# 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

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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.

Prevalence of MDR-GNB carriage observed in the 12 months before and in the 24 months after intervention were compared by chi-square test. Risk factors for MDR-GNB carriage in a subgroup of patients were identified by a multivariate logistic regression model.

**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. ESBL-KP was detected no more until June 2018. No MDR-GNB isolate was detected for three months.

Prevalence of MDR-GNB and ESBL-KP carriage after intervention significantly decreased compared to the previous year (20.6% vs 62.2 %; $p<0.001$ and 11.1% vs 57.8%; $p<0.001$). Multivariate analysis of principal exposure variables showed that admission in post-intervention period significantly reduced the risk of MDR-GNB carriage (OR=0.15, $p=0.01$).

**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 next 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 diffusion, 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 (2–6). Adherence to hand-washing protocols in hospitals, particularly in intensive care units, is one of the most important measures to prevent and control the spread of healthcare-associated infections (7). Sensitization of healthcare workers and parents to hand-washing and other hygienic preventive procedures in NICUs is essential to manage microbiological diffusion.

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 (8). Currently, active surveillance systems involve monitoring/targeting infectious disease or carriage by multidrug resistant organisms (MDROs). These systems require trained professionals, including clinical microbiologists and epidemiologists, and consent less time consumption for results.

Nowadays, active surveillance may be recognized as an effective tool for early detection of unusual patterns of microbial pathogens in a specific health-care setting and the subsequent risk of healthcare-associated infections.

Active bacteriological surveillance is therefore very important to define the local diffusion of MDROs and the relative antimicrobial resistance map in each NICU (9–12).

# Background

Based on internationally recognized prevention objectives (13), the University Hospital in Palermo, Italy, started an active surveillance program of MDRO carriage in NICU in June 2009 (14–16). In 2014 this program was extended to the four other 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 (17,18).

The main focus of the program is the analysis of epidemiological characteristics 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 coordination 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 health care

providers, together with the results of the in vitro antimicrobial sensitivity testing. The final purpose is prevention and control of infectious diseases through the early identification of new clusters of carriage 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 (53,89) | 126 (68,48) * | 71 (43,29) * | 112 (70,00) * |
| ESBL, n (%) | 92 (51,11) | 102 (55,43) | 61 (37,20) | 104 (65,00) |
| ESBL-KP, n (%) | 91(50,56) | 43 (23,37) | 40 (24,39) | 104 (65,00) |

* Significant difference between MDR-GNB carriage in 2015 vs 2016 (chi-square test, $p < 0.001$), and in 2016 vs 2017 (chi-square test, $p < 0.001$).

Based on this evidence, additional episodic control measures were performed in the NICU: an extraordinary collection of rectal and nasal swabs in November 2015, reinforcement of good clinical practices for infection control and prevention, and an informational meeting with all health-care workers in January 2016 showing available data and trends in order to identify critical points that could facilitate the diffusion of resistant microorganisms in the NICU.

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 includes an open space with 8 intensive care coats and another one with 8 sub-intensive care coats. Intensive and sub-intensive rooms are adjacent, with two hand-washing sinks located at each entrance, and measure about 60 square meters and 40 square meters respectively. Every year about 370 newborns (both inborn and outborn) are admitted.

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 the 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 hospitalized newborns, regardless of any clinical or laboratory sign 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 put in place in order 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 as follows:

- extraordinary weekly rectal samples for the first month (from 11/28/2017 to 12/19/2017);
- extraordinary sampling of other newborn colonization sites (nasal and oral mucosa) and cultures of devices and material strictly in contact with each newborn (feeding bottles and soothers, remnant milk samples after newborn feeding) every week in the first month then after four weeks;
- environmental samples (from intensive care, sub-intensive care and milk preparation room surfaces, healthcare workers hands and stethoscopes) at the beginning of the intervention and after 2 months (on 11/27/2017 and on 01/23/2018). From February 2018 monthly sample collection continued as previously including only rectal swabs.

b) Stakeholders weekly meetings

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

c) Improvement of prevention measures

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

- introduction of 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 (19);
- hand-washing sensitization posters for caregivers and parents showed in all NICU and Neonatology rooms;
- substitution of contaminated devices after feeding;
- introduction of a checklist 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 has been calculated and compared in two groups: samples collected from newborns admitted in the pre-intervention period (from November 2016 to October 2017) and samples collected from newborns admitted in the post-intervention period (from November 2017 to October 2019).

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 population and control population.

Inclusion criteria was to be admitted to the NICU and enrolled 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 (because we do not perform rectal swab on NICU admission, we cannot discriminate if colonization was already present);
- 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 exposure to risk factors.

The observation period was defined as the interval of time between NICU admission and the date of the first positive rectal swab, for colonized patients, and the interval of time between NICU admission and the

last rectal swab obtained, for non-colonized patients.

Clinical characteristics analysed were 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 and use of antimicrobials.

# Collection of Samples and Microbiological Analysis

Collected samples were enriched in liquid cultures (Brain Heart Infusion, BHI) for 24 hours at 37 °C, then plated in McConkey Agar with three antimicrobial discs (amoxicillin-clavulanate 30 µg, meropenem 10 µg, ceftazidime 30 µg) to detect multi-drug resistant bacteria. In case of positivity to the first screening, colonies were isolated in McConkey Agar, identified with standard biochemical methods and submitted for antimicrobial susceptibility testing (Kirby-Bauer Method) and ESBL detection according with the European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines (20–22).

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 (19,23,24).

# Statistical Analysis

Prevalence of MDR-GNB carriage was analysed by chi-square test. Chi-square for trend was calculated with the Mantel–Haenzsel test. Patient characteristics, procedures and clinical outcomes have been compared using chi-square test or Fisher test for categorical variables and Student's t-test for continuous variables. The association between risk factors and MDRGN carriage was determined using a bivariate logistic regression. All associated variables with p-value < 0.25 have been included in a multivariate logistic regression model. All significance tests were two-tailed, and $p < 0.05$ was considered significant.

Exposure to risk factors for MDRGN carriage was assessed during the time between admission in NICU and first positive rectal swab (for colonized newborns) or last negative rectal swab (for non-colonized newborns).

Statistical analysis was carried out by Microsoft Excel 2010 and Epi Info software (version 7.2; CDC, Atlanta, GA, USA).

## Results

# Strengthened microbiological surveillance

From November 28th 2017 to January 23rd 2018 in the strengthened microbiological surveillance (performed as described above), 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 (34.8%) rectal swabs, 17 (24.6%) nasal swabs, 8 (14.5%) swabs from oral mucosa; moreover, 9 (32.1%) milk samples and 12 (30.8%) soother swabs were positive for MDR-GNB. ESBL-KP was detected from 16 (23.2%) rectal swabs, 10 (14.5%) nasal swabs, 3 (5.4%) swabs from oral mucosa; 5 (17.9%) milk samples and 7 (17.9%) soother swabs were positive for ESBL-KP. Since January 23rd 2018 ESBL-KP was detected no more until June 2018. No MDR-GNB isolate was detected for three months. Temporal trends of MDR-GNB and ESBL-KP rectal carriage in relation to the intervention measures adopted are shown in Fig. 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 (Fig. 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 of the post-intervention period (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 (62.2%) rectal swabs, and in particular 104 (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 (20.6%) rectal swabs, and in particular 21 (11.1%) were ESBL-KP while 2 (1.1%) were other ESBL-producing GNB. Carriage by ESBL-KP accounted for 94.5% of all MDR-GNB isolated in the pre-intervention period and for 53.8% 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 25.9%; ESBL-producing GNB 8.8%; ESBL-KP 3.5%). No difference was observed in the prevalence of MDR-GNB and of 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-GN carriage in the pre- and post-intervention periods are shown in Fig. 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 (Fig. 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, mean (SD), wk | 34.8 (3.9) | 35.7 (3.8) | 33.9 (3.7) | **0.01** |
| 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, mean (SD) | 9 (1.1) | 9 (0.9) | 8.9 (1.2) | 0.46 |
| 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 |

| Variable | All patients (n = 102) | Intervention population (n = 50) | Control population (n = 52) | p |
|---|---|---|---|---|
| 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%), mean gestational age was 34.8 weeks and 61.8% of babies were born preterm. Mean birth weight was 2234 g and mean 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 indicated in 48% of patients. Amikacin (31.4%) and ampicillin (23.5%) were the drugs most frequently used. Colonization by MDR-GNB affected 28 (27.4%) patients. 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 breastfeeding 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 (OR = 0.15, $p = 0.01$). Other variables that resulted associated at bivariate analysis (being inborn, lower gestational age and birth weight, breastfeeding, use of nasogastric tube, administration of ampicillin-sulbactam and cephalosporines) were not significantly associated to MDR-GNB carriage by multivariate analysis (Table 3).

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

All variables with unadjusted p-values < 0.25 resulting from bivariate analysis have been included in multivariate analysis. Adjusted OR and p-value from multivariate analysis are shown and significant values are highlighted.

| Exposure variable | p (unadjusted) | Risk of MDR-GNB carriage (OR adjusted) | p (adjusted) |
|---|---|---|---|
| Admission in post-intervention period | **0.001** | 0.15 | 0.01 |
| Inborn | **0.02** | 1.90 | 0.55 |
| Caesarean delivery | 0.19 | 0.60 | 0.52 |
| Surgical intervention | 0.17 | 76.92 | 0.06 |
| Gestational age | **0.02** | 0.73 | 0.09 |
| Birth weight | **0.03** | 1.00 | 0.27 |
| Breastfeeding | **0.01** | 2.32 | 0.34 |
| Nasogastric tube | **0.01** | 2.68 | 0.22 |
| Endotracheal tube | 0.23 | 0.79 | 0.83 |
| Ampicillin | 0.18 | 0.32 | 0.23 |
| Ampicillin-sulbactam | **0.02** | 1.01 | 0.90 |
| Cephalosporins | **0.02** | 1.54 | 0.68 |
| Carbapenems | 0.19 | 0.91 | 0.74 |
| Glycopeptides | 0.20 | 0.61 | 0.39 |
| Fluconazole | 0.12 | 0.47 | 0.54 |
| Duration of hospital stay before colonization | **0.002** | 1.92 | 0.43 |

# 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 collection of rectal swabs, reinforcement of good clinical practice and an informational meeting involving all healthcare workers. These measures determined a short-term decrease of MDR-GNB carriage prevalence from 68.5% in 2015 to 43.3% in 2016. But after few months, prevalence of MDR-GNB carriage raised again up to 70% in 2017 (Table 1). High prevalence of MDR-GNB carriage was reported from Ecuador (56%), Philippines (61%) and Hungary (> 50%) (25,26). Nevertheless, differences in local

epidemiology, logistics and hospital organization must be considered. In our setting, the contextual rapid increase of carriage of ESBL-KP, that accounted for most MDR-GNB identified, suggested the need for a more structured and permanent intervention to achieve a long-lasting containment of carriage. This intervention strategy included a strengthening of sample collection with extraordinary clinical samples and environmental samples, frequent stakeholders' meetings and 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 (Fig. 3).

The two months strengthened surveillance of patients together with microbiological analysis of surfaces and healthcare workers' hands was useful for strictly monitoring colonization trend, tracing transmission roots and enhancing the adherence to preventive measures, first of all hand hygiene in the five key moments suggested by WHO (27–29). 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 (Fig. 2). The evidence of MDR-GNB spreading by saliva and milk increased the awareness of doctors and nurses and the adherence to hand hygiene before and after milk administration and to the immediate substitution of contaminated devices after feeding (30). 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. 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 (25). This interdisciplinary approach is aligned with ours for the 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 (31).

The role of MDR-GNB intestinal colonization as a risk factor for infection has been reported in several studies (30,32,33). Colonization by ESBL-producing GNB was a risk factor for developing ESBL infections in paediatric cardiac surgery patients (34). 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, colonization certainly represents a potential source of dissemination of microorganisms from colonized patients to other NICUs or other paediatric and community health facilities (35). 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 (36).

The present study aims to analyse the impact of the coordinated strategy of measures on the intestinal carriage of MDR-GNB, while a direct effect on MDR-GNB-related infections has not been evaluated. 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 measure 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 (37). 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(38–40). Use of appropriate audits and measures to promote adherence to infection prevention and control procedures, decision-making and feedback outcomes to stakeholders has been included among essential infection prevention strategies in the paediatric population (41). Intensification of microbiological surveillance has been used as a strategy to contain ESBL-KP outbreaks (31).

The quasi-experimental analysis was carried out in order to evaluate the eventual 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 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 (OR adjusted = 0.15, p = 0.01), 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 (42), but our data did not support this evidence (OR = 2.34, p = 0.34), so the role of this factor needs to be further elucidated. 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 (26,35,43,44). 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 (45). 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 has 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 (46). 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 present 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 diffusion 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 (17,38). This approach also allows for harmonization of procedures with the aim of optimizing assistance to the newborns (38). 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

## • Funding

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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; all authors revised it critically and approved the version to be submitted.

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# Figures

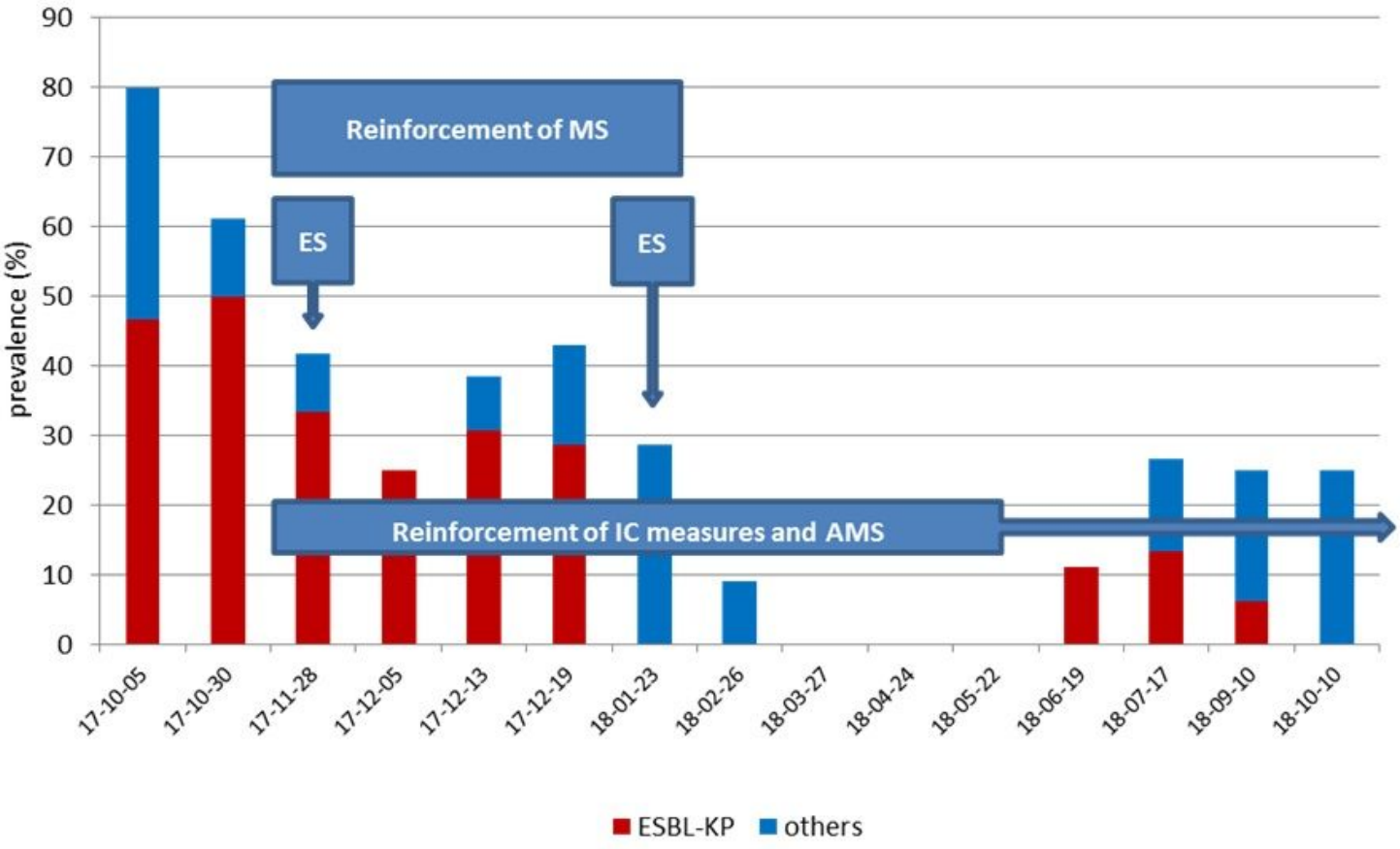
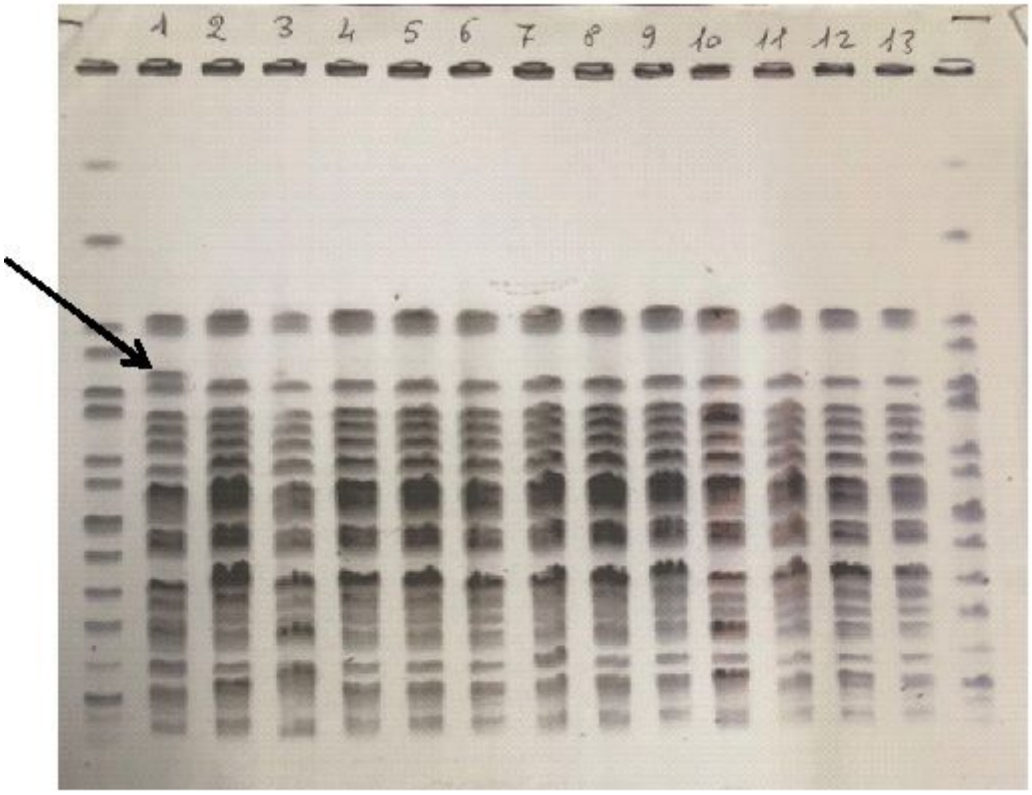


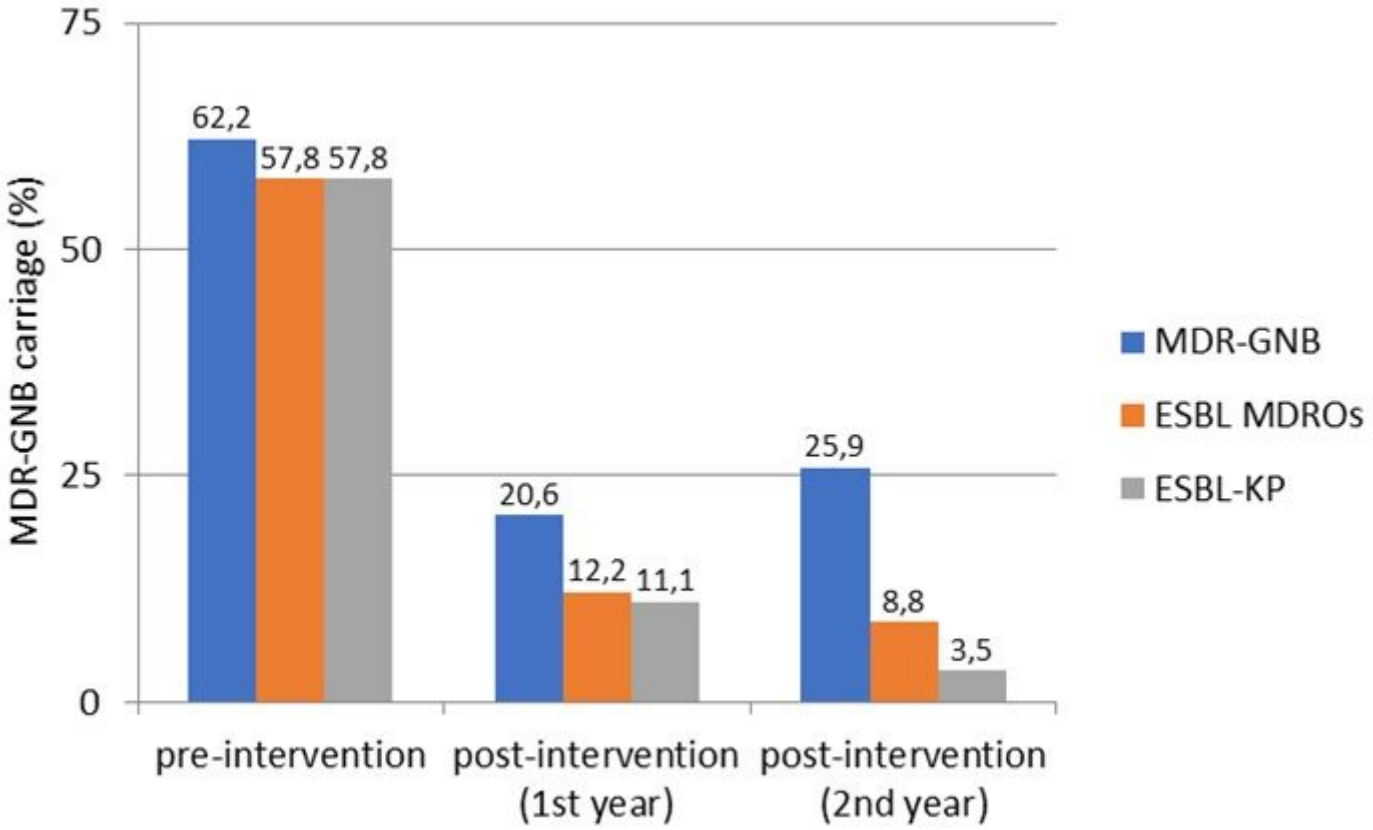

**Figure 1**

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). AMS = antimicrobial stewardship; ES =environmental sampling; IC = infection control; MS = microbiological surveillance



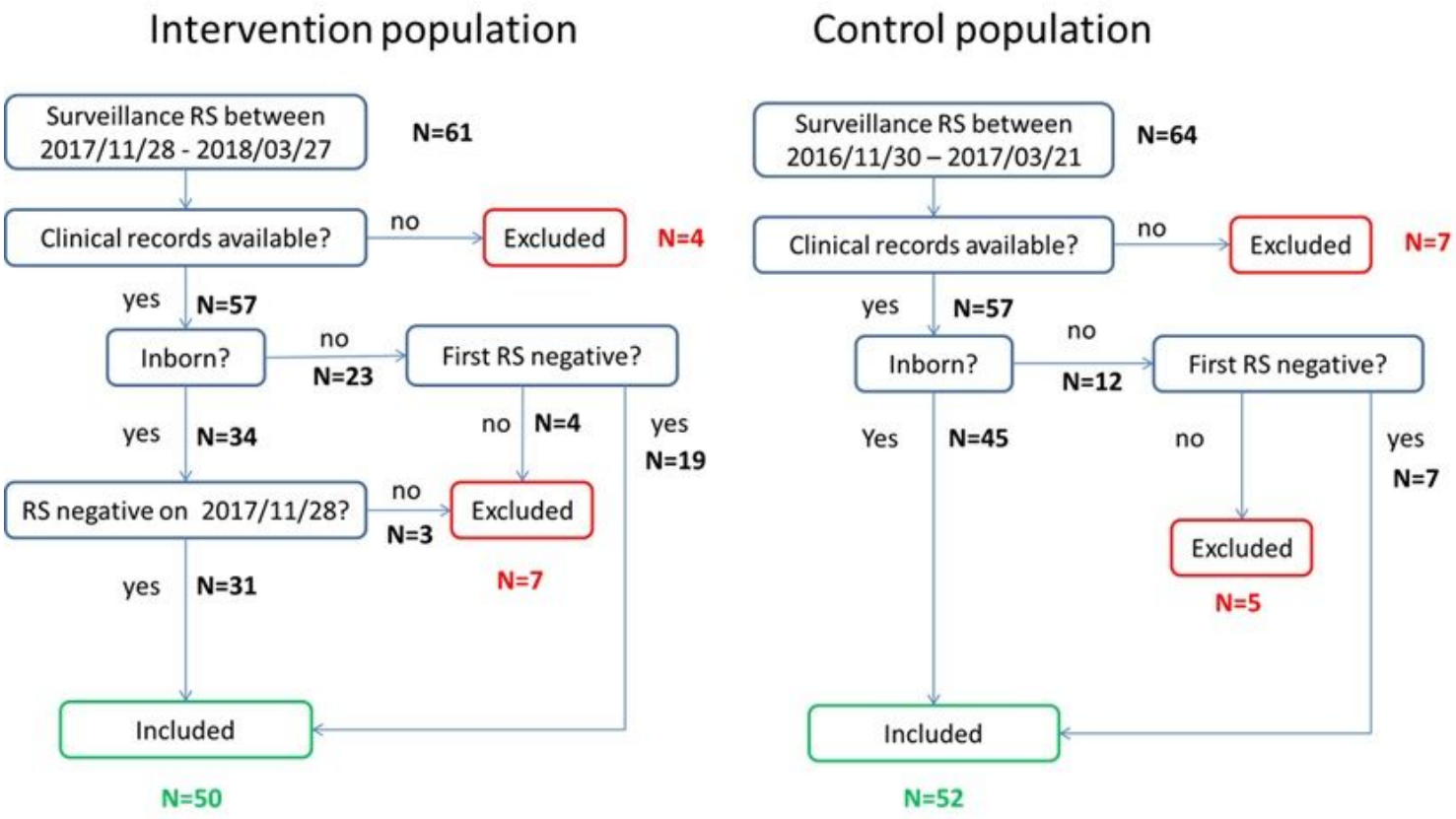

## Figure 2

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.