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**Nosocomial outbreak of monoclonal VIM carbapenemase producing *Enterobacter cloacae* complex in an intensive care unit during COVID-19 pandemic: an integrated approach.**

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**Running title.** Nosocomial VIM outbreak in an ICU

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**Background.** An outbreak of VIM carbapenemase-expressing *Enterobacter cloacae* complex occurred between March and October 2020 in an intensive care unit of a tertiary care and teaching hospital in France. At the same time, the hospital was facing COVID-19 first wave.

**Aim.** To describe the management of an outbreak caused by a VIM-producing *Enterobacter cloacae* complex strain during the COVID-19 pandemic in an ICU and to show the importance of an integrated approach.

**Methods.** A multifocal investigation was conducted including descriptive and molecular epidemiology, environmental screening, and assessment of Infection Prevention and Control measures.

**Results.** A total of 14 cases were identified in this outbreak with a high attributable mortality rate (85.7%). The outbreak management was coordinated by a crisis cell, and involved the implementation of multidisciplinary actions such as: enhanced hygiene measures, microbiological and molecular analysis of patients and environmental *E. cloacae* complex strains, and simulation-based teaching. All 23 *E. cloacae* complex strains isolated from patients and environment samples belonged to Multilocus Sequence Type ST78 and carried *bla*<sub>-VIM4</sub> gene. Using Fourier-transform infrared spectroscopy, all but two isolates were also found to belong to a single cluster. Although the source of this outbreak could not be pinpointed, the spread of the strain was controlled thanks to this multifocal approach and multidisciplinary implication.

**Conclusions.** This investigation highlighted the usefulness of Fourier-transform infrared spectroscopy in the rapid typing of outbreak strains as well as the importance of an integrated approach to successfully fight against multidrug resistant microorganism dissemination and healthcare associated infections.

**Keywords.** Outbreak; *Enterobacter cloacae* complex; healthcare associated infection; Fourier-transform infrared spectroscopy; integrated approach; carbapenemase

Multidrug Resistant (MDR) bacteria have become a major worldwide public health challenge and hospitals are now increasingly faced with management of local outbreaks involving such pathogens. Especially, intensive care units (ICU) provide an ideal background for outbreaks caused by MDR bacteria among which carbapenemase-producing Enterobacterales (CPE) can be found [1,2]. Amid CPE involved in ICU outbreaks, VIM producers have been reported worldwide, and described as especially difficult to control [2]. In France, few outbreaks involving these pathogens have been reported [3]. However, the number of VIM strains identified by the national referent centre (Centre National de Référence-CNR) has been in constant increase since 2012 [4]. In 2017, the national prevalence for *E. cloacae* with carbapenem resistance was under 0.01%, among 185 isolates [5]. Locally, few cases of infections due to VIM-producing strains were registered: one or two per year from 2016 till 2018 and five cases in 2019, without any obvious epidemiologic link found between them. Infection prevention and control (IPC) measures during the COVID-19 pandemic employed have been implicated in outbreaks with multidrug-resistant bacteria [6]. In particular, use and misuse of personal protective equipment (PPE) like gloves or gowns have been reported to be contributory factors [7]. The likely origin of outbreaks is not always easy to pinpoint, but common sources are index patients with a history of hospitalisation abroad, contaminated instruments, and/or environmental reservoirs [1]. CPE outbreaks in ICUs usually require a combination of IPC measures to achieve control, including screening of patients, use of contact precautions, staff education, enhanced environmental cleaning and disinfection, cohorting of patients and staff as well as a proper antimicrobial stewardship. Investigation and management of outbreaks require close cooperation and communication between all involved healthcare workers [8].

In this retrospective study, we describe the management of an outbreak caused by a VIM-producing *Enterobacter cloacae* complex (ECC) strain during the 2020 COVID-19

pandemic in an ICU, including the first time use of Fourier-transform infrared spectroscopy for the prospective typing/clustering of outbreak isolates.

## **METHODS**

### Case Definition and Series

From March through October 2020, patients colonized/infected with VIM-producing ECC were identified in one of the five ICUs at a tertiary care and teaching hospital in France. This care unit houses 16 beds for adult patients with an internal medicine care pathway but, at the time of this outbreak, was not a COVID-19 dedicated ward. Demographic data included age and gender. Comorbidities were collected. On the first day of ICU admission, severity and organ failure scores (Simplified Acute Physiology Score II (SAPS II) and Sequential Organ Failure Assessment (SOFA)) were computed. Organ support requirement and mortality in the ICU were recorded. Classification between infection and colonization as well as cause of death were prospectively determined in medical meetings with independent clinicians.

Initially, suspected cases were identified through perirectal screening undertaken on admission, and weekly thereafter, in accordance with French guidelines [9] as well as local routine practice. VIM-producing ECC isolates were characterized by identification of the suspected isolates using matrix-assisted laser desorption ionisation-time of flight mass spectrometry (MALDI Biotyper 2.2; Bruker Daltonik GmbH, Bremen, Germany) and carbapenemase production ascertained using Xpert carba-R<sup>®</sup> kit (Cepheid, Sunnyvale, USA) and/or Resist-5 OOKNV<sup>®</sup> immunoassay kit (Coris Bioconcept, Gembloux, Belgium). Antimicrobial susceptibility was assessed according to the EUCAST guidelines [10]. A case was defined as an ECC isolate positive for VIM-production obtained from either a clinical or a surveillance sample from a patient with a negative admission screening culture and admitted in the ICU on or after March 1<sup>st</sup>, 2020.

## Causes Analysis and Environmental Assessment

After detection of the third VIM-producing ECC isolate, the standard institutional procedure related to IPC measures was implemented and maintained until the last positive case left. This procedure includes:

- Contact precautions for colonised/infected patients and “at-risk” patients (*i.e.* patients treated in the same ward) such as allocation to single rooms, protective gowns for all staff interactions with the patient or their proximal environment, enhanced handwashing practices but no systematic glove wearing[9];
- Testing and follow-up of positive and “at-risk” patients[9];
- Terminal cleaning followed by room disinfection with hydrogen peroxide vapour after patient exit;
- Cohorting or care organisation based on a go-forward approach[9];
- Activation of a crisis cell including the general leadership of the hospital [9].

Complementary investigations were conducted to try and identify a possible contamination source and better characterise the bacterial strains involved, as described below.

### Environmental assessment following the outbreak

Following a cause analysis scheme previously set up, each patient room was investigated by multiple swabbing of surfaces (Figure 1 and Supplementary Table S1) using Sigma transwab-liquid Amies devices (Medical wire & equipment, Corsham, England) followed by seeding on Columbia Sheep blood agar (Becton-Dickinson, Le Pont de Claix, France), Drigalski (Becton-Dickinson) and Carbasmart (Biomérieux SA, Marcy l’*é*toile, France) media and an overnight incubation at  $36\pm 2^{\circ}\text{C}$ . When feasible a 25 cm<sup>2</sup> (5 x 5 cm template) surface was sampled and when not, the whole surface was sampled. Additionally, sink drains removed from the ICU rooms, samples collected from shared medical devices and

samples collected in common areas of the affected unit were examined for the presence of carbapenemase-producing bacteria (Supplementary Table S2).

*Enterobacter cloacae* complex whole-genome sequencing, MLST Sequence-type determination and Fourier-Transform InfraRed (FTIR) spectroscopy typing

Thirteen patient strains along with 10 strains recovered from environmental samples were submitted to high throughput whole-genome sequencing using the Illumina technique, as previously described [11]. Multilocus Sequence Typing (MLST) sequence-type (ST) determination was performed using the MLST scheme established by Miyoshi-Akiyama *et al.* [12]. Clustering of the same 23 isolates was also carried out using FTIR (IR-Biotyper<sup>®</sup>, Bruker Daltonik GmbH). Another ECC strain identified as only expressing an extended-spectrum  $\beta$ -lactamase was added to the panel and served as outlier. Briefly, isolates were subcultured twice for 18h at  $36\pm 2^\circ\text{C}$  on Plate Count Agar (PCA) (Biomérieux SA) and then processed using IR Biotyper kit (Bruker Daltonik GmbH) according to the manufacturer's recommendations. Spectra were acquired and processed by OPUS software v8.2 (Bruker Optics GmbH). Data from the area corresponding to polysaccharides ( $1,300\text{--}800\text{ cm}^{-1}$ ) were used for the analysis of differences between isolates. Dendrograms were built by IR Biotyper Client Software v3.0 (Bruker Daltonik GmbH) using Euclidian distance and average linkage clustering method. This method has been previously described [13] and proposed as useful in retrospectively typing outbreak isolates [13, 14].

Ethics

This study was approved by the local ethic commission and registered by the CNIL (Commission Nationale Informatique et Libertés) under the number PI2021\_843\_0187.

**RESULTS**

Case Finding and Series

Over the 6-months outbreak period, 410 patients were admitted in the ICU. Among them, 19.3% were affected by Covid-19. VIM producing ECC strains were isolated from 14 patients (3.4%); their characteristics and comorbidities are presented in Table I. Seven of them were infected by SARS-CoV2. The index case was a woman aged 59, whose admission on March 7<sup>th</sup> was due to her COVID-19-related acute respiratory distress syndrome (ARDS). For this first patient, a VIM-producing ECC strain was detected in a bronchoalveolar sample on March 24<sup>th</sup>. Given the patients' age, major comorbidities were highly prevalent. The main reason for ICU admission of these 14 patients was acute respiratory failure, specifically COVID-19-related ARDS. They had more frequently active infection (78.6%) rather than bacterial colonization (21.4%). Ventilator-associated pneumonia and septic shock were the most frequent clinical presentations in ECC positive patients (90.9% and 72.7%, respectively). The mortality rate was high (7 patients, 50%) and attributable to the nosocomial infection in 85.7% of the cases.

At the time of VIM-producing ECC-related infection, patients had previously received a median of 2 different antibiotics. Empiric antibiotic treatment was efficient in only 35.7% (Supplementary Table S3). Therefore, antibiotherapy was adapted and cefiderocol prescribed to five patients (Supplementary Table S4).

### Environmental investigation

A total of 1246 samples were analysed. The first round of sampling held in April 2020 (Figure 2) did not yield any CPE positive cultures. As for the second round of sampling (September 2020), no positive samples were recovered on medical devices shared for the care of all patients housed in the ICU. The only samples positive for VIM-producing ECC were harvested from the room housing a patient harbouring the bacteria at the time of sampling (9 samples) and an empty room at the time of sampling (1 sample). Bacterial isolates cultivated from sink drains did not include any ECC strain. However, a VIM-producing *Pseudomonas*



*aeruginosa* strain was recovered from the sink drain of a patient room. This room was not the same as the ones in which VIM-producing ECC carriers had been housed.

#### Enterobacter cloacae complex typing and clustering

With a cut-off value set at 0.159 [15], IR-biotyping results led to the assignment of all but 2 VIM-positive ECC isolates in the single cluster IV (Figure 3). The outlier strain was successfully recognized and formed a specific pure cluster (Cluster III).

MLST typing revealed that all 23 isolates belonged to ST 78 while the chosen outlier belonged to ST 66. Complete genome sequencing allowed for the identification of *bla<sub>VIM-4</sub>* carriage in all environmental and clinical isolates.

#### Outbreak management

The crisis management cell was activated after the discovery of the third case. Over the outbreak period, ten meetings were organized, including the hospital executive management board six times. These meetings allowed making adapted and concerted decisions and granting the means to implement appropriate measures. The coordinated timelines of cases and all instated measures are presented in Figure 2. Several times during this epidemic, a new case occurred while no other case was hospitalized in the unit. Sometimes, a new case was detected several weeks after the exit of the previous ECC carrier from the ICU (Figure 2).

Usual IPC measures were the first concern, including improving hand hygiene and supporting ICU staff in strict application of contact precautions. Medical and paramedical ICU teams were involved in the assessment and improvement of their own practices, daily supported by IPC staff. The results of these evaluations revealed gaps in hand hygiene practices and also misuse of PPE. These deficiencies were favoured by the heavy work load and very real lack of staff encountered in the ICU during COVID-19 epidemic. Various educational tools were used, such as observational audits, personalised infographics and simulation-based training.

A thorough cleaning and disinfection of the unit was implemented by closing successively each of its two wards and subjecting each one to terminal cleaning followed by airborne disinfection of surfaces. In September 2020, after reopening the unit, a new procedure for sink drains decontamination using white vinegar was implemented weekly. A monitoring of the procedure's efficiency was also scheduled by sampling sink drains and looking for the lasting presence of CPE every three months.

## **DISCUSSION**

In the context of the COVID-19 pandemic, we report here a monoclonal outbreak of VIM-producing ECC with a high attributable mortality rate. This high rate is likely linked to COVID-19 co-infections as it has been estimated that secondary infections account for around 50% of fatalities associated with COVID-19 [16-18]. Since the beginning of the COVID-19 pandemic (first local case on February 24<sup>th</sup>) about 15.2 % of patients with an identified CPE carriage were also infected by SARS-CoV2. The screening policy did not allow to describe the rate of COVID-19 patients colonized by CPE. However, COVID-19 and CPE coinfections have already been documented [19, 20].

Also, the length of stay in the ICU, which is a risk factor for hospital acquired infections [20], was extended and influenced by the high proportion of COVID-19-related ARDS.

From a clinical point of view, few antibiotic treatments retain activity against VIM-producing microorganisms: aminoglycosides, IV and/or nebulised colistin, tigecycline and the recently described cefiderocol [21-23]. However, the use of cefiderocol was limited by its availability as a non-marketed product at the time of the outbreak.

To better understand the transmission chain, phenotypic (IR-typing) and molecular typing of environmental and patients' isolates was undertaken. All ECC isolates but the ESBL outlier were found to belong to ST78. As for IR-typing, the ten environmental strains

clustered together with eleven clinical isolates out of the thirteen tested. Overall, 21 of the 23 (91.3%) ST78 isolates were attributed to a single cluster by IR-typing. The two remaining clinical isolates belonging to the ST78 population were found in separate pure clusters. One of those ST78 isolates (Patient 2) displayed phenotypic differences when cultivated on PCA. This could account for variations in IR absorption profiles of membrane carbohydrates between this isolate and the other ST78 ones, leading to a separate clustering by IR-typing as this technique is based on similitudes between carbohydrate absorption spectra. The last isolate (Patient 11) had a growth similar to the one of other isolates. Therefore, the same explanation cannot be put forward to explain its separate clustering by IR-typing. Nevertheless, the overall agreement between the reference typing method (MLST) and IR typing was good, as previously reported [13, 15]. This method was used prospectively for the first time in our hospital to help in clustering isolates of an on-going VIM-producing ECC outbreak. Moreover, FTIR is cheaper, faster and does not need a highly trained staff to be handled. It could therefore be a useful tool in the routine follow-up of bacterial outbreaks, providing swifter results than MLST.

ECC ST78 has previously been identified as a potential high risk international clone [24]. Several outbreaks have been reported over the last few years with ECC ST78 strains producing various carbapenemases [25, 26]. However, these outbreaks were mostly caused by IMP-positive ECC ST78 strains, while *bla<sub>VIM</sub>* genes are mostly reported in *P. aeruginosa*. To the best of our knowledge, this is the first report of an outbreak involving a VIM-positive ECC ST78 clone. A further analysis of core genome Single Nucleotide Polymorphisms could help in pointing out whether various clades of the ST78 were involved in this outbreak and in identifying multiple introductions and routes of spread, as was previously reported by Harada *et al.* [26]. It could also support or invalidate the separate clustering of two ST78 strains by IR-typing.

Several possible routes of transmission were investigated for this clonal outbreak and preventive/corrective actions implemented. The first one was faulty hand hygiene practices, probably linked to misuse of gloves and gowns, as previously described in outbreaks reports [7, 27]. An update on good hand hygiene practices was performed using risk management tools such as cause analysis, and infographics created to teach healthcare workers how to put themselves in a reflexive attitude regarding their hand hygiene practices. A simulation based approach to training in healthcare associated infection prevention and more specifically in hand hygiene was likewise included [28, 29]. Since September 2020, the monthly follow-up of an indicator of hydro alcoholic solutions consumption has also been implemented [30]. The possibility of an environmental reservoir was also examined as sinks and sink drains have been reported as possible sources of contaminations, especially in ICUs [31, 32]. Indeed, a recent study held in an ICU previously housing COVID-19 patients showed the persistence of Gram-negative bacteria such as *Pseudomonas spp.* and ECC in sinks and sink drains even after terminal cleaning and disinfection [33]. Another one also identified VIM-producing CPE in a case-patient sink drain during an outbreak [34]. Even if the data provided by the epidemiological investigation in this study do not definitely support a link between colonization/infection of the cases identified in this study and an environmental source, these observations confirm that sinks and sink drains warrant further investigations and management measures to limit MDR persistence and spread. Especially, the identification of VIM-producing *P. aeruginosa* in one sink drain led us to suspect a horizontal plasmid transmission, as previously described in the literature [35, 36]. One such management measure is the decontamination of sink drain with acetic acid which has been reported as efficient in reducing CPE contamination of sink drains as well as the number of CPE colonised/infected patients [32, 37]. After the instatement of such a procedure in the ICU,

results obtained from the first round of monitoring samples showed a persistence of VIM-producing *P. aeruginosa* in one patient room. Extended Spectrum Beta-Lactamase (ESBL) producing enterobacteria were also retrieved from 2 patient rooms as well as in the common sink used by healthcare workers of the ICU.

Upgrades including the addition of automated airborne disinfection of surfaces and medical equipment to manual cleaning to reach sites inaccessible to cleaners were instated [38]. Indeed, this technology has been previously associated with a reduction in environmental contamination and healthcare associated infections with MDR organisms [39]. The evaluation of existing cleaning practices also allowed us to upgrade the organisation between the different actors, and define the exact role of each category of healthworkers.

Ultimately, the possibility of VIM-producing ECC carriage within the nursing staff was discussed during summer 2020. According to the literature, the link between patient and healthcare worker carriages is not always obvious [40-42]. As no readily available and efficient treatment could have been provided in case of positivity and eviction was not an option during the COVID-19 pandemic, especially in an ICU, it was finally decided not to screen the entire staff for VIM-producing ECC carriage. Another limitation of the study was that the number of cases was judged too small and the patients too heterogeneous to perform a case-control study and draw valid conclusions on risk factors from comparisons.

## **CONCLUSIONS**

Our description of a monoclonal outbreak of VIM-producing ECC and its management reveals how important it is to keep a multifocal approach to prevent the spread of such strains. Despite the lack of an established origin, the containment of this outbreak was achieved through a multidisciplinary and integrated approach including the ICU clinical staff (descriptive epidemiology and implementation of upgraded IPCs), the hygiene laboratory staff (case detection, environmental sampling, phenotypic and molecular typing), and the IPC unit

staff (re-education of healthcare personnel on basic hygiene measures and implementation of upgraded IPCs). Our results also point out FTIR as an interesting alternative to MLST or whole genome sequencing to quickly, prospectively and cheaply type outbreak isolates. This integrated approach and the associated multidisciplinary management are keys to a rapid and successful control of outbreaks.

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#### **CONFLICTS OF INTEREST**

All authors report no potential conflicts of interest relevant to the content of the manuscript.

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#### **SUPPLEMENTARY DATA**

Supplementary materials are available at Journal of Hospital Infection online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

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## Figures legends

Figure 1. Spatial distribution of sampling points in ICU room.

- ★ No germ found
- ★ *E. cloacae* VIM
- ★ Other germs found

Figure 2. Description of the integrated approach implemented to control this outbreak. Cases are represented in green, with their first positive sample as a red line. Actions are classified in two categories: linked to the control of possible environmental source or to the prevention of cross-transmission. The chronology of the implementation of all these actions is represented following the timeline on the left.

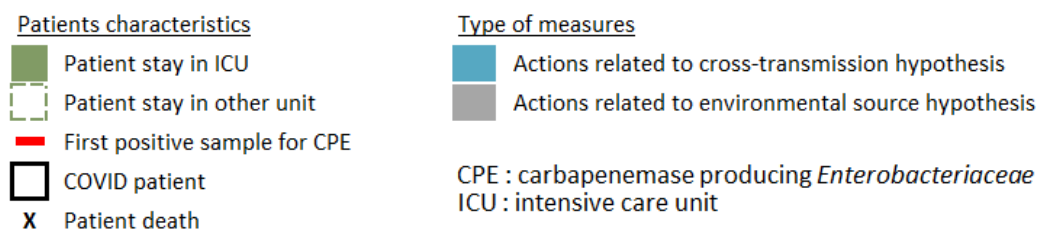


Figure 3. Dendrogram obtained by clustering the FTIR spectra of 23 isolates of *E. cloacae* complex using the Euclidian distance and average linkage clustering method. For each isolate, source identification is given along with the isolation date. The vertical blue line represents the cut-off value chosen to determine clusters of isolates. Clusters were numbered arbitrarily from top to bottom of the figure.

Table I. Clinical characteristics of VIM-producing ECC cases.

Variable	Case Patients (n=14)
<b>Male, n (%)</b>	9 (64%)
<b>Median age, years [IQ]</b>	62 [58.3 - 67]
<b>Median Body Mass Index, kg/m<sup>2</sup> [IQ]</b>	30.9 [29.4-35]
<b>Comorbidities, n (%)</b>	
Obesity	9 (64.3)
Hypertension	8 (57.1)
Diabetes	4 (28.6)
Cardiovascular disease	3 (21.4)
Chronic obstructive pulmonary disease	2 (14.3)
Chronic kidney disease	1 (7.1)
Malignancies	1 (7.1)
<b>SAPSII (median [IQ])</b>	44 [36.8-50]
<b>ARDS n (%)</b>	8 (57.14)
Severe	7 (87.5)
Moderate	1 (12.5)
<b>Support organ failure n (%)</b>	
Invasive mechanical ventilation	14 (100)
Vasopressor	12 (85.7)
RRT	6 (42.9)
<b>Mechanical ventilation, days (median [IQ])</b>	27.5 [21-30]
<b>Length of stay in ICU, days (median [IQ])</b>	30 [24.8-35]
<b>Length of stay in Hospital, days (median [IQ])</b>	32 [26-36.3]
<b>Median delay between ICU admission and rectal carriage, days [IQ]</b>	17 [14.5-27.3]
<b>Median delay between ICU admission and infection, days [IQ]</b>	17 [10-22.5]

SAPSII : Simplified Acute Physiology Score 2

ARDS : Acute Respiratory Distress Syndrome

RRT : Renal Replacement Therapy

Figure 1

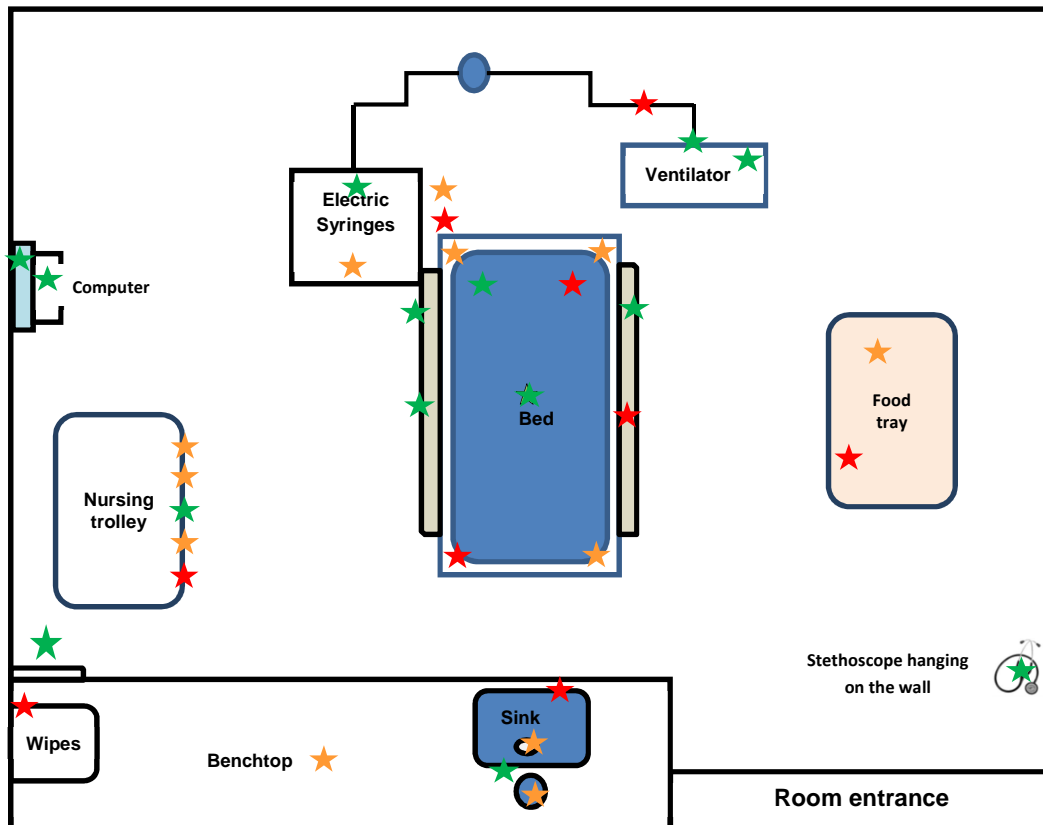


Figure 2 : Description of the integrated approach implemented to control this outbreak

