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Nosocomial outbreak of extended-spectrum β-lactamase-producing *Enterobacter cloacae* among cardiothoracic surgical patients: causes and consequences

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SUMMARY

Background: Enterobacteriaceae are recognized as leading pathogens of healthcare-associated infections.
Aim: To report the investigation of a nosocomial outbreak of extended-spectrum β-lactamase-producing *Enterobacter cloacae* affecting cardiothoracic surgery patients in a Belgian academic hospital.
Methods: Cases were defined based on epidemiological and microbiological investigations, including molecular typing using repetitive element-based polymerase chain reaction and multi-locus sequence typing. Case–control studies followed by field evaluations allowed the identification of a possible reservoir, and the retrospective assessment of human and financial consequences.
Findings: Over a three-month period, 42 patients were infected or colonized by CTX-M-15-producing *E. cloacae* strains that belonged to the same clonal lineage. Acquisition mainly occurred in the intensive care unit ($N = 23$) and in the cardiothoracic surgery ward ($N = 16$). All but one patient had, prior to acquisition, undergone a cardiothoracic surgical procedure, monitored by the same transoesophageal echocardiography (TOE) probe in the operating room. Despite negative microbiological culture results, the exclusion of the suspected probe resulted in rapid termination of the outbreak. Overall, the outbreak was associated with a high mortality rate among infected patients (40%) as well as significant costs (€266,550).

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Conclusion: The outbreak was indirectly shown to be associated with the contamination of a manually disinfected TOE probe used per-operatively during cardiothoracic surgery procedures, because withdrawal of the putative device led to rapid termination of the outbreak.

Introduction

Enterobacteriaceae are recognized as leading bacterial pathogens of healthcare-associated infections, and they have been repeatedly involved in nosocomial outbreaks [1–7]. The proportion of extended-spectrum β-lactamase (ESBL)- and/or carbapenemase-producing Enterobacteriaceae has significantly increased over the last decade [8]. These multidrug-resistant organisms (MDROs) are known to be responsible for life-threatening infections, as well as more expensive outbreaks [6,7,9,10]. Hence, they constitute a major challenge for infection control teams (ICTs) all over the world.

This article describes the control process of a three-month outbreak of ESBL-producing *E. cloacae* which affected patients hospitalized in the intensive care units (ICUs) and the cardiothoracic surgery ward of an academic hospital; and the human and financial consequences of these events are analysed.

Methods

Setting

The outbreak occurred at the CHU UCL Namur Mont-Godinne site (CHUMG), a 370-bed tertiary hospital with four intensive care units (total of 30 beds) and with a 120-bed surgical department. The CHUMG is part of a larger multi-site institution which arose in January 2016 from the amalgamation of three hospitals (CHU UCL Mont-Godinne, Centre Hospitalier de Dinant, and Clinique et Maternité Sainte-Elisabeth Namur). This merged entity serves a regional population (490,000 inhabitants) primarily from the province of Namur in the southern part of Belgium. The CHUMG surgical department includes all surgical specialties including a cardiovascular, thoracic and lung transplantation, representing an important part of its activity (~300 cardiopulmonary bypass interventions per year: unpublished institutional data).

The probable index case was transferred from another hospital to the ICU of CHUMG on May 25th, 2016 for surgical treatment of a postoperative sternitis caused by an ESBL-producing *E. cloacae*. Upon admission and following surgery, this patient was isolated in a single room with contact precautions reinforced based on the Centers for Disease Control and Prevention recommendations [11]. Despite the early implementation of these measures, two other patients hospitalized in the same ICU ward also developed an infection (one pneumonia and one mediastinitis with septicaemia) due to an ESBL-producing *E. cloacae* isolate within the five days following the index case admission. These clustered cases were considered as an outbreak by the ICT.

From June 1st, 2016 onwards, systematic screening for ESBL carriage was initiated (three times a week), and strict isolation and contact precautions were implemented for all identified carriers. The ICT organized numerous informational rounds and follow-up meetings emphasizing the importance of hand hygiene compliance and antibiotic pressure reduction. Nursing staff on the affected units were cohorted. Despite timely application of those measures, the outbreak persisted during the months of June and July 2016, mainly affecting patients on the ICU and the cardiothoracic surgery ward. The possible occurrence of an environmental reservoir or source was therefore hypothesized, and microbiological and field investigations were performed.

Microbiological investigations

Outbreak control required the prompt identification of all epidemic strain carriers. At first, ESBL-carriage screening was performed on rectal swabs (ESwab; Copan Italia S.p.A, Brescia, Italia). From July 26th, 2016 onwards, due to the high proportion of pneumonia among infected patients, an additional screening was performed using oropharyngeal swabs, using the same methodology.

Samples were directly cultured on chromogenic selective agar medium (ChromID ESBL; bioMérieux SA, Marcy l'Etoile, France) and read after a 24 h incubation period at 37°C. Bacterial identification was performed using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS, Microflex LT; Bruker Daltonik GmbH, Bremen, Germany) on growing coloured colonies. Antimicrobial susceptibility testing was performed by disc diffusion methodology whereas the presence of ESBLs was assessed by combination disc synergy tests based on Clinical and Laboratory Standards Institute guidelines [12]. A home-made multiplex end-point polymerase chain reaction (PCR) targeting TEM, OXA and CTX-M β-lactamases was performed on isolates displaying a resistance phenotype compatible with the occurrence of an ESBL [13].

The outbreak-related strains were differentiated from other unrelated ESBL-producing *E. cloacae* by molecular typing using repetitive extragenic palindromic PCR (repPCR, Diversilab; bioMérieux) [14]. Results were retrospectively confirmed by multi-locus sequence typing (MLST), performed on eight presumed epidemic strains and on two presumed non-epidemic strains. For this purpose, the bacterial genome was sequenced using the MiSeq platform (Illumina, San Diego, CA, USA). Raw data were processed using the CLC Genomics Workbench version 11.0.1 (Qiagen, Hilden, Germany). Sequence types were assigned based on the PubMLST database (http://pubmlst.org/ecloacae/) [15]. Raw sequence data were introduced in the NCBI database (GenBank accession numbers: epidemic strains: SRR8302291-296; SRR8302301-302).

Environmental samples were collected using ESwabs pre-moistened in Amies transport medium. Cultures were performed in Letheen broth (Becton Dickinson GmbH, Heidelberg, Germany) incubated at 37°C for seven days with terminal subculture on MacConkey agar plates.

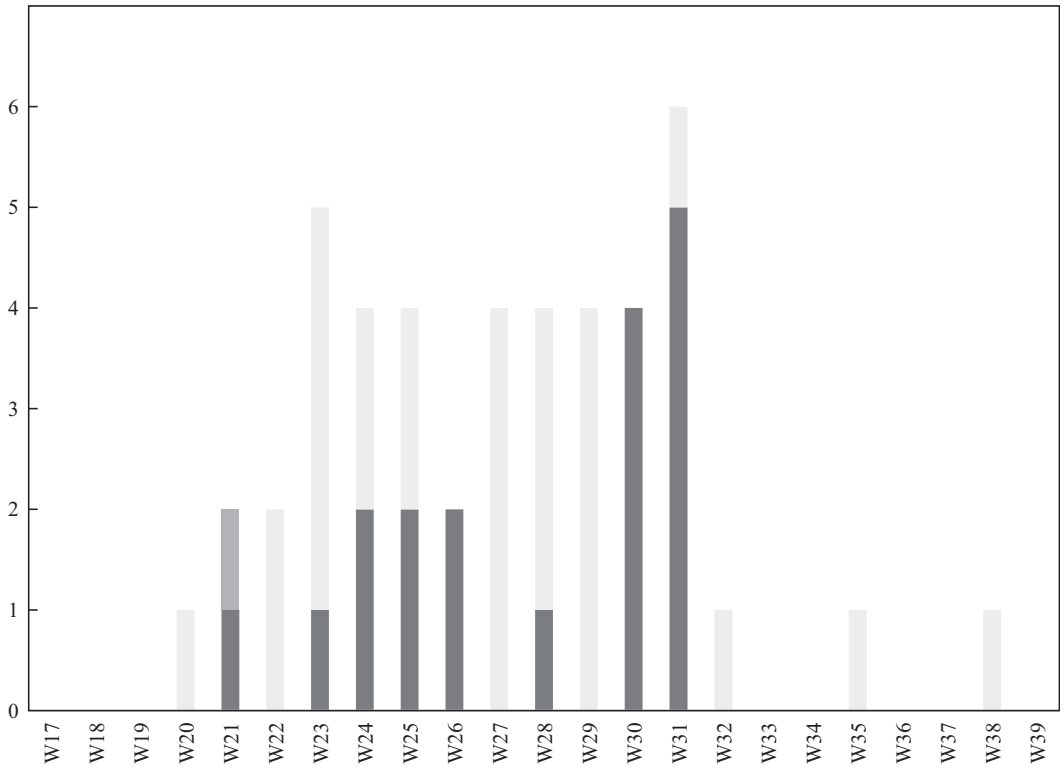

Figure 1. Absolute number of new (one per patient) extended-spectrum β-lactamase (ESBL)-producing *E. cloacae* (including index-case: week 21; and two non-outbreak related cases reflecting the baseline: weeks 20, 38) isolated among patients hosted at the CHU UCL Namur Mont-Godinne site from April 25th to October 2nd, 2016, classified according to the sampling site. ESBL-carriage screening using oropharyngeal sample swabs was implemented on July 26th, 2016 (week 30). The suspected transoesophageal probe and its associated ultrasound unit were withdrawn from clinical usage at the end of week 31. Other: intraoperative sternal biopsies, sampled from index case; W: week.

Reservoir investigation

Once the outbreak-related cases were correctly defined, an initial case—control study was initiated, with the control group being defined as patients hospitalized in the same units over the same period but not colonized/infected by ESBL *E. cloacae*. Metadata (including date and diagnosis upon admission, major comorbidities, caregivers tracking, operating room (OR) in case of surgery, type of surgical intervention, date and type of ESBL-producing *E. cloacae* positive sample, per-operative transoesophageal echocardiography (TOE) and type of TOE probe used, number and date of chest physiotherapy sessions and internal transfers) were collected from medical record charts. This study enabled the listing of potential contamination reservoirs that were further investigated by the ICT.

Outbreak impact analysis

A second case—control study was retrospectively performed after the discharge of the last outbreak patient from the institution (October 15th, 2016), to evaluate the impact of colonization/infection in terms of length of hospital stay, mortality rate, and hospital readmission rates. In this study, the control group included patients who underwent coronary artery bypass graft or valve replacement surgery during the eight weeks preceding the outbreak. Those patients were compared with infected and colonized patients who underwent the same surgeries during the outbreak period. Statistical analysis was performed using MedCalc® software version 18.2.1.

A cost evaluation was performed (in euros) including:

- Expenses and costs associated with the measures that had been implemented for controlling the outbreak, from the day the ICT started to implement those control measures (May 29th, 2016) to the day the ICT deemed that they were no longer necessary (September 12th, 2016). Data collected included laboratory expenditures, costs pertaining to additional precautions and isolation of patients in single room, environmental cleaning costs, staff time, bed closures, as well as purchase of new equipment. Data were collected from the laboratory and from the logistics department (isolation and cleaning costs). Additional time spent by healthcare workers on patient isolation was estimated with the method proposed by Wassenberg *et al.* (30 min/day for nurses, 10 min/day for physicians) [16].
- Expenses associated with the medical management of infected patients (antimicrobial therapy and additional length of stay). Additional length of stay (LOS) for surgical patients was evaluated by subtracting the average LOS depending on surgical procedure (institutional data) from the actual LOS of infected patients. The costs of additional bed-days were extracted from federal records [17].

Results

From May 30th to August 7th, 2016, a mean of four new cases were weekly identified among patients staying at CHUMG; the non-outbreak incidence in the institution was inferior to one new case per two weeks (Figure 1). In total, 42 patients were either infected ($N = 9$; including eight pneumonias and one mediastinitis; Table I) or colonized ($N = 33$) by the epidemic strain over a 107-day period. Acquisition occurred mainly in patients during their stay in the ICU ($N = 23$) and in the cardiothoracic surgery ward ($N = 16$), as well as, to a lesser extent, on the vascular surgical ward ($N = 2$) and on the haematological unit ($N = 1$). All outbreak-related *E. cloacae* isolates displayed the same antimicrobial resistance pattern (cross-resistance to third-generation cephalosporins, fluoroquinolones, gentamicin, and trimethoprim–sulfamethoxazole) and produced a CTX-M-15 ESBL. They also presented a high degree of relatedness (>95% similarity) by Diversilab repPCR and clustered in the same repPCR type (Diversilab type 11). Eight *E. cloacae* isolates from the outbreak were further sequenced, demonstrating belonging to MLST type ST190.

The case–control study revealed that 41 out of 42 patients had undergone cardiothoracic surgery under cardiopulmonary bypass prior to acquisition, including aortocoronary bypass ($N = 13$), valve replacement with or without bypass ($N = 18$), pulmonary transplant ($N = 2$) and various other cardiothoracic or vascular surgical procedures including, among others, atrial tumour excision, sternal revision, or aortic replacement ($N = 8$). Median time elapsed between surgery and acquisition of the outbreak-related *E. cloacae* isolate was 5.5 days (range: 3–64) when considering the time to positivity of rectal swabs but only 1.3 days (range: 0–3) when analysing culture results from throat specimens.

The early colonization of the upper respiratory tract suggested the possible existence of a reservoir located in the operating rooms with entry through the patient's respiratory mucosae at the time of surgery. Repeated visits to the operating room highlighted the transoesophageal echocardiography (TOE) probe as a possible reservoir candidate, since this examination was the only procedure noted to be specific to cardiothoracic surgery patients and because it remained in direct and prolonged contact with patient's upper respiratory tract mucosa during the whole period of surgery. Three TOE probes were used in the operating room. The case–control study demonstrated that all colonized/infected surgical patients had been exposed to the three-dimensional ultrasound probe (X7-2t, iE33; Philips Healthcare, Inc.; Andover, MA, USA), which, due to its higher resolution, was used more frequently than the two others (especially for hemodynamically unstable patients). However, repeated samplings of the ultrasound machine ($N = 4$) and of the TOE probe ($N = 6$, swabbing of the probe handle, the shaft and the tip) were negative on microbiological culture. In order to control the outbreak, and despite the lack of microbiological proof, it was decided to withdraw the suspected TOE probe and its associated ultrasound machine from clinical usage on August 8th, 2016. This resulted in a termination of the outbreak (Figure 1).

The second case–control study revealed that the outbreak was associated with a slight trend towards increased mortality (odds ratio (OR): 2.69; 95% confidence interval (CI): 0.26–27.48) (Table II). Mortality was significantly increased

Table I
Infected patients' characteristics

Case no.	Sex	Age	Surgical procedure	Date of surgery	Characteristics of first positive sample	Infection	Treatment	Total postoperative length of stay	Outcome
1	M	62	Sternum debridement	May 26th, 2016	May 26th, 2016, sternal sample	Mediastinitis	Surgical debridement, MEM	12	Discharge
2	F	61	Aortic replacement for type A dissection	May 26th, 2016	May 29th, 2016, sputum	Pneumonia	PTZ, MEM	27	Discharge
3	F	87	VR	May 26th, 2016	May 30th, 2016, rectal swab	Mediastinitis + sepsis	MEM	55	Discharge
4	M	82	VR	Jun 10th, 2016	Jun 13th, 2016, sputum	Pneumonia	PTZ, MEM	64	Discharge
5	H	71	CABG + VR	Jun 23rd, 2016	Jun 26th, 2016, sputum and blood culture	Pneumonia + sepsis	MEM	57	Death
6	F	49	None	—	Jun 29th, 2016, sputum	Pneumonia	MEM	—	Death
7	H	50	Aortic replacement for abdominal aneurism	Aug 2nd, 2016	Jul 3rd, 2016, sputum	Pneumonia	MEM	3	Death
8	H	66	CABG	Jul 29th, 2016	Aug 1st, 2016, throat sample	Pneumonia	PTZ	15	Death
9	H	58	Sternal revision for cardiac tamponade	Aug 1st, 2016	Aug 3rd, 2016, throat sample	Pneumonia	PTZ, MEM	52	Discharge
10	F	78	CABG + VR	Aug 1st, 2016	Aug 3rd, 2016, throat sample	Pneumonia	PTZ	16	Discharge

CABG, coronary artery bypass grafting; F, female; M, male; MEM, meropenem; PTZ, piperacillin–tazobactam; VR, valve replacement.
Case 1 is the index case.

Table II
Influence of colonized/infected status on patient's outcome

	Control group ($N = 37$)	Total outbreak group ($N = 32$)	Colonized ($N = 27$)	Infected ($N = 5$)	Statistics
Mean (range) age (years)	70.7 (48.6–84.6)	72.46 (37.9–88.9)	70.8	78.0	
Sex ratio (M/F)	3.1	2.6	2.9	1.5	
Type of surgery					
CABG	10	13	12	1	
VR	17	14	12	2	
CABG + VR	10	5	3	2	
Mean (range) surgery time (min)	286.3 (210–618)	296.7 (188–450)	289.9	333.4	
Mean postoperative LOS (days)					
ICU	4.1 (2–13)	7.6 (2–57)	4.1	26.4	$P = 0.107$
Total	12.5 (7–33)	15.9 (6–63)	12.4	41.2	$P = 0.146$
In-hospital revision surgery rate (%)	5.4	6.25	3.7	20	OR: 1.17
					95% CI: 0.15–8.79
In-hospital mortality rate (%)	2.7	9.4	3.7	40	OR: 2.69
					95% CI: 0.26–27.49
Three-month readmission rate (%)[a]	11.1	17.2	15.4	33.3	OR: 1.67
					95% CI: 0.40–6.88

CABG, coronary artery bypass grafting; F, female; ICU, intensive care unit; M, male; VR, valve replacement; LOS, length of stay; OR, odds ratio; CI, confidence interval.
[a] Denominator excluding in-hospital deceased patients.

when comparing infected patients (colonized patients excluded) with the control group (OR: 24; 95% CI: 1.66–347.91). Similarly, ICU and total LOS were significantly increased for infected patients ($P < 0.001$ for both) but not among colonized patients ($P = 0.997$ and 0.915 respectively) compared to the control group. Among cases, mean age ($P = 0.205$) and length of surgery ($P = 0.174$) were higher in infected than in colonized patients but those differences were not statistically significant.

The global outbreak-related expenses were estimated to amount to €266,550 (Table III). From May 29th, 2016 to September 12th, 2016, more than 1800 screening swabs were processed by the laboratory. A total of 359 Gram-negative bacterial isolates were identified and 75 of these were analysed for antimicrobial susceptibility testing (total estimated routine laboratory costs excluding technician time: €11,500). During the same period, the institution spent an extra €5300 in personal protective equipment (including 16,250 isolation gowns, 13,400 gloves, and 1460 masks) compared to the previous years. Extra cleaning costs included 133 additional room cleanings and daily operating room decontamination with hydrogen peroxide in which cardiothoracic surgery procedures were performed. Finally, the institution decided to acquire a new TOE probe as well as two new automated washer-disinfectors (plus transport and storage systems) dedicated for the washing and disinfection of TOE probes (one for the

Table III
Cost evaluation summary

	Details	Costs (€)
Outbreak control measures	Laboratory costs including:	
	Screening swabs processing (technician time included)	14,500
	Resistance mechanisms investigation and typing procedures	2500
	Personal protective equipment	5300
	Cleaning costs	
	Technician time	5500
	Products (including 150 L of hydrogen peroxide)	5500
	Healthcare teams, time	34,150
	Infection control experts, time	11,500
	Bed closure	None retained
	Newly acquired materials (one TOE probe, two automated washer-disinfectors with associated transport and storage systems)	88,000
Infected patient's management	Antibiotics	4500
	Additional length of stay:	
	ICU wards	73,450
	Non-ICU wards	21,650
Total		266,550

ICU, intensive care unit; TOE, transoesophageal echocardiography.
Expenses associated with index case management were not included in this evaluation.

operating room, one for the cardiac ward), which accounted for €88,000.

The nine infected patients required treatment with high dosage of costly broad-spectrum antibiotics (i.e. 104 days of meropenem and 19 days of piperacillin–tazobactam). When evaluating the impact of increased LOS, we found that 140 additional days were spent in ICU (€522.95 per day) whereas 44 additional days were spent in non-ICU wards (€496.05 per day) by infected cases [17].

Discussion

Our institution faced a large nosocomial outbreak among cardiothoracic surgery patients that was caused by an ESBL-producing *E. cloacae* strain. Despite the lack of direct microbiological evidence (none of the environmental culture specimens was positive; molecular detection on environmental surfaces and materials not performed) it was demonstrated indirectly that a specific TOE probe was associated, because exclusion led to the immediate termination of cases. However, we acknowledge that this lack of microbiological documentation constitutes a weakness of the present report.

Only one onco-haematology patient with acute myeloid leukaemia not exposed to cardiac surgery, nor to any TOE procedure, was found to be colonized by the ESBL-positive *E. cloacae* epidemic strain. However, this patient had close contact with a colonized patient and had been nursed by the same healthcare staff during a seven-day stay in a unit with positive pressure rooms hosting mainly haematology patients but also occasional pulmonary transplant patients. The multiple negative results of cultures support the limited sensitivity of microbiological culture techniques (especially on swab samples) from inanimate material and environmental surfaces [18].

TOE probes have been reported previously as a source of contamination associated with nosocomial outbreaks among cardiac surgery patients [3,5,19]. These lumen-free endoscopes are classified as semi-critical material according to Spaulding classification, indicating that they should undergo a high-level disinfection [20,21].

Before this adverse event, we performed manual high-level disinfection using chlorine dioxide wipes. Disposable disinfectant wipes offer several advantages such as a short turnaround time (2–5 min), ease of use, safety for workers, and affordable costs [22,23]. Moreover, unlike automated washing-disinfection, the use of wipes does not require any maintenance contracts nor microbiological water controls. However, one of the drawbacks is the operator-dependent character of the manual processing, and several field audits during the outbreak indeed highlighted a lack of standardization and of traceability associated with cleaning/disinfection wipes. It was clear that healthcare staff had been insufficiently informed and trained about the importance of the procedure. The French Hygiene Society recommended in 2013 using protective sheets in case of wiping disinfection, but those sheets were rejected by anaesthesiologists due to their impact on ultrasound image quality [24]. Furthermore, although contact time between chlorine dioxide and probe is theoretically minimal (30 s if rinsing procedure is accurately performed), the possibility of a compatibility issue between chlorine dioxide (known to be an oxidizing agent) and polymer components of the TOE probe (such as silicone bead around the transducer lens) was also raised [25,26]. Unfortunately, this hypothesis could not be further investigated as the discarded TOE probe could not be retrieved for thorough examination.

Altogether, those concerns led to the decision to switch from the use of wipes to automated reprocessors using 5% peracetic acid. TOE probes and machine disinfection procedures were audited by the ICT, and an on-field educational programme was set in order to improve the level of staff knowledge. A computerized traceability system was also implemented, in partnership with anaesthesiologists, in order to facilitate future TOE-related investigations. Finally, since these events, ICU patients have been screened on a weekly basis for MDRO carriage by rectal swabbing.

The total cost of this outbreak was estimated at €266,550. Cost per patient was increased by a coefficient of 3.78 among infected patients, compared to colonized patients (€3975 per colonized patient and €15,040 per infected patient, excluding index case). The largest part of the expenses was associated with increased LOS, newly purchased equipment and healthcare workers' time dedicated to the outbreak management (included as an opportunity cost). However, despite extensive listing and calculation of additional costs, the outbreak-related expenses were probably underestimated.

First, as we neither closed wards nor discontinued the surgical programme (except for pulmonary transplant), no bed closure costs were included. Previously published cost evaluations, reporting that bed closures and/or interruption of new admissions accounted for major expenses, led to the ICT and institution favouring other measures, although such decisions were considered at different time-points of the outbreak [6,7,10,27]. However, a significant but multi-factorial decrease in the number of cardiothoracic interventions was recorded during the outbreak period. Also, the overcrowding of ICU with isolated/infected patients could have had an impact on other (non-related to cardiothoracic surgery) hospital admissions that we were not able to assess retrospectively. Second, certain losses of income associated with specific Belgian legislation regarding the hospitalization charges were not considered. In Belgium, additional incomes can be charged for patients requesting a private room, but not when the patient has to be isolated in a private room for medical reasons. Knowing those private incomes concern usually 20% of cardiac surgery patients, their loss would also represent one of the largest revenue shortfalls due to this outbreak for the institution. Finally, another pitfall of this evaluation is the absence of the long-term outbreak-related expenses that could have included expenditures related to long-term follow-up of affected patients and therefore impact on the cardiothoracic surgical activity.

Conflict of interest statement
None declared.

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