

Manuscript Number: AJIC-D-18-00336R1

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

Article Type: Brief Report

Keywords: Children; Nasal saline irrigation; Bacterial contamination.

Corresponding Author: Dr. Sara Torretta,

Corresponding Author's Institution:

First Author: Sara Torretta

Order of Authors: Sara Torretta; Roberto Mattina; Francesco Talloru;
Giuliana Sala; Elena Cornelli; Serena Bezze; Paola Marchisio

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

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

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

American Journal of Infection Control

Highlights (mandatory)

Highlights consist of a short collection of bullet points that convey the core findings of the article and should be submitted in a separate file in the online submission system. Please use 'Highlights' in the file name and include 3 to 5 bullet points (maximum 85 characters, including spaces, per bullet point). See the following website for more information

<http://www.elsevier.com/highlights>

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.

1 **TITLE PAGE**

2 **Title**

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

5 ¹Sara Torretta, MD; ²Roberto Mattina, MD; ³Francesco Talloru, MD; ²Giuliana Sala, MD;
6 ³Serena Cornelli, MD; ³Elena Bezze, MD; ⁴Paola Marchisio, MD.

7

8 ¹Otorhinolaryngological Unit, Department of Clinical Sciences and Community Health;
9 Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Università degli Studi di
10 Milano, Milan, Italy.

11 ²Department of Biomedical, Surgical, and Odontoiatric Sciences; Università degli Studi di
12 Milano, Milan, Italy.

13 ³Neonatal Intensive Care Unit, Department of Clinical Sciences and Community Health;
14 Fondazione IRCCS Cà Granda Ospedale Maggiore Policlinico, Università degli Studi di
15 Milano, Milan, Italy.

16 ⁴Paediatric Highly Intensive Care Unit, Department of Patophysiology and Transplantation,
17 Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Università degli Studi di
18 Milano, Milan, Italy.

19

20 **Corresponding author and address for reprints:**

21 Sara Torretta, MD
22 Department of Clinical Sciences and Community Health,
23 Università degli Studi di Milano,
24 Fondazione IRCCS Cà Granda Ospedale Maggiore Policlinico,
25 Via F. Sforza 35,
26 20122 Milano,
27 Italy
28 Tel.: +39 0250320245; Fax: +39 0250320248;
29 E-mail: sara.torretta@unimi.it

1	TITLE PAGE
2	Title
3	BACTERIAL CONTAMINATION OF SALINE NASAL IRRIGATIONS IN CHILDREN: AN
4	ORIGINAL RESEARCH

5 **Abstract**

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

8 24.9% out of 253 collected samples were positive; the number of positive samples
9 significantly (p-value < 0.001) increased over time. *Staphylococcus aureus* was the most
10 frequently detected bacterium (28.6%); none of the 118 patients receiving NSIs developed
11 nasosinusal infection.

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

14 | **Key words:** Children; Nasal saline irrigation; Bacterial contamination.

15

16

Formatted

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

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

3
4
5 **BACKGROUND**

6 Nasal saline irrigations (NSIs) are used in patients with upper respiratory tract infections
7 and allergic rhinitis.¹⁻⁴ A national survey documented that the majority of Italian
8 paediatricians consider them effective and well tolerated.³ Different devices are actually
9 available³ and NSIs performed by repeatedly resampling from a saline solution bottle by
10 means of a bulb syringe is probably the easiest and most inexpensive approach.
11 Despite they are generally considered safe, bacterial contamination of the device may
12 occur, as saline bottle bacterial contamination has been described.⁵⁻²⁰
13 The aim of this study was to evaluate bacterial colonization of saline solution in children
14 daily undergoing NSIs by the use of a syringe bulb and saline solution bottle.

15 **METHODS**

16 *Materials*

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

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

22 *Interventions*

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

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

37 *Microbiological evaluation*

38 Each specimen was immediately vortexed and cultured on Mueller Hinton agar,
39 MacConkey agar, Mannitol Salt Agar (Difco) under aerobic condition and on Columbia
40 blood agar (Difco) in a 5%CO₂ atmosphere at 37°C. The plates were firstly examined after

41 18-24 hours of incubation and furtherly checked for the presence of bacterial colonies after
42 48 hours in order to detect the slow-growing microorganisms. The Microbial identification
43 was performed at genus and species level according to their typical colony morphology,
44 Gram stain, standard rapid tests and finally confirmed by biochemical tests (API -
45 BioMérieux).

46 *Statistical analysis*

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

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

53

54 **RESULTS**

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

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

60 24.9% of samples were positive at microbiological assessment. Bacterial contamination in
61 at least one sample was detected in 22.0% of patients, and no significantly difference in
62 the number of patients with at least one positive sample was found when NSIs performers
63 were separately considered (healthcare professionals= 21.5% vs. children's parents=
64 21.5%). Bacterial contamination occurred significantly (p -value= 0.003) earlier when NSIs
65 were administered by the healthcare professionals compared to the parents, as 59.2% of
66 positive samples among those collected by healthcare professionals were taken within 24
67 hours after the bottle opening, while only 17.4% of positive samples among those collected
68 by the children's parents were taken within 24 hours after the bottle opening.

69 The number of positive samples at microbiological assessment significantly (p -value <
70 0.001) increased over time, with a mean 14.3% (standard error= 0.1; p -value < 0.001)
71 daily increase (Figure 2).

72 *Staphylococcus aureus* was the most frequently detected one (28.6%) (Table 1).

73 Polymicrobial contamination was found in 2.4% (6/253) samples.

74 None of the patients developed signs of acute nasosinus infection.

75

76 **DISCUSSION**

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

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

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

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

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

99

100

101 **CONCLUSIONS**

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

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

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

117

118

119 **Funding:** This research did not receive any specific grant from funding agencies in the
120 public, commercial, or not-for-profit sectors.

121 **Conflict of interest statement:** Nothing to declare.

TABLE

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

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

FIGURES LEGEND

Figure 1: Rate of samples collected over time

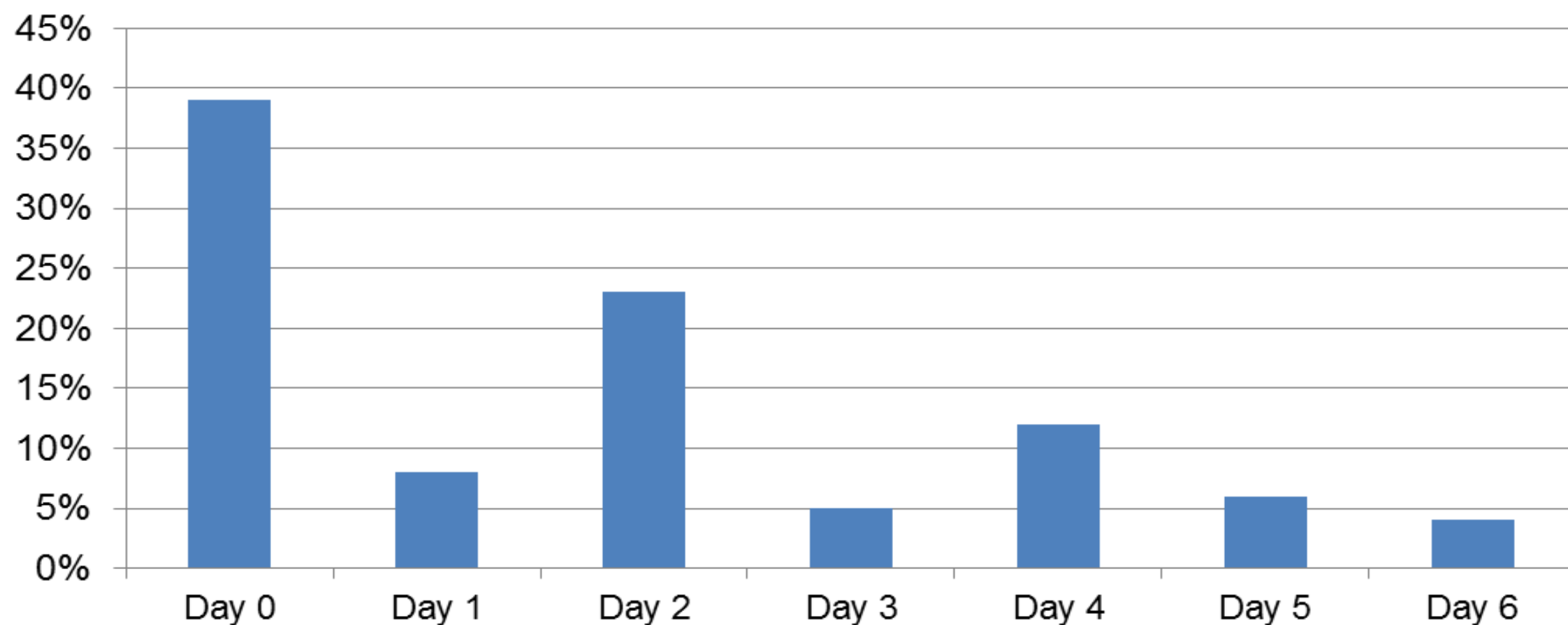
Figure 2: Rate of positive samples over time

References

1. Shaikh N, Wald ER, Pi M. Decongestants, antihistamines and nasal irrigation for acute sinusitis in children. *Cochrane Database Syst Rev* 2010;CD007909.
2. Kassel JC, King D, Spurling GK. Saline nasal irrigation for acute upper respiratory tract infections. *Cochrane Database Syst Rev* 2010;CD006821.
3. Marchisio P, Picca M, Torretta S, Baggi E, Pasinato A, Bianchini S, Nazzari E, Esposito S, Principi N. Nasal saline irrigation in preschool children: a survey of attitudes and prescribing habits of primary care pediatricians working in northern Italy. *Ital J Pediatr* 2014;40:47.
4. Hardy ET, Stringer SP, O'Callaghan R, Arana A, Bierdeman MA, May WL. Strategies for decreasing contamination of homemade nasal saline irrigation solutions. *Int Forum Allergy Rhinol* 2016;6:140-142.
5. Shargorodsky J, Lane AP. What is the best modality to minimize bacterial contamination of nasal saline irrigation bottles? *Laryngoscope* 2015;125:1515-1516.
6. Psaltis AJ, Foreman A, Wormald PJ, Schlosser RJ. Contamination of sinus irrigation devices: a review of the evidence and clinical relevance. *Am J Rhinol Allergy* 2012;26:201-203.
7. Foreman A, Wormald PJ. Can bottle design prevent bacterial contamination of nasal irrigation devices? *Int Forum Allergy Rhinol* 2011;1:303-307.
8. Lee JM, Nayak JV, Doghramji LL, Welch KC, Chiu AG. Assessing the risk of irrigation bottle and fluid contamination after endoscopic sinus surgery. *Am J Rhinol Allergy* 2010;24:197-199.

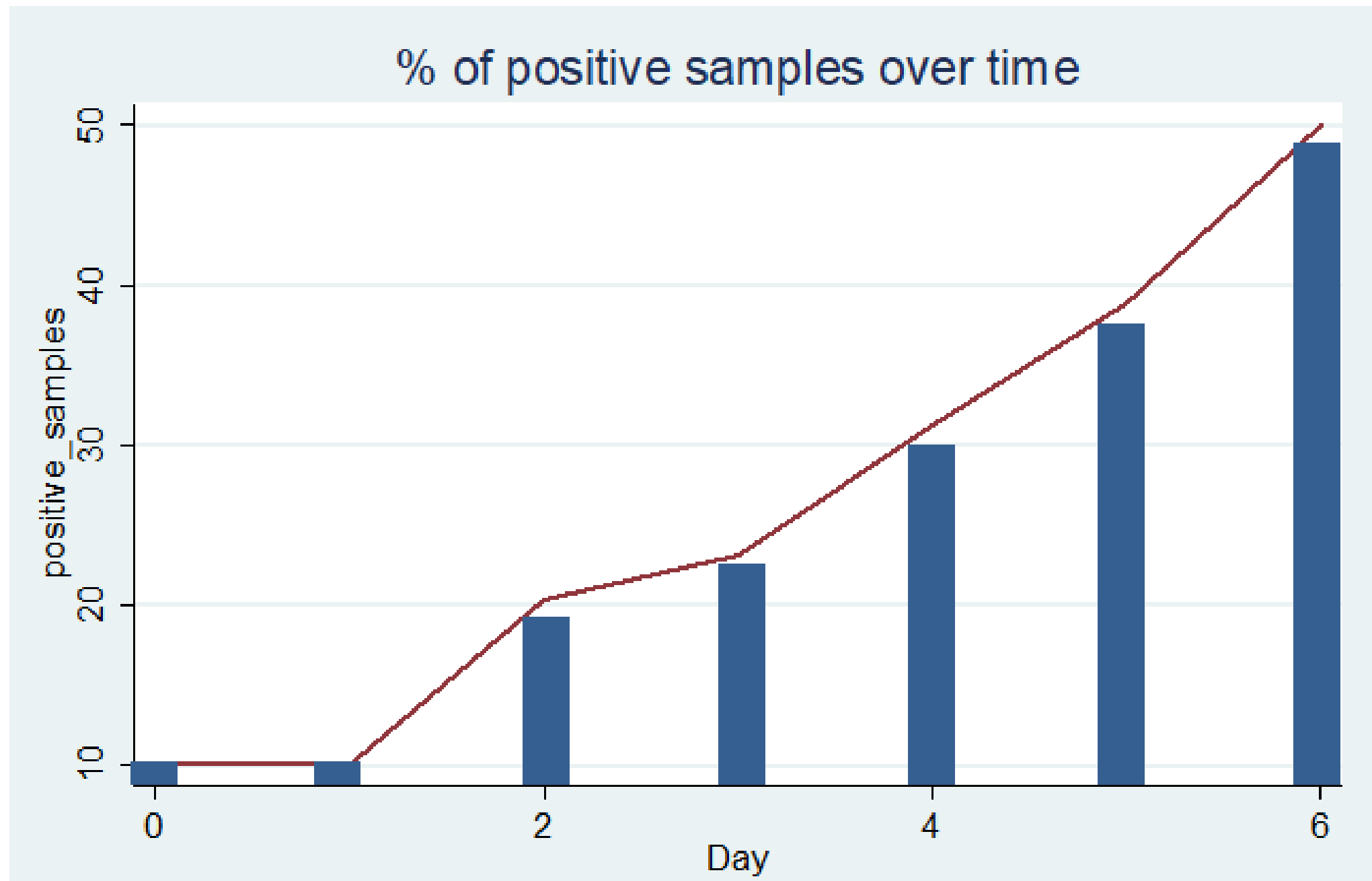
9. Kofonow JM, Bhuskute A, Doghramji L, Palmer JN, Cohen NA, Chiu AG. One-way valve bottle contamination rates in the immediate post-functional endoscopic sinus surgery period. *Am J Rhinol Allergy* 2011;25:393-396.

% of collected samples



Figure(s)

[Click here to download Figure\(s\): fig2.pdf](#)



***Conflict of Interest Form**

[Click here to download Conflict of Interest Form: coi_disclosure-3.pdf](#)