Ambulance sanitation in Italy: a pilot study

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Abstract: Introduction: the assessment of biological agents’ exposure in the work environment is an employer’s duty. Air and surfaces could be transmission’s vehicles of pathogens. We consider the ambulance as the work environment where, respecting hygienic targets of low risk, we can prevent out-of-hospital infections for workers and patients. Our study analyzes standard sanitation and fumigation; the aim is to reach the lower level of surfaces’ contamination.

Materials and Methods: we chose, thanks to bibliography, three points of sampling: stretcher, oxygen knob and backdoor’s handle. The points were tested before sanitation on three different ambulances. We made thus standard sanitation with Sodium Hypochlorite on two ambulances and fumigation on the other one. After that, we sampled the same three points on each ambulance. Samples were analyzed with standard culture methods after 72 hours.

Results: first samples showed a high bacterial load on every surface. Both standard sanitation and fumigation showed success in decreasing bacterial load. Without approved rating standards for ambulance’s hygiene we refer to ISPESL’s Guidelines about safety and hygiene in surgical wards. Here the standard is 50 CFU per sample, referred to a surface of 20 cm², without pathogens.

Discussion and Conclusions: our analysis is just quantitative, further analysis should be conducted in order to assess the presence of pathogens on ambulances. Both standard sanitation and fumigation show success in decreasing bacterial load however with higher levels than those indicated by guidelines. Further analysis would be needed to define load standards in order to ensure a safe environment for workers and patients.

Keywords: Pathogens; Ambulance; Fumigation; Biological Exposure; Hygiene; Sodium hypochlorite.
exposure by inhalation or contact, monitoring the microbial contamination in the air and/or on the surfaces is a mandatory step to assess the general hygiene level and environmental healthiness. The air and surfaces of equipment, floors, apparatus and work clothing, as well as the hands of workers can represent important vehicles of microbiological contamination and potential sources of transmission of infectious agents [2]. The focus of this work is on the measurement of total bacterial concentrations and the detection of specific microbial indicators and pathogens, related to the performance of the work activity under consideration.

In the case of activities with potential or accidental exposure to biological agents, knowing the levels and types of contaminants and their temporal and spatial variation allows to detect the presence of possible sources of contamination or microbial amplification, intervening promptly with appropriate measures of prevention or containment. Microbiological contamination of surfaces can occur through contact with other contaminated surfaces (objects, tools, worker’s hands, etc.) and through sedimentation.

An analysis of the literature shows that, in Europe and abroad, numerous studies have been carried out on care-related infections, especially in the intra-hospital setting [3],[4],[5]; in Italy, only one study [6] has been carried out, which examines the knowledge of hygiene practices regarding emergency vehicles by health care personnel and volunteers. However, the correlation between the hygiene of emergency vehicles, the presence of pathogens on them and the transmission of nosocomial infections to patients is not highlighted; in fact, little attention is paid to infections acquired by patients or operators outside the hospital: emergency vehicles used to transport people come into daily contact with potentially infected subjects, who can infect both operators and other users of the emergency vehicle. The selected literature highlights that this problem has been partially investigated, especially abroad. In Denmark several studies have been carried out on the identification of pathogens on emergency vehicles [7], [8] as well as on the uniforms of the operating personnel [9], in particular to try to understand if the adherence to the national guidelines on the sanitization of the vehicles actually led to a reduction of the potentially pathogenic bacterial load on them. The findings highlight that adherence to the guidelines is far from what is considered “optimal” and this leaves room for contamination levels to be higher than the standard, although still low to be a cause of infection for people transported or working on them [7].

Another study conducted in Saudi Arabia, however, focuses on the use of the fumigation technique [10]. Fumigation is a sanitization technique used for vehicles and environments. It is implemented thanks to a device capable of transforming a chemical substance into a gas. In our case the device is able to transform, thanks to a UV (ultraviolet) lamp, Oxygen into Ozone. Ozone is an element with strong antioxidant action that has been used since the early twentieth century for its bactericidal, fungicidal and inactivating action of viruses [11].

This technique requires a certain amount of time depending on the airspace of the environment to be effective (in the case of the sanitary compartment of the ambulance it requires a treatment of 22 minutes), so the vehicle engaged in sanitization will not be available for service. The procedure, to be effective, however, requires that a mechanical cleaning of the surfaces be carried out beforehand to remove any contaminants, so the total time for a complete treatment is high, which is why it isn’t a technique used routinely, although very valuable.

**Objectives**

The primary objective is to detect the microbial load inside the patient transport compartment of ambulances, before and after different types of sanitization.

**Materials and methods**

**Sampling, culture analysis of samples, quantitative analysis.**

The study was carried out in October 2019 at the Territorial Emergency Service of the AUSL (Italy) in the headquarters of Bologna; for the sampling, authorization was requested from the managers of the Emergency-Urgency service of the Company. The samplings were carried out on three different days. Based on the literature reviewed, we chose to perform sampling on 3 surfaces of the ambulance. In particular, considering the amount of manipulation or contact with the patient and the operators of the vehicle, we chose the following points:

1. Backrest of the transport stretcher
2. Knob of one of the O2 tanks connected to the internal distribution system
3. Internal handle for opening the rear door

The samples were taken by a person previously trained by the laboratory that provided us with the material. Three ambulances from the AUSL of Bologna were tested, as indicated as available for the study and which had not been sanitized in the last 48 hours prior to our measurements. The sample considered is “of convenience” since the primary objective of the study is to evaluate the actual presence of bacterial load on emergency vehicles. The choice of vehicles was made through a randomization process. The samples were collected using sterile kits “SANSPONGE” (Steroglass S.r.l - Perugia Italy), the kit is designed to detect the presence of microbial contamination on work surfaces, equipment, carcasses of slaughtered animals. Depending on the sowing technique and culture media used, it is possible to conduct non-selective and broad-spectrum microbiological research (for example, determine the aerobic mesophilic load) or research aimed at highlighting the presence of specific microorganisms, such as Listeria, Salmonella, E. coli. We chose to evaluate the aerobic mesophilic load in this study. These kits are widely used in the food, medical, public health and cosmetic industries. The sampling kit consisted of a sponge of size 4.5 x 9 cm, contained by an envelope of size 114x229 mm and with volume equal to 450 mL. Samples were collected by sterile method.

We collected samples prior to sanitization on all 3 ambulances at the 3 chosen locations to assess bacterial load on vehicles considered “dirty.”

We then sanitized 2 vehicles with a standard technique, i.e. using an electrolytic chloride (Sodium Hypochlorite).

The choice of disinfectant was dictated by the guidelines provided by the Italian Society of 118 Systems on the “Manual for operators of emergency vehicles: control, verification and procedures for cleaning and disinfection of emergency vehicles” in 2009 [12], where the stabilized Sodium Hypochlorite is considered a high-level disinfectant agent effective against Gram + and Gram -, fungi, mycobacteria, lipophilic viruses, hydrophilic viruses and spores. The above operational indication is also confirmed by the recent literature review by the Italian Society of Territorial Emergency Nurses [13].

Following cleaning with Sodium Hypochlorite, we carried out new surveys on the sanitized surfaces to evaluate the effectiveness of the technique.

On the third vehicle, the first three samples were performed without sanitization, the other three samples after fumigation.

Fumigation was carried out thanks to a device, Ozone Air 80® (by the company Bertin srl, marketed by Tecnolife srl). The product allows users to choose between three programs that affect different airspace (10, 25, 50 m³) by varying the treatment time. We have calculated that the sanitary compartment of an ambulance consists of approximately 10 m³ and we have therefore chosen the first treatment for a total of 22 minutes of fumigation after which the side door of the vehicle was opened to allow an adequate exchange of air. After this treatment, we carried out the measurements on the three chosen points.

In total, we collected 18 samples, 9 before the sanitization procedures and 9 afterwards.

Samples were stored in a cooler bag with a temperature between +4°C and +8°C during transport to the laboratory.

The laboratory analyzed the samples by microbiological method ISO 4833-1:2013: this analysis is aimed at the quantitative determination of aerobic mesophilic microorganisms (also called total aerobic mesophilic load) and is based on the colony count (CFU) grown in Petri dishes containing Plate Count Agar medium and seeded according to the inclusion technique (germ agar) after aerobic incubation at 30°C for 72 h.

Results

Since it is not known what levels of contamination could be expected in emergency vehicles in service at the AUSL considered and not having available reference values that would give us indications in this sense, a preliminary survey of the aerobic mesophilic load present on some surfaces of a typical ambulance was conducted. The purpose of this first survey was twofold. On the one hand, it was useful to the laboratory to adapt the sensitivity of the method to the real conditions of contamination to be detected. On the other hand, the aim was to verify if there was a significant microbial contamination on non-sanitized surfaces, because if this was not the case, it would not be possible to evaluate the effectiveness of the sanitization methods adopted. The preliminary survey showed an average contamination by aerobic mesophilic microorganisms on the sampled surfaces of the order of magnitude of 103 CFU.

A contamination level of the same order of
magnitude, or even higher, was then confirmed in the samplings performed on the non-sanitized surfaces of the 3 ambulances under study, as can be seen in Table 1, which reports the results of the aerobic mesophilic load (expressed in CFU/sample) found in the different sampled points.

<table>
<thead>
<tr>
<th>SAMPLE ID</th>
<th>CFU PRE SANIFICATION</th>
<th>CFU POST SANIFICATION</th>
<th>CFU POST FUMIGATION</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ambulance 1 - stretcher</td>
<td>6.200 cfu/sample</td>
<td>estimated 500 cfu/sample</td>
<td></td>
</tr>
<tr>
<td>Ambulance 1 - O2 knob</td>
<td>4.800 cfu/sample</td>
<td>910 cfu/sample</td>
<td></td>
</tr>
<tr>
<td>Ambulance 1 - rear handle</td>
<td>1.100 cfu/sample</td>
<td>&lt; 400 cfu/sample</td>
<td></td>
</tr>
<tr>
<td>Ambulance 2 - stretcher</td>
<td>2.600 cfu/sample</td>
<td>estimated 800 cfu/sample</td>
<td></td>
</tr>
<tr>
<td>Ambulance 2 - O2 knob</td>
<td>1.000 cfu/sample</td>
<td>&lt; 400 cfu/sample</td>
<td></td>
</tr>
<tr>
<td>Ambulance 2 - rear handle</td>
<td>1.300 cfu/sample</td>
<td>&lt; 400 cfu/sample</td>
<td></td>
</tr>
<tr>
<td>Ambulance 3 - stretcher</td>
<td>6.900 cfu/sample</td>
<td>&lt; 400 cfu/sample</td>
<td></td>
</tr>
<tr>
<td>Ambulance 3 - O2 knob</td>
<td>25.000 cfu/sample</td>
<td>&lt; 100 cfu/sample</td>
<td></td>
</tr>
<tr>
<td>Ambulance 3 - rear handle</td>
<td>2.500 cfu/sample</td>
<td>&lt; 400 cfu/sample</td>
<td></td>
</tr>
</tbody>
</table>

A certain degree of variability in the levels of microbial contamination present in the three ambulances before the sanitization procedure is evident, as is the constant reduction in the level of contamination of the surfaces obtained after sanitization. This reduction appears to be significant (p<0.05), as verified with Student’s t-test, the details of which are reported in Table 2.

It was not possible to establish whether there are significant differences in the degree of effectiveness of the two sanitization treatments applied in this study, due, on the one hand, to the low sensitivity of the counting method used, which, having been “calibrated” on an order of magnitude of 103 CFU per sample, has a detection threshold of 100 CFU per sample, clearly too high for the levels of contamination that characterize the sanitized surfaces, and, on the other hand, to the excessively small number of tests collected. It emerges, however, that both the procedure of manual sanitization by electrolytic chloride and fumigation are valid techniques for the reduction of microbial load on surfaces, confirming what are the current recommendations.
All calculations are performed after transformation of all values found into the respective decimal logarithms, following the usual practice in microbiological testing based on the colony counting technique. Samples with values below or close to the detection limit of the method (<100 CFU and <400 CFU respectively) were arbitrarily considered as equal to 100 CFU (2.00 Log) or equal to 400 CFU (2.60 Log). This is a detrimental choice but, given the purpose of the investigation, aimed at demonstrating the effectiveness of sanitization treatments, and the small number of samples examined, it adds a large margin of safety to the possible demonstration of significance of the result.

### Table 2. Testing of the significance of the differences found between the two sets of surveys

<table>
<thead>
<tr>
<th>SAMPLE ID</th>
<th>Log (CFU) pre sanitization</th>
<th>Log (CFU) post sanitization</th>
<th>Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ambulance 1 - stretcher</td>
<td>3,79</td>
<td>2,70</td>
<td>1,09</td>
</tr>
<tr>
<td>Ambulance 1 - O2 knob</td>
<td>3,68</td>
<td>2,96</td>
<td>0,72</td>
</tr>
<tr>
<td>Ambulance 1 - rear handle</td>
<td>3,04</td>
<td>2,60</td>
<td>0,44</td>
</tr>
<tr>
<td>Ambulance 2 - stretcher</td>
<td>3,41</td>
<td>2,90</td>
<td>0,51</td>
</tr>
<tr>
<td>Ambulance 2 - O2 knob</td>
<td>3,00</td>
<td>2,60</td>
<td>0,40</td>
</tr>
<tr>
<td>Ambulance 2 - rear handle</td>
<td>3,11</td>
<td>2,60</td>
<td>0,51</td>
</tr>
<tr>
<td>Ambulance 3 - stretcher</td>
<td>3,84</td>
<td>2,60</td>
<td>1,24</td>
</tr>
<tr>
<td>Ambulance 3 - O2 knob</td>
<td>4,40</td>
<td>2,00</td>
<td>2,40</td>
</tr>
<tr>
<td>Ambulance 3 - rear handle</td>
<td>3,40</td>
<td>2,60</td>
<td>0,80</td>
</tr>
</tbody>
</table>

One-tailed Student’s t-test for paired data

Null hypothesis $H_0$: the mean of the differences (d mean) between the pre- and post-sanitization value pairs is not significantly different from 0 (H 0: d mean = 0).

Alternative hypothesis $H_1$: the mean of the differences (d mean) between the pairs of pre and post sanitization values is significantly greater than 0 (H 1: d mean > 0).

The test, performed with Real Statistic Pack for Excel©, gives the result $t = 4.271132515$, which, in Student’s t distribution for 8 degrees of freedom, corresponds to a probability $p = 0.00136$, thus lower than the significance level $\alpha = 0.05$. It is therefore possible to exclude the null hypothesis and to state that, on average, the levels of aerobic mesophilic charge found after sanitization are significantly lower than those obtained from non-sanitized surfaces.

### Discussion

The study performed showed the presence of microbial contamination inside rescue vehicles. The levels of contamination found, although variable on the different surfaces and on the different vehicles considered, are in the order of magnitude of 103 CFU per sample or even higher. Further investigation is needed to understand if the microbial contamination found is the result of randomness and if, above all, it could represent a real risk of transmission of care-related infections. To explore this possibility, it will be necessary to broaden the field of investigation, including, in addition to the determination of the levels of broad-spectrum microbial contamination, the detection, both qualitative and quantitative, of specific groups of microorganisms (or individual species) to which is recognized particular pathogenic relevance (for example, streptococci, staphylococci, Pseudomonas, enterobacteriaceae carbapenemase-producers, Salmonella, Staphylococcus aureus methicillin-resistant).

The study demonstrated the effectiveness of current sanitation measures in reducing microbial contamination levels. It was not possible to accurately measure the extent of the reduction achieved at all sampling points performed, due to the too low sensitivity of the microbiological method used. For a survey of this type, it would be necessary to adjust the sensitivity to the area of the surface actually sampled, lowering the detection threshold to at least 1 CFU/cm². However, it is more than likely, given the penalizing choice made for samples at or below the detection limit, that the average reduction in microbial contamination actually achieved by the sanitization measures under study is even better than that which emerges from the analyses performed.
In addition to being a health problem, care-related infections also have a significant socioeconomic impact [14].

There is a possibility that emergency vehicles are a vehicle for transmission of pathogens to both staff and transported patients if not properly sanitized. In particular, surfaces may represent the main mean of contamination. The analysis carried out by us shows the presence of microorganisms on the vehicles analyzed, further investigation is necessary to understand whether this microbial load is the result of randomness and whether it represents a real pathogenic risk.

In order to understand if the amount of CFU constituted a real risk, we relied on some general indicators related to hospital environments. In fact, there are no specific indicators for emergency vehicles in literature, as this area is still little investigated and not regulated by specific guidelines.

The ISPESL guidelines “Guidelines on standards of safety and hygiene at work in the operating department” [15], in the paragraph concerning the safety of the operating department [15], in the section concerning the microbiological contamination of surfaces, cites as reference parameters the French guidelines “C.Clin-Ouest: recommandations pour les contrôles d’environnement dans les établissements de santé” [16], (TABLE 3).

Table 3. Reference values reported by ISPESL Guidelines

<table>
<thead>
<tr>
<th>STRUCUTRES</th>
<th>OBJECTIVES</th>
<th>TECHNIQUES</th>
<th>EXPECTED RESULTS</th>
<th>MEASURES IF RESULTS</th>
<th>MEASURES IF RESULTS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Operating rooms</td>
<td>Compliance of disinfection and air treatment</td>
<td>Contact</td>
<td>&lt; 5 cfu/plate</td>
<td>If 5 &lt; X ≤ 15:</td>
<td>accetable.</td>
</tr>
<tr>
<td>Other critical environments</td>
<td>Compliance of disinfection and air treatment</td>
<td>Contact</td>
<td>≤ 50 cfu/plate</td>
<td>If &gt; 50 revise the protocol.</td>
<td></td>
</tr>
<tr>
<td>(rooms for invasive</td>
<td></td>
<td></td>
<td></td>
<td>if &gt; 50 revise the protocol.</td>
<td></td>
</tr>
<tr>
<td>examinations in sterile</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>cavities etc.)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Referring to table 3, the sanitary compartment of the emergency vehicle could be compared to a hospital area (in the case of the table, an area adjacent to the operating room is taken into consideration, which will have minimum requirements certainly stricter than an ordinary hospital ward for which, however, there are no precise reference values). An acceptable result is indicated as a number of CFU ≤ 50 per plate, a result obtained thanks to sampling by contact method, or using kits such as those we have chosen, and analyzing a surface of 20 cm². The surfaces we analyzed can be approximated as follows:

- Backrest of the transport stretcher → area of about 40 cm²
- Knob of the O2 tank → area of about 19 cm².
- Internal handle for opening the rear door → area of about 25 cm²

As our data are approximate, they are not
directly comparable to those indicated by the guidelines, which can however give us at least an idea of the contamination/area ratio. Our measurements show a bacterial load higher than that indicated here. It is important to specify that the samples were taken on vehicles that had not been sanitized or used in the last 48 hours, it would be desirable to perform further studies by analyzing the bacterial load after an ordinary transport and immediately after the standard sanitization practices implemented by staff. The guidelines may also provide a baseline for further study, particularly with regard to the possible presence of pathogens. Further studies could contribute to the definition of hygiene standards for emergency vehicles, which do not exist to date.

However, the results obtained after both manual sanitization and fumigation suggest that careful and precise hygiene of the vehicle can lead to very low standards of contamination that reduce the risk of transmission and proliferation of potentially pathogenic bacteria.

Study limitations
The main limitation of the study is the impossibility of generalizing the results obtained, taking into account the small sample size used.

Conclusions
The SIIET (Italian Society of Territorial Emergency Nurses) has recently published recommendations [13] regarding the sanitization of emergency vehicles: the authors agree that the vehicles should be sanitized every 24 hours; with regard to the treatment with Ozone there are no specific indications for Covid-19; in any case in a 2006 study [17] Ozone treatment was found to be effective in the treatment of room sanitation during the SARS epidemic in Beijing.

It is clear that the issue of reducing transmission by indirect contact is complex and, in part, still to be studied in depth.

It is worth reflecting on some solutions to improve the quality of sanitation and, therefore, to determine a reduction of indirect contact transmission. Despite the fact that the level of contamination of the emergency vehicles we analyzed seems to be high, the sanitization techniques used are effective. Manual sanitization with Sodium Hypochlorite is a procedure that must be performed at the end of each transport, especially on surfaces and devices that come into contact with the patient.

The use of fumigation can also be useful as a procedure as it significantly decreases surface and air contamination levels.

Further future investigations would be desirable to compare the two methodologies on a larger quantity of samples, in order to evaluate if they are equivalent or if one is superior to the other.

The cost/benefit ratio has to be assessed by each company, as well as to consider the time needed for its implementation that forces the vehicle concerned to a suspension of service not always possible.

The presence of potential pathogens still remains to be analyzed.

Certainly, an implementation of sanitation measures and a more careful adherence to hygiene protocols can prevent and interrupt the transmission of microorganisms, making the environment of emergency vehicles safer.

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References