coming into contact with patients, thus making them potential dispersers of microbes to patients. As a consequence, these data indicate that successive training interventions focused on the importance of preventing cross-infections through HH performed at WHO moments 1 and 2 176 (those that constitute a greater risk of infection to patients) should be emphasised [8]. In general, 177 the increase in HH during the COVID-19 pandemic had a positive impact on the reduction of 178 HAIs and infection resistance rates, as seen in other studies [28-30]. This justifies the 179 implementation of strategies aimed at markedly improving HH compliance to levels ranging 180 from 89.8–97.1% [5, 7, 16, 17, 31]. From this perspective, it is of interest to define accurately the 181 key factors which influence the compliance in the healthcare setting so that strategies which 182 coincide with the most critical moments for patient safety can be implemented. 183 Factors such as training session attendance, knowledge of the HH leaflet, and carrying pocket-184 sized hand sanitizer were also independently and significantly associated with HH compliance. 185 These results corroborate the data obtained in a previous study [32] which recorded compliance 186 rates of 66% after carrying out educational and awareness-raising interventions for professionals. 187 Of note, in general, it is difficult to compare the results between studies due to differences in the 188 methodology employed or the specific study period. Thus, the results reported here should be 189 considered with caution as it is too early to know if they will be maintained over time once the 190 COVID-19 pandemic has ended. 191 The outcomes seen in the data we report here can have an impact on infection prevention and 192 control strategies for COVID-19 and inform practice in future microbial epidemics. Indeed, 193 increased information in the media about the importance of HH, and the greater perception of 194 risk by staff following after the COVID-19 pandemic was declared led to greater awareness of

the value of these measures [33]. Even so, some authors, including Wong *et al.* [19] and Ragusa *et al.* [34], observed maximum compliance with HH prior to the pandemic, which may suggest a
prior awareness of its importance by healthcare-personnel —a very encouraging finding when

198 considering how to continue promoting these measures. There was a marked improvement in

199 hand hygiene practice in our hospital which indicates that the strategies implemented were

200 effective. In future work, the main objective will be to maintain the improvement over time and

201 focus on factors that can potentially be improved, such as enhancing the hand hygiene

202 knowledge, awareness and motivation of health care personnel.

203 The limitations of this study were inherent in its design and objective. For example, the decrease 204 in patients seen in surgical areas during the COVID-19 pandemic (because of the suspension of 205 non-urgent operations) may have impacted on the number of observation opportunities in these 206 areas. In addition, the Hawthorne effect – modification of behaviour due to awareness of 207 observation - and the possible resulting overestimation of compliance must also be considered. 208 Nonetheless, this effect remained constant in all the study observations. Another possible 209 limitation was that of selection, but as recorded in a previous study on HH compliance [17], the 210 rate of non-participation by staff did not exceed 1.2%. The use of multiple observers may have 211 led to increasing variability in recording and and collection of data. To minimise this effect, all 212 observers were trained using the same methodology and were all members of the Preventive 213 Medicine Service.

214 CONCLUSION

The COVID-19 pandemic promoted an increase in the HH compliance reflecting a very positive 215 change in this practice by healthcare-personnel. Monitoring and evaluation of compliance 216 217 allowed us to communicate results to staff, thereby generating active feedback for the 218 development of strategies to improve the quality of patient care and HH compliance. 219 Considering the role that healthcare-personnel play as health agents both in centres and the 220 community, we must take advantage of this momentum and direct our efforts towards new 221 continuous improvement objectives. These include (i) achieving the same or higher HH 222 compliance in WHO moments 1 and 2 as already recorded for moments 3, 4, and 5; and (ii)

- 223 maintaining the outcomes from the study to ensure that the healthcare environment is a safe
- 224 place for both staff and patients.
- 225 **Conflicts of Interest:** The authors declare no conflict of interest.
- Author Contributions: Conceptualization, M.G.S, P.G.V. and J.S.P.; methodology, M.G.S. and
- 1.L.G.S.; software, P.C.S; validation, J.S.P.; formal analysis, M.G.S. and I.L.G.S.; investigation,
- 228 G.P.A and R.F.S.A.; resources, C.M.B.M., L.C.L. and M.S.V.; data curation, G.P,A. and
- 229 R.F.S.A.; writing-original draft preparation, M.G.S.; writing-review and editing, P.G.V., J.S.P.
- and M.G.S.; visualization, C.M.B.M..; supervision, M.F.P.; project administration, P.G.V. All
- authors have read and agreed to the published version of the manuscript.
- 232 Data Availability Statement: Data that are not presented in the article are available upon
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- 234 **Ethical standard:** The study was conducted in accordance with the Declaration of Helsinki and
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Figure 1. Evolution of the Degree of Compliance on Hand Hygiene Recommendations pre-371 pandemic period and during the COVID-19 pandemic period.



375 Table 1. Period percentage change (PPC) in the Degree of Compliance on Hand Hygiene

# **Recommendations.**

PERIOD	PPC	p value
From 2005 to 2013	68,60	0.040
From 2013 to 2014	-26,35	0.049
From 2014 to 2015	26,35	0.030
From 2015 to 2016	-14,78	0.063
From 2016 to 2019	4,56	0.035
From 2019 to 2021	15,16	0.033
From 2021 to 2022	-11,12	0.135

•	DCR during the pandemic (June 2020 to April 2022)	DCR pre-pandemic (years 2018/2019) Ref.	OR (95%CI)	<i>p</i> -value
	% ( <i>n</i> / <i>N</i> )	% ( <i>n</i> / <i>N</i> )		
Total	70.1 (4,079/5,821)	54.5 (4,949/9,083)	2.0 (1.8–2.1)	< 0.001
Age				
< 35 years	71.4 (1,471/2,061)	54.4 (1,584/2,914)	2.1 (1.9–2.4)	< 0.001
$\geq$ 35 years	69.4 (2,608/3,760)	54.5 (3,365/6,169)	1.9 (1.7–2.1)	< 0.001
Sex				
Men	68.0 (646/950)	55.1 (879/1,596)	1.7 (1.5-2.1)	< 0.001
Women	70.5 (3,433/4,871)	54.4 (4,070/7,487)	2.0 (1.9-2.2)	< 0.001
Sector		ſ		
Physicians	73.4 (224/305)	62.8 (589/938)	1.6 (1.2–2.2)	0.001
Nurses/	72.7 (2,459/3,382)	55.4 (3,086/5,571)	2.1 (2.0–2.4)	< 0.001
Physiotherapists				
Auxiliary nurses	66.8 (1,289/1,929)	50.2 (1,158/2,309)	2.0 (1.8–2.3)	< 0.001
Others	52.2 (107/205)	43.8 (116/265)	1.4 (1.0-2.0)	0.070
Health care area				
Medical area	70.2 (2,155/3,068)	54.8 (2,610/4,759)	1.9 (1.8–2.1)	< 0.001
Surgical	67.7 (1,041/1,538)	50.5 (1,207/2,391)	2.1 (1.8–2.3)	< 0.001
Critical care	72.7 (883/1,215)	58.6 (1,132/1,933)	1.9 (1.6–2.2)	< 0.001
WHO HH activity				
Moment 1	67.3 (833/1,238)	47.3 (1,088/2,302)	2.3 (2.0-2.7)	< 0.001
Moment 2	55.4 (240/433)	43.2 (390/902)	1.6 (1.3–2.1)	< 0.001
Moment 3	66.4 (413/622)	60.7 (693/1,141)	1.3 (1.0–1.6)	0.019
Moment 4	65.4 (663/1,013)	66.0 (1,247/1,890)	1.0 (0.8–1.1)	0.774
Moment 5	76.7 (1,923/2,506)	53.7 (1,526/2,842)	2.8 (2.5–3.2)	< 0.001
Knows the HH leaflet			× ,	
Yes	70.5 (4,032/5,717)	54.8 (4,924/8,986)	2.0 (1.8–2.1)	< 0.001
No	45.2 (47/104)	25.8 (25/97)	2.4 (1.3-4.3)	0.004
Training sessions		· · · ·	× ,	
Yes	71.5 (3,737/5,227)	56.3 (4,539/8,063)	1.9 (1.8–2.1)	< 0.001
No	57.6 (342/594)	40.2 (410/1,020)	2.0 (1.6–2.5)	< 0.001
PSAS	× /			
Yes	74.3 (1,758/2,365)	59.1 (1,913/3,239)	2.0 (1.8–2.3)	< 0.001
No	67.2 (2.321/3.456)	52.0 (3.036/5.844)	1.9 (1.7-2.1)	< 0.001

Table 2. Hand hygiene compliance during the COVID-19 pandemic and in the pre-pandemic
 period.

Ref.: reference category for calculating the magnitude of the association; DCR: degree of compliance with the
recommendations; PSAS: pocket-sized hydroalcoholic solution; HH: hand hygiene; OR: odds ratio; 95% CI:
95% confidence interval; *p*: level of statistical significance; *N*: number of opportunities to perform hand
hygiene; *n*: number of times hand hygiene was performed; Moment 1: before contact with the patient; Moment
2: before carrying out an aseptic technique; Moment 3: after contact with biological fluids; Moment 4: after
contact with the patient; Moment 5: after contact with the patient's environment. Note: Boldface indicates
statistical significance (p<0.05)</li>

	DCR with HH $\%(n)$	OR (95%CI)	<i>p</i> -value	ORa (95%CI)	<i>p</i> -value
Period				()0/001)	
Pandemic	70.1 (4.079/5.821)	2.0(1.8-2.1)	< 0.001	2.0(1.8-2.1)	< 0.001
(Years 2020-22)	, ( 1,07570,021)	2.0 (1.0 2.1)	(0.001	2.0 (1.0 2.1)	
Pre-pandemic	54.5 (4.949/9.083)				
(Years 2018-19)		1		1	
Age					
< 35 years	61.4 (3.055/4.975)	1.1 (1.0–1.1)	0.141	1.0(0.9-1.1)	0.796
> 35 years	60.2 (5.973/9.929)	1			
Sex					
Men	59.9 (1,525/2,546)	1.0 (0.9–1.1)	0.443	1.0(0.9-1.1)	0.872
Women	60.7 (7,503/12,358)	1			
Sector			C		
Physicians	65.4 (813/1,243)	2.1 (1.7–2.6)	< 0.001	1.9 (1.5–2.4)	< 0.001
Nurses/	61.9 (5,545/8,953)	1.8 (1.5–2.2)	< 0.001	1.4 (1.2–1.8)	0.001
Physiotherapists					
Auxiliary nurses	57.7 (2,447/4,238)	1.5 (1.3–1.8)	< 0.001	1.0 (0.8–1.3)	0.699
Others	47.4 (223/470)	1		1	
Health care area					
Medical area	60.9 (4,765/7,827)	1.2 (1.1–1.3)	< 0.001	1.2 (1.1–1.3)	< 0.001
Surgical	57.2 (2,248/3,929)	1		1	
Critical care	64.0 (2,015/3,148)	1.3 (1.2–1.5)	< 0.001	1.4 (1.3–1.5)	< 0.001
WHO HH activity					
Moment 1	54.3 (1,921/3,540)	1.3 (1.2–1.5)	< 0.001	1.4 (1.2–1.6)	< 0.001
Moment 2	47.2 (630/1,335)	1		1	
Moment 3	62.7 (1,106/1,763)	1.9 (1.6–2.2)	< 0.001	1.9 (1.6–2.2)	< 0.001
Moment 4	65.8 (1,910/2,903)	2.2 (1.9–2.5)	< 0.001	2.3 (2.0-2.6)	< 0.001
Moment 5	64.5 (3,449/5,348)	2.0 (1.8–2.3)	< 0.001	2.1 (1.8–2.4)	< 0.001
Knows the HH leaflet					
Yes	60.9 (8,956/14,703)	2.8 (2.1–3.7)	< 0.001	1.8 (1.3–2.5)	< 0.001
No	35.8 (72/201)	1		1	
Care training sessions					
Yes	62.3 (8,276/13,290)	1.9 (1.7–2.1)	< 0.001	1.6 (1.4–1.8)	< 0.001
No	46.6 (752/1,614)	1		1	
PSAS					
Yes	65.5 (3,671/5,604)	1.4 (1.3–1.5)	< 0.001	1.4 (1.3–1.5)	< 0.001
No	57.6 (5,357/9,300)	1		1	

#### 391 Table 3. Factors associated with HH compliance according to the characteristics of the health 392 professionals and activity.

393 DCR: degree of compliance with the recommendations; PSAS: pocket-sized hydroalcoholic solution; HH:
 394 hand hygiene; OR: odds ratio; 95% CI: 95% confidence interval; p: level of statistical significance; ORa:
 395 adjusted odds ratio; pa: adjusted level of statistical significance; Moment 1: before contact with the patient;
 396 Moment 2: before carrying out an aseptic technique; Moment 3: after contact with biological fluids; Moment
 397 4: after contact with the patient; Moment 5: after contact with the patient's environment. Note: Boldface
 398 indicates statistical significance (p<0.05)</li>