Efficacy of a coordinated strategy for containment of multidrug-resistant Gram-negative bacteria carriage in a Neonatal Intensive Care Unit in the context of an active surveillance program

Laura Saporito (laura.sapo@tin.it)
University of Palermo, Italy https://orcid.org/0000-0002-9756-0957
Giorgio Graziano
University of Palermo
Federica Mescolo
University of Palermo
Emanuele Amodio
University of Palermo
Vincenzo Insinga
University of Palermo
Grazia Rinaudo
University of Palermo
Aurora Aleo
University of Palermo
Celestino Bonura
University of Palermo
Marcello Vitaliti
University of Palermo
Giovanni Corsello
University of Palermo
Francesco Vitale
University of Palermo
Carmelo Massimo Maida
University of Palermo
Mario Giuffrè
University of Palermo

Research

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Abstract

Background: Antimicrobial resistance in Neonatal Intensive Care Unit (NICU) patients is a threat, due to the large use of antimicrobial treatment and invasive devices in fragile babies.

Since 2014 an active surveillance program of multidrug-resistant Gram-negative bacteria (MDR-GNB) carriage is in place in the five NICUs of Palermo, Italy. In 2017 an increase in the prevalence of MDR-GNB and in particular of extended-spectrum β-lactamases-producing *Klebsiella pneumoniae* (ESBL-KP) was observed in "Civico" hospital NICU.

Aim: To estimate the impact of a coordinated intervention strategy in achieving long-lasting reduction of MDR-GNB prevalence in the NICU.

Methods: Rectal swabs were obtained monthly and processed to detect MDR-GNB using standard methods. MDR-GNB were characterized by pulsed-field gel electrophoresis (PFGE). From November 2017 the following intervention measures were applied: a) two-months strengthening of sample collection; b) stakeholders' meetings; c) improvement of prevention measures and antimicrobial policy.

Findings: During the strengthened microbiological surveillance MDR-GNB and ESBL-KP were detected in rectal swabs (34.8%; 23.2%), nasal swabs (24.6%; 14.5%), oral swabs (14.5%; 5.4%), milk samples (32.1%; 17.9%), soother swabs (30.8%; 17.9%) and from a sub-intensive room surface. Thirteen ESBL-KP strains isolated from clinical and environmental samples showed identical PFGE patterns.

Prevalence of MDR-GNB and ESBL-KP carriage significantly decreased in the year after intervention compared to the previous year (20.6% vs 62.2 %; $p<0.001$ and 11.1% vs 57.8%; $p<0.001$). MDR-GNB were not detected for three months and ESBL-KP for five months. Multivariate analysis of principal exposure variables showed that admission in post-intervention period significantly reduced the risk of MDR-GNB carriage (adj-OR=0.21, 95% CI=0.076-0.629; $p<0.001$).

Conclusions: MDR-GNB broadly circulate in NICU setting and can colonize different body sites and spread by various vehicles. A coordinated strategy of multiple interventions with active cooperation between epidemiologists and clinicians in the NICU can effectively reduce their circulation and in particular the carriage of most dangerous ESBL-KP strains.

Introduction

The World Health Organization has declared antimicrobial resistance as one of the most dangerous public health threats of the last ten years with an increasing impact in the future (1).

Control and prevention of antimicrobial resistance and healthcare-related infections have been included in the Italian National Prevention Plan of Antimicrobial Resistance 2017-2020.

Neonatal Intensive Care Units (NICUs) are complex assistive settings, heavily burdened by antimicrobial resistance, due to the large use of antimicrobial treatments in critical patients exposed to invasive devices and procedures. In these settings, prevention and control of infections and drug resistance can play a crucial role in the outcome of critical newborns with limited therapeutic options (2,3). Adherence of healthcare workers and parents to hand-washing protocols and other hygienic preventive procedures in hospitals, particularly in intensive care units, is essential to manage the spread of healthcare-associated infections. (4)

Surveillance is commonly defined as the on-going and systematic collection, analysis and interpretation of health data essential to the planning, implementation and evaluation in public health practice (5). Nowadays, active surveillance of infections or carriage by multidrug resistant organisms (MDROs) may be recognized as an effective tool for early detection of unusual patterns of microbial pathogens in a specific health-care setting (6–8) and to define the local epidemiology of MDROs and the relative antimicrobial resistance map in each NICU (8–11).

Background

Based on internationally recognized prevention objectives (12), the University Hospital in Palermo, Italy, started an active surveillance program of MDRO carriage in NICU in June 2009 (13–15). In 2014 this program was extended to the other four NICUs in the metropolitan area to define antimicrobial resistance patterns in different settings, monitoring eventual microbial circulation related to patient movements inside the network of the local health system (16,17).

The main focus of the program is the epidemiological analysis of MDROs circulation, seasonal variability, associated risk factors, molecular typing of isolated bacteria and evaluation of antimicrobial resistance related to bacterial carriage. This active surveillance program involves an epidemiological team with its laboratory, a neonatal team and NICU healthcare providers. Each stakeholder participated in regular meetings, in order to identify shared goals and methodology and to provide periodic feedback of their activities. Collection of specimens (nasal and rectal swabs) from each hospitalized newborn is performed every 4 weeks (for the purposes of this study, each 4-week interval was defined as a month) in every NICU and processed in order to identify carriage by MDROs. Complete figures and trends of microbial isolations are registered and periodically reported to healthcare providers, together with the results of the in vitro antimicrobial sensitivity testing. Final purpose is prevention and control of infectious diseases through an early identification of new carriage clusters or changes in the time-line pattern of colonization in a specific setting.

During the first four years of the program, we observed a higher prevalence of multi-drug resistant Gram-negative bacteria (MDR-GNB) carriage in the "Civico" hospital NICU compared to the others in Palermo. An accurate examination of the annual prevalence of MDR-GNB carriage in rectal swabs in this health-care setting pinpointed a high percentage of MDR-GNB colonized newborns in 2014 and an increasing trend in 2015 (Table 1).

Table 1. Number and percentage of rectal samples positive for MDR-GNB, ESBL-producing GNB and ESBL-KP during four years surveillance in “Civico” Hospital NICU.

period of surveillance	2014 (feb - dec)	2015 (jan – dec)	2016 (jan – dec)	2017 (jan – oct)
tested samples, n	180	184	164	160
MDR-GNB, n (%)	97 (53.9)	126 (68.5) a	71 (43.3) a, b	112 (70.0) a,c
ESBL, n (%)	92 (51.1)	102 (55.4)	61 (37.2) a, b	104 (65.0) a, c
ESBL-KP, n (%)	91(50.6)	43 (23.4) a	40 (24.4) a	104 (65.0) a, b, c
	a p<0.05 with respect to 2014			
	b p<0.05 with respect to 2015			
	c p<0.05 with respect to 2016			

Based on this evidence, few additional episodic control measures were performed: an extraordinary collection of rectal swabs in November 2015 and a meeting with all health-care workers in January 2016 to inform about worrying colonization data and trend and to reinforce good clinical practices for infection control and prevention.

Despite a significant reduction of MDR-GNB carriage in 2016 (p<0.001), the annual prevalence showed a new increase in 2017 (p<0.001, Table 1), mainly involving Extended-spectrum β-lactamases-producing *Klebsiella pneumoniae* (ESBL-KP). This evidence led to the introduction in November 2017 of a more effective and long-lasting approach to reinforce the implementation of the measures already adopted.

Aim of the study

The aim of this study was to estimate the impact of a coordinated intervention strategy in achieving more effective and long-lasting reduction of MDR-GNB colonization prevalence in “Civico” hospital NICU, comparing the “pre-intervention” period (from November 2016 to October 2017) with the “post-intervention” period (from November 2017 to October 2019) and evaluating the role of multiple clinical risk factors.

Patients And Methods

Setting and population

The “Civico” hospital NICU is set in the adult hospital campus and includes two nearby open spaces of 60 and 40 square meters respectively. The NICU has 16 beds, which consist of 8 intensive care coats and 8 sub-intensive care beds. A hand-washing sink is located at each entrance. Around 370 term and preterm newborns are admitted annually.

Two neonatologists and two nurses are dedicated in each area, intensive and sub-intensive room respectively, while another nurse is responsible for feeding-preparing.

All newborns included in our monthly surveillance program between November 2016 and October 2019 have been enrolled in this study. Rectal swabs for detection of MDR-GNB carriage were collected every 4 weeks from all admitted newborns in NICU, regardless of any clinical or laboratory signs of infection. Carriage was defined as a positive culture of MDR-GNB from at least one rectal swab collected during the NICU stay.

From November 2017, a strategy of multiple coordinated intervention measures was introduced to reduce the prevalence of MDR-GNB carriage.

Description of Intervention Measures

The intervention strategy included:

a) Strengthening of sample collection

Microbiological surveillance was reinforced for 2 months (11/27/2017 - 01/03/2018) as follows:

- extraordinary weekly sampling of rectal, nasal, oral mucosa swabs, devices and material strictly in contact with each newborn (feeding bottles and soothers, remnant milk after newborn feeding) for the first month and then after four weeks;
- environmental samplings (room surfaces including milk preparation room, healthcare workers hands and stethoscopes) at the beginning of the intervention and after four weeks. From February 2018 monthly sample collection continued as previously including only rectal swabs.

b) Stakeholders weekly meetings

During the first two months of intervention, weekly meetings of NICU healthcare workers were performed with experts from surveillance team, sharing surveillance program results, pinpointing adherence of healthcare workers to standard precautions and discussing possible critical points and preventive strategies. Subsequently, these meetings were scheduled monthly, involving only NICU staff.

c) Improvement of prevention measures

The following changes in NICU organization and patient management were introduced:

- a new standard protocol for antimicrobial therapy approved by the Hospital Health Management, which defined new guidelines regarding standardized start and stop timing in suspected sepsis, duration of therapy in confirmed infections and sepsis, stop timing after the first negative culture (18);
- hand-washing sensitization posters for caregivers and parents showed in all NICU and Neonatology rooms;

- substitution of contaminated devices after feeding;
- introduction of bundles for common procedures, such as blood-sample collection, diaper change, milk preparation or fridge sanitation.

Evaluation of the impact of intervention measures

Prevalence of MDR-GNB, ESBL-producing GNB, ESBL-KP carriage was assessed and compared in two groups: newborns admitted in the pre-intervention period and newborns admitted in the post-intervention period.

In addition, in order to evaluate the impact of intervention measures and to identify possible changes in risk factors for MDR-GNB carriage, we conducted a quasi-experimental study comparing clinical features in two subgroups of patients: intervention and control populations.

Inclusion criteria were the admission in the NICU and enrolling in the surveillance program for MDR-GNB from November 2017 to March 2018 (intervention population) or from November 2016 to March 2017 (control population).

Exclusion criteria were:

- clinical records not available;
- outborn patients with MDR-GNB colonization in the first rectal swab (we do not perform rectal swab on NICU admission so we cannot discriminate if MDR-GNB carriage was already present before the admission in the ward);
- patients with a positive rectal swab already before the implementation of preventive measures (November 28th 2017).

Outcome variable was detection of MDR-GNB in at least one rectal swab obtained for the surveillance program during the observation period of risk factors exposure.

The observational period was defined as the range between NICU admission and the date of the first positive rectal swab in colonized patients, and the range between NICU admission and the last rectal swab obtained in non-colonized patients.

We analysed the following clinical characteristics: type of delivery, sex, gestational age, birth weight, APGAR score at 5', presence of malformations, feeding (breast milk and/or formulas), use of nasogastric tube, parenteral nutrition, use of invasive devices (central and peripheral venous access), invasive or non-invasive ventilation, surgical treatment, use of antimicrobials and hospital discharge (alive, dead or moved to another hospital).

Collection of Samples and Microbiological Analysis

Collected samples were enriched in liquid cultures (Brain Heart Infusion, Oxoid) for 24 hours at 37°C, then plated in McConkey Agar with three antimicrobial discs (amoxicillin-clavulanate 30µg, meropenem 10µg, ceftazidime 30µg) to detect multi-drug resistant bacteria (13). After overnight incubation, suspected MDR colonies were isolated for identification (standard biochemical methods), susceptibility testing and ESBL detection according with the European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines (19–21).

MDR-GNB were defined as Gram-negative bacteria non-responders in vitro to at least three different classes of antimicrobials under testing (aminopenicillins, third-generation cephalosporins, monobactams, aminoglycosides and carbapenems).

Molecular characterization of MDR-GNB was performed using pulsed-field gel electrophoresis (PFGE) after DNA-cutting with restriction enzymes and electrophoretic profiles were interpreted according to standard procedures (18,22,23).

Statistical Analysis

Prevalence of MDR-GNB carriage, in relation to patient characteristics, procedures and clinical outcomes have been compared using the chi-square test or Fisher test for categorical variables and Student's t-test or the Mann-Whitney U test for continuous variables.

A multivariate analysis by backward stepwise logistic regression was carried out to determine variables significantly associated with MDR-GNB carriage. All variables that did differ between subjects with MDR-GNB carriage ($p < 0.10$) were initially entered in the model, and the least significant variable was removed one at a time. Goodness of fit of the logistic models was assessed using the Hosmer and Lemeshow test. Several multiple logistic regression models were tested in order to determine the most significant and simplest model with the best available fit for the data.

All significance tests were two-tailed, and $p<0.05$ was considered significant.

Statistical analysis was carried out by using the R statistical software package (version 3.6.1) and Microsoft Excel 2010.

Results

Strengthened microbiological surveillance

During the strengthened microbiological surveillance (performed as described above from November 28th 2017 to January 23rd 2018) 69 rectal and nasal swabs, 55 oral swabs, 39 samples from feeding bottles and soothers and 28 milk samples were collected. MDR-GNB were detected from 24 rectal swabs (34.8%), 17 nasal swabs (24.6%), 8 swabs from oral mucosa (14.5%); moreover, 9 milk samples (32.1%) and 12 soother swabs (30.8%) were positive for MDR-GNB. ESBL-KP was detected from 16 rectal swabs (23.2%), 10 nasal swabs (14.5%), 3 swabs from oral mucosa (5.4%); 5 milk samples (17.9 %) and 7 soother swabs

(17.9%) were positive for ESBL-KP. At last, MDR-GNB were significantly reduced during the months post-intervention and ESBL-KP was not detected in the ward from January 23rd to June 2018. Temporal trends of MDR-GNB and ESBL-KP rectal carriage in relation to the intervention measures adopted are shown in Figure 1.

Environmental testing yielded 104 samples: 45 NICU surface samples, 23 sub-intensive room surface samples, 11 specimens from feeding-preparing surfaces, 15 from caregivers' hands, 5 from stethoscopes, 5 from baby cots, 2 from laminar-flow hoods. One powdered and one liquid feeding formula sample have also been analysed.

MDR-GNB isolates were detected in one sample from sub-intensive room (ESBL-KP) on the first environmental sampling day and in one sample from feeding-preparing room (*Stenotrophomonas maltophilia*) on the second environmental sampling day. In the same data, non-MDR *Pseudomonas aeruginosa* was isolated in two samples from sub-intensive and feeding-preparing room. No MDR-GNB were isolated from healthcare workers hands.

Thirteen ESBL-KP strains isolated from rectal, nasal, oral swabs, milk, soothers and environmental surfaces were submitted to molecular characterization by PFGE. Analysis of electrophoretic profiles showed identical or closely related patterns suggesting a common origin for all tested strains (Figure 2).

Comparison between pre- and post-intervention periods

In the study period (November 2016 - October 2019) 419 patients have been enrolled in the surveillance, 142 in the pre-intervention period (November 2016 - October 2017), 143 and 134 respectively in the first and in the second year after the intervention (November 2017 - October 2019). A mean of 15 (SD = 2.76) patients were screened each month during the pre-intervention period. There was no significant difference in the post-intervention period when 14.9 patients (SD=2.61) were tested each month (p=0.96).

A total of 539 rectal swabs were analysed. In the pre-intervention period 180 rectal swabs were collected and analysed: MDR-GNB were detected from 112/180 (62.2%) rectal swabs, and in particular 104/180 (57.8%) were ESBL-KP. In the first year of the post-intervention period 189 rectal swabs were analysed: MDR-GNB were detected from 39/189 (20.6%) rectal swabs, and in particular 21/189 (11.1%) were ESBL-KP while 2/189 (1.1%) were other ESBL-producing GNB. Carriage by ESBL-KP accounted for 92.8% (104/112) of all MDR-GNB isolated in the pre-intervention period and for 53.8% (21/39) of those isolated in the first year of the post-intervention period. Prevalence of MDR-GNB, ESBL-producing GNB and ESBL-KP carriage between the two periods was significantly different ($p<0.001$). Chi square for trend was also significant ($p< 0.001$). In the second year of the post-intervention period (November 2018 - October 2019) 170 samples were analysed. Prevalence of MDR-GNB carriage remained low (MDR-GNB 44/170, 25.9%; ESBL-producing GNB 15/170, 8.8%; ESBL-KP 6/170, 3.5%). No significant differences were observed in the prevalence of MDR-GNB and ESBL-GNB with the previous year (p=0.24 and p=0.30, respectively). Prevalence of ESBL-KP was significantly lower in the second post-intervention year (p=0.006). Trends of MDR-GNB carriage in the pre- and post-intervention periods are shown in Figure 3.

Quasi-experimental study: comparison between intervention population and control population

One-hundred and two patients fulfilled the criteria to be included in the quasi-experimental study: 50 patients admitted from November 2017 to March 2018 (intervention population) and 52 patients admitted from November 2016 to March 2017 (control population), according to the inclusion/exclusion algorithm (Figure 4).

Characteristics of patients, medical devices and antimicrobial treatment are summarized in Table 2.

Table 2. Comparison between different variables in intervention population and control population in quasi-experimental study population.

Variable	All patients (n=102)	Intervention population (n=50)	Control population (n=52)	p
Characteristics of patients				
Male gender, n (%)	54 (52.9%)	29 (58%)	25 (48.1%)	0.31
Twin birth, n (%)	15 (15.5%)	6 (12.5%)	9 (18.4%)	0.42
Inborn, n (%)	76 (74.5%)	31 (62%)	45 (86.5%)	**0.004**
Admission to NICU >24 h after birth, n (%)	9 (8.9%)	7 (14%)	2 (3.9%)	0.09
Birth through caesarean section, n (%)	65 (65%)	30 (60%)	35 (70%)	0.29
Gestational age, median (IQR), wk	34.5 (32-38)	36.5 (33-39)	33 (32-37)	**0.014**
Preterm birth (<37wk), n (%)	63 (61.8%)	25 (50%)	38 (73.1%)	**0.02**
Birth weight, mean (SD), g	2234 (865)	2509 (844)	1980 (811)	**0.002**
Apgar score at 5 min, median (IQR)	9 (8-10)	9 (8-10)	9 (8-10)	0.71
Malformation, n (%)	11 (10.8%)	6 (12%)	5 (9.6%)	0.70
Nutrition and devices				
Breast milk feeding, n (%)	75 (73.5%)	32 (64%)	43 (82.7%)	**0.03**
Formula feeding, n (%)	101 (99%)	49 (98%)	52 (100%)	0.49
Nasogastric tube, n (%)	43 (42.2%)	19 (38%)	24 (46.1%)	0.40
Parenteral nutrition, n (%)	60 (58.8%)	29 (58%)	31 (59.6%)	0.87
Central venous access device, n (%)	44 (43.1%)	20 (40%)	24 (46.1%)	0.53
Peripheral venous access device, n (%)	81 (79.4%)	37 (74%)	44 (84.6%)	0.18
Endotracheal tube, n (%)	19 (18.6%)	6 (12%)	13 (25%)	0.09

Noninvasive ventilation, n (%)	41 (40.2%)	22 (44%)	19 (36.5%)	0.44
Surgical procedure, n (%)	3 (2.9%)	2 (4%)	1 (1.9%)	0.61
Antimicrobial therapy				
Antibiotics, any, n (%)	49 (48%)	21 (42%)	28 (53.8%)	0.23
Ampicillin, n (%)	24 (23.5%)	16 (32%)	8 (15.4%)	0.048
Ampicillin - Sulbactam, n (%)	11 (10.8%)	0	11 (21.1%)	**<0.001**
Cephalosporines, n (%)	21 (20.6%)	7 (14%)	14 (26.9%)	0.11
Carbapenems, n (%)	18 (17.6%)	9 (18%)	9 (17.3%)	0.93
Amikacin, n (%)	32 (31.4%)	16 (32%)	16 (30.8%)	0.89
Glycopeptides, n (%)	17 (16.7%)	8 (16%)	9 (17.3%)	0.86
Metronidazole, n (%)	8 (7.8%)	5 (10%)	3 (5.8%)	0.48
Fluconazole, n (%)	20 (19.6%)	6 (12%)	14 (26.9%)	0.06
Rectal swab colonization				
MDR-GNB, n (%)	28 (27.4%)	6 (12%)	22 (42.3%)	**<0,001**
ESBL, n (%), all ESBL-KP	23 (22.5%)	1 (2%)	22 (42.3%)	**<0,001**

Among 102 patients, 54 (52.9%) were male, 15 (15.5%) were twins, 76 (74.5%) were inborn. Caesarean section was the most frequent type of delivery (65%), median gestational age was 34 weeks and 61.8% of babies were born preterm. Mean birth weight was 2234 g and median APGAR score was 9. Eleven (10.8%) patients had some kind of malformations. Nine (8.9%) patients were admitted to the NICU more than 24 hours after birth. Almost all newborns (99%) were given formula complemented by breast milk in 73.5% of cases. Use of medical devices ranged between 18.6% for endotracheal tube and 79.4% for peripheral venous catheter. Three (2.9%) patients had surgery during their hospitalization. Antimicrobial therapy was administered in 48% of patients. Amikacin (31.4%) and ampicillin (23.5%) were the most frequently used drugs. MDR-GNB carriage affected 28 patients (27.4%). In particular, all ESBL-producing GNB were ESBL-KP (23 patients, 22.5%). *Enterobacter* spp. and *Escherichia coli* carriage accounted for 3.9% and 1% of

patients respectively. Infection was diagnosed in 28 patients (27.7%) and 1 (1%) patient died. Mean duration of stay was 24 days.

Distribution of some characteristics was significantly different between control and intervention population (Table 2). In particular, proportion of inborn, preterm infants and breast milk feeding was significantly higher in control population (p=0.004, p= 0.02 and p=0.03 respectively), while birth weight was significantly lower (p= 0.002). Mean duration of hospital stay markedly decreased from 28.4 days (SD 17.3) in the control population to 20 days (SD 19.4) in the intervention population (p=0.02). In the intervention population ampicillin-sulbactam was replaced by ampicillin.

Prevalence of MDR-GNB carriage was significantly lower in the intervention population compared with controls (12% vs. 42.3%, p <0.001), namely ESBL-KP positivity on rectal swabs showed a significative reduction (2% vs. 42.3%, p<0.001).

Multivariate analysis showed that intervention population had a significantly reduced risk of MDR-GNB carriage compared to control population (adj-OR=0.21, 95% CI 0.076-0.629; p=0.0047), whereas breast milk feeding was associated with an increased risk of MDR-GNB carriage (adj-OR=11.9, 95% CI=1.49-95.9; p-value=0.019) (Table 3).

Table 3. Association between MDR-GNB carriage and multiple exposure variables in quasi-experimental study population.

Exposure variable	OR (95% CI)	p-value	Risk of MDR-GNB carriage (adj-OR – 95% CI)	Adj-p*
Sex, ref. F				
Admission in post-intervention period, ref. No	0.19 (0.06-0.51)	0.001	0.21 (0.076-0.629)	0.0047
Inborn, ref. No	6.1 (1.5-41.3)	0.02		
Caesarean delivery, ref. No	1.9 (0.72-5.4)	0.19		
Surgical intervention, ref. No	5.6 (0.49-64.5)	0.18		
Gestational age, per week increase	0.87 (0.77-0.98)	0.023		
Birth weight, per 100 gr increase	0.98 (0.97-0.99)	0.027		
Breast milk feeding, ref. No	14.4 (2.5-313.5)	0.001	11.9 (1.49-95.9)	0.019
Nasogastric tube, ref. No	2.82 (1.15-7.1)	0.02		
Endotracheal tube, ref. No	1.7 (0.56-4.9)	0.31		
Ampicillin, ref. No	0.45 (0.12-1.4)	0.18		
Ampicillin-sulbactam, ref. No	3.7 (0.98-14.4)	0.07		
Cephalosporins, ref. No	3.1 (1.1-8.8)	0.02		
Carbapenems, ref. No	1.02 (0.3-3.13)	1		
Glycopeptides, ref. No	1.12 (0.35-3.5)	1		
Fluconazole, ref. No	2.7 (0.94-7.6)	0.051		
Duration of hospital stay before colonization, per day increase	0.96 (0.92-0.99)	0.04		

*Hosmer-Lemeshow Goodness-of-Fit Test p-value=0.94

Discussion

After the identification of a high MDR-GNB carriage in a specific NICU during the first two years of surveillance, an episodic implementation of control strategy had been organized through an extraordinary sampling of patients and an informational meeting involving all healthcare workers of the ward in order to reinforce adherence to hand hygiene and good clinical practices. These measures determined a short-term decreasing of MDR-GNB carriage prevalence from 68.5% in 2015 to 43.3 % in 2016. However, after few months, prevalence of MDR-GNB carriage raised up again until 70% in 2017 (Table 1). High prevalence of MDR-GNB carriage was reported from Ecuador (56%), Philippines (61%) and Hungary (>50%) (24,25). Nevertheless, differences in local epidemiology, logistics and hospital organization must be considered. In our setting, the contextual rapid increasing of ESBL-KP carriage, that accounted for most MDR-GNB identified, suggested the need for a more structured and permanent intervention to achieve a long-lasting containment of prevalence. This intervention strategy included a strengthening of sample collection with extraordinary clinical and environmental samples, frequent stakeholders' meetings and an improvement of prevention measures regarding the correct use of antibiotic therapy, sensitization to hand-washing, implementation of checklists for common procedures and invasive procedure management.

The impact of this multiple and coordinated intervention strategy for the reduction of MDR-GNB carriage has been statistically significant and sustained (especially related to ESBL-KP) even in the second year after intervention, with a further reduction of ESBL-KP prevalence despite a modest increase of MDR-GNB carriage (Figure 3).

The two months strengthened surveillance of patients together with microbiological analysis of surfaces and healthcare workers' hands was useful for strictly monitoring carriage trend, tracing transmission roots and enhancing the adherence to preventive measures, first of all hand hygiene in the "five key moments" suggested by WHO (26–28). During this period, MDR-GNB were detected not only from rectal swab, but also from nasal and oral mucosa. In addition, feeding bottles, soothers and milk samples were analysed because, when contaminated after feeding, they represent a further source of MDR-GNB environmental spreading. The role of cross-transmission of MDR-GNB was confirmed by the presence of identical or closely-related ESBL-KP strains in rectal, oral, nasal swabs, milk and environmental samples (Figure 2). The evidence of MDR-GNB spreading by saliva and milk increased the awareness of physicians and nurses and the adherence to hand hygiene before and after milk administration and to the immediate substitution of contaminated devices after feeding (29). In our experience, the finding of environmental contamination by intestinal bacteria highlighted the role of healthcare workers in the prevention of such spreading and prompted the implementation of a detailed procedure for diaper change and a better compliance to correct actions. Environmental contamination by ESBL-KP in NICU has been reported by Szél and colleagues, and has been associated to high prevalence of MDR-GNB carriage and infection (24). Successful elimination of ESBL-producing nosocomial bacteria was obtained thanks to the implementation of a multidisciplinary intervention based on reduction of invasive procedures, changes of the antibiotic policies, microbiological screening at short intervals, progressive feeding, safer

bathing protocol, staff hand hygiene training and continuous monitoring of the number of newly infected and newly colonized patients. This interdisciplinary approach is aligned with ours for most of the measures taken and our results are comparable. In 2018 in Montpellier, a hospital surveillance program revealed an outbreak of ESBL-KP infection/colonization related to incubators as probable pathogen reservoir: ESBL-producing strains from 19 patients displayed the same molecular profile between them and a strain isolated from an incubator after cleaning. In accordance with our data, the introduction of new preventive hygiene measures stopped the outbreak pinpointing the fundamental role of environmental colonization management (30).

The role of MDR-GNB intestinal carriage as a risk factor for infection has been reported in several studies (29,31,32). Colonization by ESBL-producing GNB was a risk factor for developing ESBL infections in paediatric cardiac surgery patients (33). Cross-transmission of colonization, however, is above all a sign of poor adherence to infection prevention measures and, therefore, the prevalence of colonization could be used as an indicator of health workers' compliance with standard and contact precautions in patient care. Furthermore, carriage certainly represents a potential source of dissemination of microorganisms from colonized patients to other NICUs or other paediatric and community health facilities (34). Recent studies have shown the persistence of MDR-GNB colonization even up to 2-5 years after NICU discharging, emphasizing the impact of the problem (35).

Our analysis considers the role of the whole set of control measures performed at the same time but we are not able to identify the single contribution of each group of actions (strengthening of sample collection, improvement of prevention measures or stakeholders weekly meeting) on the reduction of MDR-GNB and ESBL-KP carriage. Different studies analysed the impact of single measures on microbial colonization or infection. The correct management of central venous accesses proved to be effective against related infections (36). Antimicrobial stewardship for the correct use of antibiotics in term of doses, duration of therapy and administration route is a key point for prevention and control of drug-resistance (37–39). Appropriate audits and feedback outcomes to stakeholders have been included among essential infection prevention strategies in the paediatric population (40). Intensification of microbiological surveillance has been used as a strategy to contain ESBL-KP outbreaks (30).

The quasi-experimental analysis was carried out in order to evaluate a possible interference of different clinical characteristics of patients in determining the reduction of MDR-GNB carriage after introduction of coordinated intervention measures. Defined selection criteria have been used for both intervention and control populations in order to minimize selection bias. Possible confounders related to different structural, organizational and seasonal settings were ruled out by choosing as controls a group of patients admitted in the same hospital ward in the same season of the preceding year and cared by the same healthcare personnel. Prevalence of MDR-GNB and ESBL-KP carriage significantly reduced in the intervention population compared to controls (12% vs 42.3% and 2% vs 42.3%, respectively). The two populations have been compared in accordance to clinical features, use of medical devices (invasive and non-invasive) and type of treatment: differences for specific variables were observed and statistically investigated (Table 2). Gestational age, birth weight, being inborn, feeding, antibiotics and length of stay

in NICU resulted associated to MDR-GNB carriage at univariate analysis and were included in the multivariate model in order to analyse their possible contribution to the global risk of colonization. The risk of colonization by MDR-GNB in patients admitted during the intervention period was nearly fifth fold significantly lower than that observed in patients admitted during the control period and multivariate analysis confirmed the main significative role of introduction of coordinated intervention measures in reducing bacterial circulation, regardless of patient characteristics and procedures (Table 3).

Being inborn seemed to increase the risk of colonization (OR=1.90) even if this association was not significant (0.55).

Cassettari *et al.* observed a possible protective role of breastfeeding against ESBL-KP colonization in newborns (41), but our data did not support this evidence (adj-OR=11.9, p=0.019), so the role of this factor needs to be further elucidated. The intrinsic protective role of human milk against infection is reported in several studies (42–44) but some authors reported an increased risk of colonization in breast milk fed newborns in intensive settings (45,46), accordingly to our data. Possible biases in our population related to the large amount of newborns fed with both maternal milk and formula and to the manipulation and storage of expressed breast milk before administration should be further evaluated.

Previous studies reported the role of low gestational age and birth weight, mechanical ventilation, parenteral nutrition, invasive devices and use of antibiotics as risk factors associated to MDR-GNB carriage (25,34,44,46). In our experience these associations were confirmed but not significant, probably because of the small number of patients examined: the risk of colonization increased with lower gestational age and with the use of nasogastric tube (OR=0.73, p=0.09 and OR=2.68, p=0.22 respectively). Moreover, lower birth weight was not associated to the outcome probably because the mean weight in our population was high. First-line empiric treatment was significantly different between the two groups. In particular, ampicillin-sulbactam was replaced by ampicillin in the intervention period, as suggested by international guidelines (47). Univariate analysis showed a significant association between the use of cephalosporins and the risk of MDR-GNB carriage (OR=1.54, p=0.02), however, this association was not confirmed by multivariate analysis (p=0.68).

Our study could have some limitations. The complexity of the preventive intervention did not allow evaluating the specific contribution of each measure implemented towards the reduction of the prevalence of MDR-GNB carriage. Some patients were excluded from the analysis because of the lack of clinical records; we cannot know if some other risk factors would result significantly associated with MDR-GNB carriage including such patients. Moreover, we did not perform any screening on the mothers, so we could not rule out this possible source of colonization, as previously demonstrated (48). Finally, in our five-year active surveillance program (2014-2019), collection of rectal swabs was scheduled only every four weeks due to organizational needs, including all the newborns in the NICU at the time of sample collection. Because of this schedule, newborns hospitalized in the period between monthly samplings were not tested.

On the other hand, network-based approach is essential for the management of the circulation of multi-resistant pathogens, as patients move between one hospital and another and their microorganisms move together with them. This is particularly important for neonatal patients in an interconnected area, where they are frequently transferred from one NICU to another to undergo specialized procedures (16,37). This approach also allows for harmonization of procedures with the aim of optimizing assistance to the newborns (37). With these goals, several neonatal networks have emerged in the world for the surveillance of care-related infections. The presence of an established surveillance program and working group allows the identification of epidemiological changes in colonization trend and the implementation of related control measures.

Conclusions

This study shows the impact of a coordinated strategy of implementation of control measures on MDR-GNB carriage in NICU patients and reveals its efficacy in obtaining a long-lasting reduction of prevalence of MDR-GNB in an endemic setting.

The active surveillance program in the NICUs of Palermo metropolitan area was useful to discover the high-prevalence circulation of ESBL-KP in one NICU and to evaluate the efficacy of adopted measures. Preventive interventions implemented proved to be effective, thanks to a cooperative and participatory approach between different professional profiles, facing the problem of circulation and spread of antibiotic-resistant bacteria.

Periodical meetings were essential for sharing surveillance data, increasing the awareness on the relevance of the problem and discussing critical points and possible solutions. Sharing of protocols, information and experiences in the NICUs network of a metropolitan area could be a further important goal of improvement.

Abbreviations

ESBL-KP: Extended-spectrum β-lactamases-producing *Klebsiella pneumoniae*

MDR-GNB: multi-drug resistant Gram-negative bacteria

MDRO: multi-drug resistant organism

NICU: Neonatal Intensive Care Unit

PGFE: pulsed-field gel electrophoresis

Declarations

- **Ethics approval and consent to participate**

Informed consent was obtained from patients' parents.

- **Consent for publication**

Not applicable

- **Availability of data and materials**

The datasets used and analysed during the current study are available from the corresponding author on reasonable request.

- **Competing interests**

The authors declare that they have no competing interests

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- **Authors' contributions**

LS, GG, GC, FV, CMM and MG were responsible for the conception and design of the study; LS, GG,VI, GR and MV were involved in the acquisition and analysis of data; AA and CB were in charge of molecular typing of ESBL-KP isolates; LS, GG, FM and MG interpreted the data and drafted the article; EA revised statistical analysis and participated in interpretation of the data and global revision of the article; all authors revised it critically and approved the version to be submitted.

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Figures

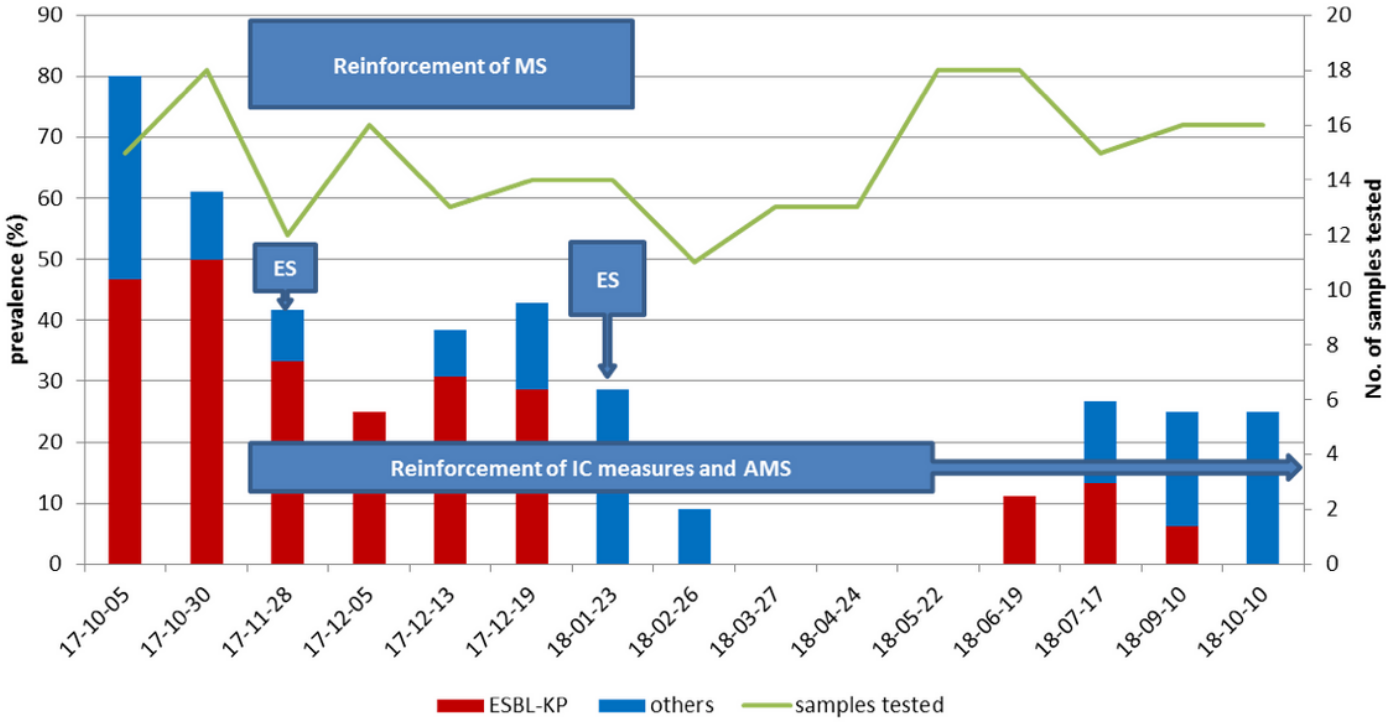

Figure 1

Temporal trend of the prevalence of MDR-GNB rectal carriage and combined measures of infection control adopted. Column color shows the proportion of ESBL-KP positive samples (red) vs other MDR-GNB (blue). Green line represents the number of samples tested. AMS = antimicrobial stewardship; ES =environmental sampling; IC = infection control; MS = microbiological surveillance

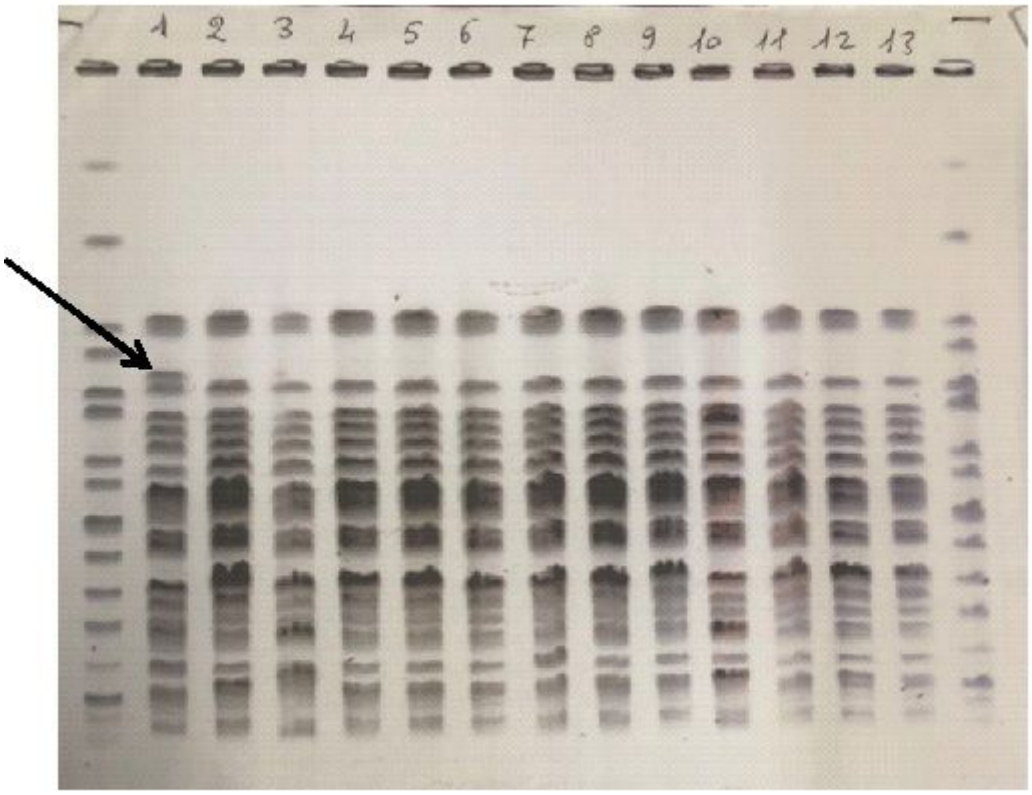

Figure 2

PFGE profile of 13 ESBL-KP strains isolated from rectal swab (1-4-6-10), oral swab (3-7), nasal swab (13), soothers (2-5-8-9), milk (11), belonging to 5 patients and one environmental sample obtained from the changing table of the intermediate care room (12). Samples 2 to 13 show identical pulsotype. Sample 1 differs by the presence of one band of about 350 Kb (arrow).

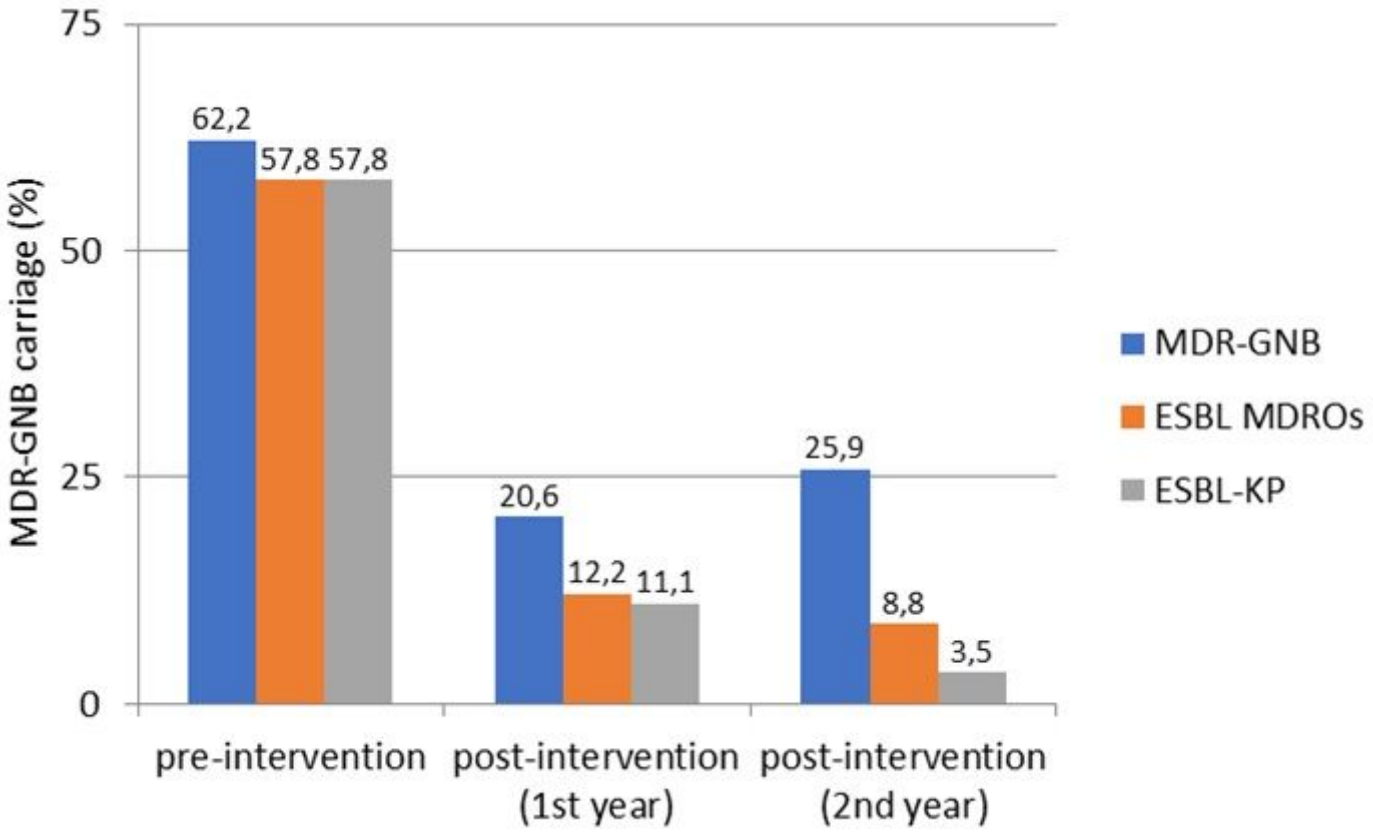

Figure 3

MDR-GNB carriages in 539 rectal swabs collected in the pre- and post-intervention periods showing a significative and persistent decrease in prevalence of all MDR-GNB positives, and in particular of ESBL-KP positive ($p<0.001$ between pre-intervention and 1st year post-intervention; $p=0.006$ between 1st and 2nd year post-intervention).

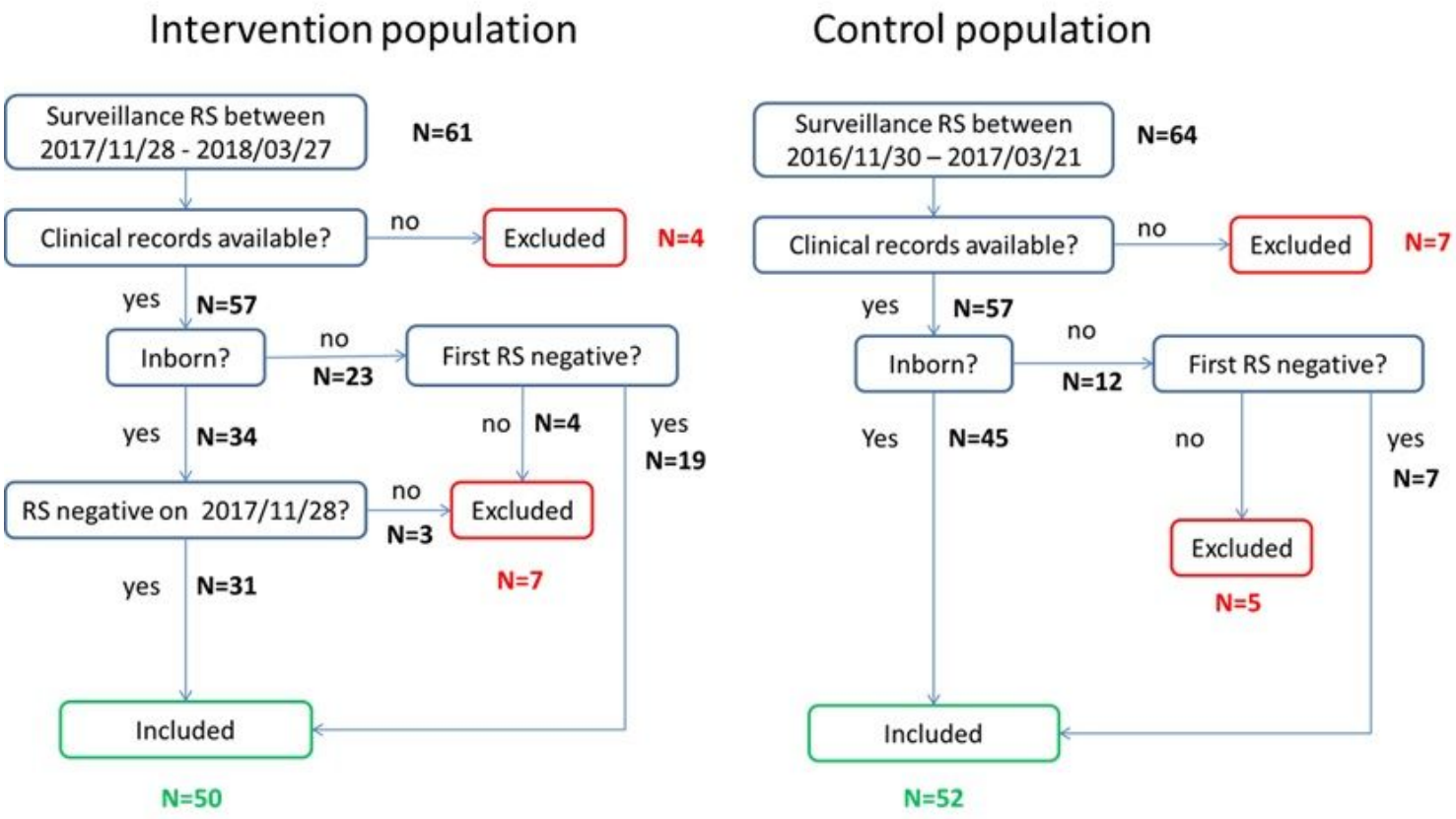

Figure 4

Algorithm for selection of intervention population and control population. RS = rectal swab.