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Title: BACTERIAL CONTAMINATION OF SALINE NASAL IRRIGATIONS IN CHILDREN: AN ORIGINAL RESEARCH

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Abstract: Microbiological analysis on nasal saline irrigations (NSIs) used in hospitalized children was performed.
24.9% out of 253 collected samples were positive; the number of positive samples significantly (p-value < 0.001) increased over time.
Staphylococcus aureus was the most frequently detected bacterium (28.6%); none of the 118 patients receiving NSIs developed nasosinusal infection.
Colonization by cutaneous and environmental germs is frequent and precocious; the respect of hygienic measure should be advocated in order to reduce contamination.

Cover Letter

Milan, May 7nd 2018

Dear Editor,

this paper reports and original research assessing the risk of bacterial contamination of saline solution in hospitalized children daily undergoing nasal saline irrigations by the use of a syringe bulb and saline solution bottle.

We hope it will be considered of interest.

Yours faithfully,

Sara Torretta

ANSWER RO REVIEWER

Ms. Ref. No.: AJIC-D-18-00336
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Highlights (mandatory)

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Highlights have been uploaded.

Reviewers' comments:
This is an interesting study but am not certain it supports the conclusion. I think that it should focus on NSIs being effective treatment and potentially cutting down on other therapies such as antihistamines in young children and improper use of antibiotics both of which are important. It also supports some infection control aspects such as good hand hygiene and single patient use. It also supports the conclusion that there is excessive bacterial flora in hospital settings and that despite instruction, translocation is possible. My concern about the conclusion the way it exists now is that despite not seeing colonization progress to infection we should use new supplies and equipment every day. This could translate into many other practices that have no benefit to preventing infection when it is very unlikely to occur.

This should be revised as a Brief Report of a maximum of 1000 words, a 2-3 sentence unstructured abstract.

Tables and figures should have a full legend since they should be interpretable without referring to the article.

We thank the Reviewer for these suggestions and the chance to improve our manuscript. The paper has been shortened into a brief report, conclusions have been changed, abstract and legends have been modified accordingly.

TITLE PAGE

Title

BACTERIAL CONTAMINATION OF SALINE NASAL IRRIGATIONS IN CHILDREN: AN ORIGINAL RESEARCH

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***Abstract**

1 **TITLE PAGE**

2 **Title**

3 **BACTERIAL CONTAMINATION OF SALINE NASAL IRRIGATIONS IN CHILDREN: AN**

4 **ORIGINAL RESEARCH**

Abstract

Microbiological analysis on nasal saline irrigations (NSIs) used in hospitalized children was performed.

24.9% out of 253 collected samples were positive; the number of positive samples significantly (p-value < 0.001) increased over time. *Staphylococcus aureus* was the most frequently detected bacterium (28.6%); none of the 118 patients receiving NSIs developed nasosinusal infection.

Colonization by cutaneous and environmental germs is frequent and precocious; the respect of hygienic measure should be advocated in order to reduce contamination.

Key words: Children; Nasal saline irrigation; Bacterial contamination.

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HIGHLIGHTS

- Nasal saline irrigations are widely used in clinical practice
- Bacterial contamination is frequent and precocious, but not associated with infection
- Non-respiratory bacteria are generally involved
- Bacterial translocation from healthcare professionals may be a source
- Respect of hygienic measures could decrease the risk of bacterial contamination

BACTERIAL CONTAMINATION OF SALINE NASAL IRRIGATIONS IN CHILDREN: AN ORIGINAL RESEARCH

BACKGROUND

Nasal saline irrigations (NSIs) are used in patients with upper respiratory tract infections and allergic rhinitis.[1-4] A national survey documented that the majority of Italian paediatricians consider them effective and well tolerated.[3] Different devices are actually available[3] and NSIs performed by repeatedly resampling from a saline solution bottle by means of a bulb syringe is probably the easiest and most inexpensive approach.

Despite they are generally considered safe, bacterial contamination of the device may occur, as saline bottle bacterial contamination has been described. [5-20]

The aim of this study was to evaluate bacterial colonization of saline solution in children daily undergoing NSIs by the use of a syringe bulb and saline solution bottle.

METHODS

Materials

Samples of saline solution taken from the bottles used for NSIs in children admitted to our hospital for lower respiratory tract infections and candidates to daily NSIs.

Exclusion criteria were: acute nasosinusal infection; severe systemic diseases (cystic fibrosis, Kartagener syndrome); neuromuscular, immunological, syndromic or genetic abnormalities, parents refusal.

Interventions

Before the first use, the syringe needle was used to pierce the rubber bottle cap by the paediatric nurse, then the needle was removed and the syringe bulb was placed and left inside the pierced rubber bottle cap. Care givers were instructed about the modality to perform NSIs as it follows: after washing his\her hands before each use, the syringe should be filled up with saline solution and used for irrigation. Then it should be placed inside the pierced rubber bottle cap. NSIs were performed by the children's parents, or by the healthcare professionals.

Paediatric nurses were instructed to periodically pick up 5 ml samples of saline solution from the bottle by means of the syringe used for NSI (after hands washing and putting disposable gloves on) just after the bottle opening (day 0), and then the day after (day 1, within 24 hours), and two (day 2, 48 hours after the bottle opening), three (day 3, 72 hours), four (day 4, 96-120 hours), five (day 5, 120-144 hours), and six (day 6, 140-168 hours) days later. The samples were moved into sterile phials and delivered to the Microbiological Laboratory to be analysed within two hours.

Microbiological evaluation

Each specimen was immediately vortexed and cultured on Mueller Hinton agar, MacConkey agar, Mannitol Salt Agar (Difco) under aerobic condition and on Columbia blood agar (Difco) in a 5%CO_2 atmosphere al 37°C. The plates were firstly examined after

18-24 hours of incubation and furtherly checked for the presence of bacterial colonies after 48 hours in order to detect the slow-growing microorganisms. The Microbial identification was perfomed at genus and species level according to their typical colony morphology, Gram stain, standard rapid tests and finally confirmed by biochemical tests (API - BioMérieux).

Statistical analysis

Descriptive statistics was used to report the main results (given as absolute numbers and percentages, or as arithmetical mean values ± standard deviation).

The dichotomous outcomes were analysed using contingency table analysis by means of Fisher's exact test; time-series regression analysis was used to evaluate the statistical trend of the percentage of positive samples over time. The characteristic of NSIs performer were tested as possible confounders.

RESULTS

The final analysis was based on 253 samples collected from bottles used for administering NSIs to 118 children (66, 55.9% males; mean age 17.0 ± 15.9 months).

The mean samples for each patient was 2.1 ± 2.8 (Figure 1), and NSIs were performed by respectively the healthcare professionals and the children's parents in 43.5% and 56.5% of cases.

24.9% of samples were positive at microbiological assessment. Bacterial contamination in at least one sample was detected in 22.0% of patients, and no significantly difference in the number of patients with at least one positive sample was found when NSIs performers were separately considered (healthcare professionals= 21.5% vs. children's parents= 21.5%). Bacterial contamination occurred significantly (p-value= 0.003) earlier when NSIs were administered by the healthcare professionals compared to the parents, as 59.2% of positive samples among those collected by healthcare professionals were taken within 24 hours after the bottle opening, while only 17.4% of positive samples among those collected by the children's parents were taken within 24 hours after the bottle opening.

The number of positive samples at microbiological assessment significantly (p-value < 0.001) increased over time, with a mean 14.3% (standard error= 0.1; p-value < 0.001) daily increase (Figure 2).

Staphylococcus aureus was the most frequently detected one (28.6%) (Table 1). Polymicrobial contamination was found in 2.4% (6/253) samples.

None of the patients developed signs of acute nasosinusal infection.

DISCUSSION

Bacterial contamination of saline solution bottles used for NSIs in children is not a rare event, as it occurred in about 25% of samples. Although no comparable studies have been previously performed in the paediatric age, our findings lines with literature, as a recent review of contamination in sinus irrigation device used after functional nasosinusal surgery documented that the overall prevalence of positive samples ranged between 25-100%,[7] with S. aureus being detected as the main pathogen.

We documented a progressive significant increase in the number of contaminated samples over time, as a not negligible percentage of samples was found to be positive within 24 hours from bottle opening, suggesting that bacterial contamination occurs very precociously, and confirming a previous report.[10]

No bacteria involved in upper airway infections have been isolated, as only germs generally located at the cutaneous or enviromental surfaces have been discovered, with S. aureus and Neisseria spp. being the most frequently detected ones.

The absence of any sign suggestive for the development of acute nasal or nasosinusal infections in this cohort of patients seems to suggest that lack of a direct link between saline solution contamination and the occurrence of any infectious process.

Microbiological results make us reflect about the importance of strictly respecting hygienic measures including accurate hand-washing before NSIs administration in order to reduce the rate of bacterial contamination resulting from germs spreading from the caregivers' hands and the surrounding environment. This derives from the observation that positive cultures were found significantly earlier when NSIs were administered by the healthcare professionals compared to the parents.

CONCLUSIONS

This study confirms the safety of NSIs in children, and advocate their use as preventive and therapeutic means in patients with upper airway disease, as they could possibly cut down on other therapies such as antihistamines and improper antibiotics.

Moreover, our results document the presence of excessive bacterial flora in hospital settings, and the possibility of bacterial translocation from caregivers and healthcare professionals, therefore advocating the importance of infection control aspects including good hand hygiene and single patient use in order to reduce the rate of bacterial contamination.

~~This is the first study documenting that colonization of nasal saline solution by cutaneous and environmental but not respiratory germs is possible, frequent, and precocious in children undergoing NSIs by means of repeated aspiration of sterile saline solution with a burb syringe from an irrigation bottle, but there is no evidence that this condition would facilitate the development of any nasosinusal infection. The respect of hygienic measure, correct patients education, and daily bottle changing should be advocated in order to reduce the rate of bacterial contamination, and improved the safety of nasal irrigations.~~

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Conflict of interest statement: Nothing to declare.

TABLE

Table I: Number and rate of samples with isolated bacterial ~~Bacterial~~ strains.

Bacterial strain	**~~No.~~ Number of samples (%)**
Staphylococcus aureus	18/63 (28.6%)
Neisseriae spp.	11/63 (17.5%)
Klebsiella pneumoniae	9/63 (14.3%)
Stenotrophomonas maltophilia	9/63 (14.3%)
Alcaligenes xylosoxidans	5/63 (7.9%)
Staphylococcus xylosus	3/63 (4.8%)
Escherichia coli	1/63 (1.6%)
Acinetobacter lwoffii	1/63 (1.6%)
Aeromonas	1/63 (1.6%)
Forme difteroidi	1/63 (1.6%)
Ocromobactum anthropi	1/63 (1.6%)
Pseudomonas aeruginosa	1/63 (1.6%)
Serratia liquefaciens	1/63 (1.6%)
Staphylococcus warneri	1/63 (1.6%)

FIGURES LEGEND

Figure 1: Rate of samples collected over time

Figure 2: Rate of positive samples over time

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Figure(s)

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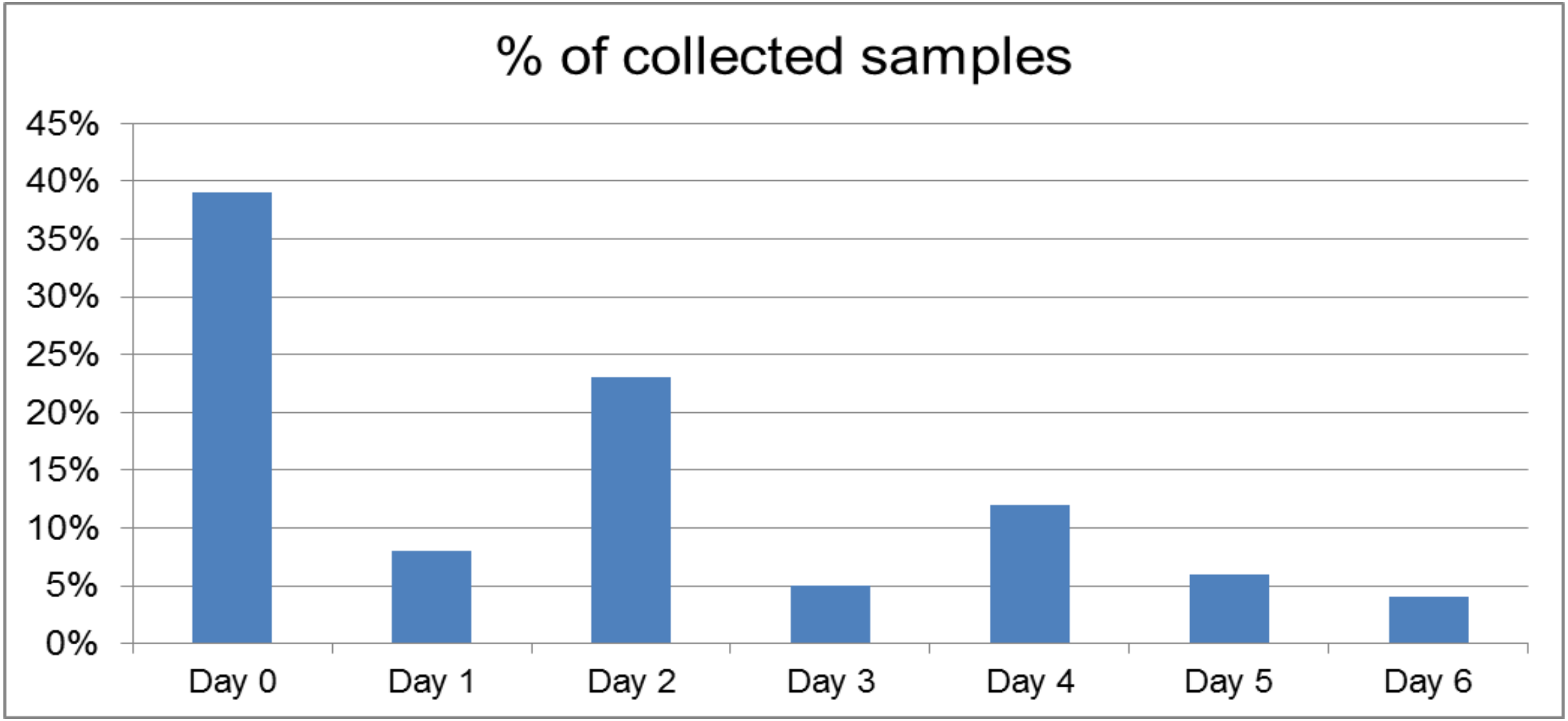

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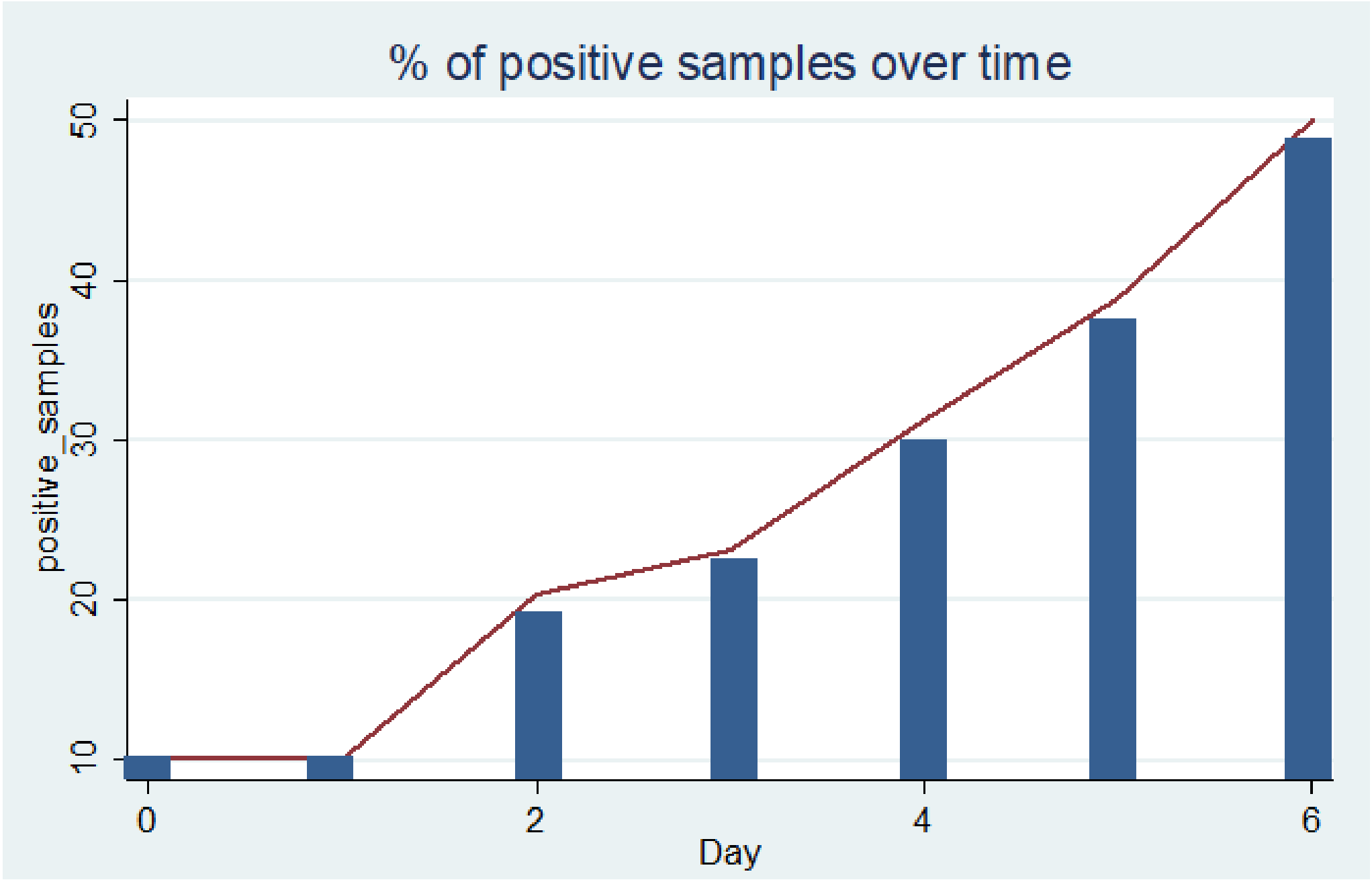

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