

Stool culture in pediatrics: an evaluation focused on its positivity

Coprocultivo en pediatría: una evaluación centrada en su positividad

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Received: April 22, 2025; Approved: August 8, 2025

What do we know about the subject matter of this study?

Stool culture is a microbiological test with low sensitivity in detecting bacterial causes of acute diarrhea. In addition, there is little information on its positivity rate in pediatrics at the national level, where viral causes predominate in younger children.

What does this study contribute to what is already known?

We report a 5-year review of 1,301 stool cultures in the pediatric population of a public hospital. It confirms a low overall positivity rate (2.2%), but with higher positivity in schoolchildren and adolescents, providing information to rationalize its use in infants, especially in cases of diarrhea in specific populations and/or clinical conditions, and reaffirming the low usefulness of indicating its use in patients hospitalized for more than 72 hours.

Abstract

Diarrheal disease is a common cause of pediatric morbidity of varied etiologies, with stool culture being a tool for bacterial diagnostic identification. **Objective:** To evaluate stool culture positivity in pediatrics according to age, request source, relationship with hospitalization length, and costs. **Patients and Method:** A 5-year review (2018 to 2022) evaluated the positivity of stool cultures collected from pediatric patients in the Pediatric Emergency Department and inpatient units of a public hospital, as well as the costs associated with the test. **Results:** 1,301 stool cultures were included, 81% of them collected in the Emergency Department and 37.3% in hospitalized infants. The overall positivity rate was 2.2%, with 29 positive results, and a predominance of *Salmonella* spp. ($n = 21$) and *Shigella* spp. ($n = 6$). *Campylobacter* was not detected using Hucker staining. Positivity was low in infants, whereas schoolchildren and adolescents showed significantly higher positivity rates ($p = 0.001$). Among hospitalized patients, there was a significant difference in positivity when the test was requested within 72 hours from admission. It is noteworthy that in the Emergency Department, most of the patients with positive stool cultures were not hospitalized. **Conclusion:** The test yield was low, especially in infants, which calls into question the actual usefulness of performing stool cultures in all patients with gastroenteritis, given its low clinical impact and increased costs. It is also reiterated that requesting this test in hospitalized patients after 72 hours from admission should be avoided, and that specific methods for *Campylobacter* detection should be routinely incorporated.

Keywords:

Diarrhea;
Gastroenteritis;
Feces;
Stool Testing;
Campylobacter

Introduction

Acute intestinal tract infections in the pediatric population, also known as diarrhea or acute gastroenteritis (AGE), are a frequent cause of medical consultation and may require hospitalization, and their etiology presents some epidemiological variations according to geography, socioeconomic status, environmental hygiene, season of the year, or vaccination status, among others¹. However, in recent decades, a clear predominance of viral etiologies has been identified, particularly in infants and preschoolers²⁻⁴. At the outpatient level, there is little epidemiological information on the etiology of diarrhea in Chile, except for the rotavirus surveillance network.

Usually, the clinical presentation of diarrhea does not provide enough information to determine an etiological diagnosis, and, regardless of the pathogen, there are liquid stools, vomiting, fever, general malaise, and possibly dehydration with acid-base or electrolyte imbalances⁵. In cases presenting with dysentery, a bacterial etiology is more likely⁶. Studying the etiology of diarrhea points to appropriate clinical management, avoiding unnecessary use of antimicrobials, and implementing appropriate control measures.

Diagnostic methods for diarrhea include stool culture for the detection of enteropathogenic bacteria, which is primarily aimed at screening for *Salmonella* spp, *Shigella* spp, *Vibrio* spp, and *Yersinia* spp^{7,8}. *Campylobacter* spp, an important bacterial pathogen in diarrhea with a high incidence in many countries, is recognized as a fastidious pathogen because it is difficult to isolate using standard methods, requiring specific thermal and microaerophilic conditions for its culture, with other techniques being used for its identification^{9,10}. However, for this pathogen, many laboratories routinely apply only direct Hucker staining to the stool sample.

Stool culture is a qualitative microbiological diagnostic technique that is laborious, has direct economic and personnel costs, and generally has a low yield, between 1.4% and 3.8%^{11,12}. A study using *Campylobacter* culture media and specific incubation conditions, which included pediatric and adult patients, reported an overall positivity rate of 12.6% and 6.1%, respectively, for *Campylobacter*, with only 0.4% positivity for Hucker staining¹⁰.

Furthermore, in relation to this traditional diagnostic method, there are recommendations specifying the low yield of stool culture in patients already hospitalized for more than 72 hours (acute hospital-acquired gastroenteritis)^{13,14}.

The objective of this study is to evaluate the performance of stool culture, regarding positivity, as a method for the etiological diagnosis of AGE in pediatrics in

a public hospital in Santiago, Chile, according to patient age, clinical unit where the sample was collected, and time since admission, as well as an evaluation of the costs associated with processing this test.

Patients and Method

Design

Retrospective study based on all stool cultures collected from pediatric patients over 5 years, from 2018 to 2022, processed and recorded in the Microbiology Laboratory of the *Hospital Clínico San Borja Arriarán*.

The following data were collected: patient age, place of sampling, differentiating between hospitalized patients (pediatric general wards, intensive care unit, intermediate care unit) and pediatric emergency department (PED), date of sampling, and length of hospital stay. Age groups were classified as follows: *new-born* (NB) under 1 month old, *infant* from 1 month to under 1 year old, *toddler* from 1 year to under 2 years old, *preschooler* from 2 years to under 6 years old, *schoolchild* from 6 years to under 11 years old, and *adolescent* from 11 to 17 years old.

One test per patient per episode of diarrhea associated with the site of stool sample collection was considered, and second stool tests processed according to the date of consultation or hospitalization were excluded.

Microbiological techniques

Samples were collected directly from fresh patient stools using a swab and transported to the microbiology laboratory in Cary Blair medium. Cultures were performed on MacConkey agar, *Salmonella-Shigella* (SS) agar, and TCBS agar (for *Vibrio* spp) plates and incubated aerobically at 35°C, with bacterial growth evaluated after 24 hours.

According to pediatric institutional protocol, in cases of dysenteric syndrome or suspected hemolytic uremic syndrome, and upon medical request, stool samples are processed using molecular methods (FilmArray®).

Between 2018 and 2020, standardized biochemical tests were performed on colonies suspected of *Salmonella*, *Shigella*, or *Vibrio*, and when *Shigella* or *Salmonella* was suspected after further incubation, slide agglutination for serological tests were performed. Since the end of 2020, suspected colonies have been processed using MALDI-TOF mass spectrometry for identification, and for *Shigella*, serological agglutination tests were used for confirmation and to differentiate from *E. coli*. During the five years included, VITEK® was used for susceptibility study, except for *Salmonella*, where a manual susceptibility test was performed. In addition, all stool culture samples underwent Hucker staining to

search for *Campylobacter* forms. For *Yersinia* in particular, incubation was performed at room temperature, and after 48 hours, a set of biochemical tests was performed to search for lactose-negative colonies. If no suspicious colony growth was observed after 72 hours of incubation, the stool culture was reported as negative for *Salmonella spp*, *Shigella spp*, *Vibrio spp*, or *Yersinia spp*. After validation, the stool culture results were entered into the Biomerieux® KernMIC® database, from which the data for this study were obtained.

Clinical and economic variables

Additionally, other data sources related to hospitalization were reviewed, including the inpatient care platform, discharge records, and the Infectious Diseases Unit's consultation log, in order to compare admission dates and sample collection dates. The patient information to be specifically considered corresponds to their National Identification Number (RUN) and age.

To assess the cost of stool culture, the value of the test in Chilean pesos was applied, according to the fee established by the National Health Fund (FONASA) for institutional care for the corresponding year¹⁵.

Statistical analysis

The data were entered into an Excel spreadsheet to calculate absolute and relative frequencies, mean,

and minimum and maximum range. For the statistical study, the data were analyzed using STATA version 16 software. The Shapiro-Wilks normality test was performed for quantitative variables, expressed as median and interquartile range (IQR: p25-75). Categorical variables were expressed in absolute and relative frequency. Association analyses were performed using the chi-square test and Fisher's exact test. The Kruskal-Wallis test was used to analyze differences between days of hospitalization after 72 hours. A p-value < 0.05 was considered significant.

Ethical considerations

This research was approved by the Scientific Ethics Committee of the Central Metropolitan Health Service and the Scientific Committee of the *Hospital Clínico San Borja Arriarán*.

Results

A total of 1,301 stool cultures of pediatric patients were included in this study. Table 1 describes the general characteristics of the sample, including the distribution of the test by year, clinical unit where the sample was collected, age group, and test positivity. Among other things, it highlights the low number of

Table 1. Characteristics of stool examinations analyzed by year, and stool culture positivity rates, stratified by age group, sample collection units, and patient destination (n = 1,301)

Variable	Stool cultures requested n (%)	Positive stool cultures n (%)	p
Year			----
2018	428 (32,9)	7 (1,6)	
2019	375 (28,8)	10 (2,7)	
2020	268 (20,6)	7 (2,6)	
2021	40 (3,1)	0	
2022	190 (14,6)	5 (2,6)	
Age group			0,001*
Newborns and young infants	163 (12,5)	1 (0,6)	
Toddler	323 (24,8)	3 (0,92)	
Preschooler	543 (41,7)	9 (1,7)	
Schoolchildren	169 (13)	10 (5,9)	
Adolescent	103 (7,9)	6 (5,8)	
Sample collection unit			0,633
Pediatric Emergency Department	1.054 (81)	25 (2,4)	
Inpatient units	247 (19)	4 (1,6)	
General ward	156 (12)	4 (1,6)	0,510
Intermediate Care Unit	71 (5,5)	0 (0)	
Intensive Care Unit	20 (1,5)	0 (0)	
Disposition from the Emergency Department			0,047*
Home	841 (65)	16 (1,9)	
Hospital admission	213 (16)	9 (4,2)	

*Statistically significant

tests requested in 2021, the high proportion of samples collected in the Emergency Department, and the lower percentage of stool cultures from schoolchildren and adolescents. In addition, the distribution of positive stool cultures is presented, highlighting the low positivity in infants and the statistically significant difference when analyzed by age group, with schoolchildren and adolescents having a higher percentage of positivity ($p = 0.001$), as well as those who were hospitalized from the emergency department ($p = 0.047$). There were no positive stool cultures from those samples collected in pediatric critical care units, and of the four tests that were reported as positive in infants, all were performed in the emergency department, and none of these patients were hospitalized.

Stool cultures positivity for AGE

29 stool cultures were reported positive, resulting in an overall positivity rate of 2.2% over five years. When evaluating the positivity of stool cultures requested in the PED for patients who were hospitalized with a diagnosis of gastroenteritis, a cumulative total of 213 tests were performed over the five years, with a positivity rate of 4.2%. Table 2 shows bacterial identification according to age, highlighting the identification of *Yersinia* in two 11-month-old infants (December 2020 and February 2022), with a clear predominance of *Salmonella* and *Shigella* in preschool, school-age, and adolescent children.

Table 2. Distribution of bacterial etiologies isolated in 1,301 stool cultures from pediatric patients according to patient age (n = 29)

Variables	Identified bacteria		
Age	<i>Salmonella</i> spp	<i>Shigella</i> spp	<i>Yersinia enterocolitica</i>
Newborns and young infants	2	0	2
Toddlers	0	0	0
Preschooler	7	2	0
Schoolchildren	9	3	0
Adolescent	3	1	0
Total	21	6	2

Agents identification

Of the 29 bacterial agents isolated, *Salmonella* spp was identified in 21 tests, *Shigella* spp in 6, and *Yersinia enterocolitica* in 2, with different distribution by year (Figure 1). In 2021, no isolation of any of the enteropathogens studied in the laboratory was reported, and there was never more than one isolation per epidemiological week. In the 5 years evaluated, *Campylobacter* was not identified in stool samples using Hucker staining.

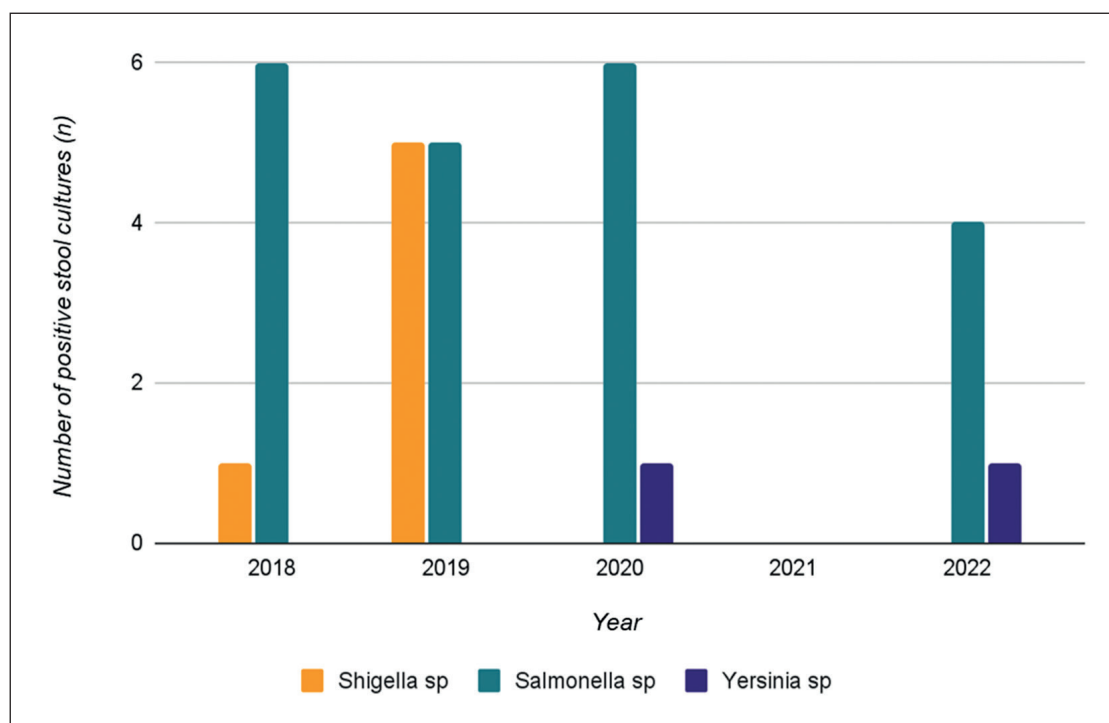


Figure 1. Bacterial species detected in pediatric stool cultures during the years 2018-2022

Stool cultures positivity in hospitalized patients undergoing etiologic investigation for hospital-acquired acute diarrhea

A total of 247 stool cultures were studied in patients already hospitalized, 140 of which (56.7%) were collected within 72 hours of admission. Table 3 shows the median and range of days on which stool cultures were collected for this group of patients. The median time between admission and sample collection was more than one week, and the upper value of the IQR was greater than three weeks in all units; there was no significant difference between positivity and the origin of the sample collection ($p = 1.00$), nor between the number of days of hospitalization at the time of sample collection and the unit of hospitalization ($p = 0.09$). However, it is noteworthy that in the intensive and intermediate care units, a higher percentage of requests were made after 72 hours of hospitalization than in the general care ward ($p = 0.002$).

107 stool cultures were performed from patients hospitalized for more than 3 days, with a median of 10 days (IQR 6 to 28). Of the total, bacterial pathogen iso-

lation was demonstrated in only one test, determining an overall positivity of 0.9% for stool samples collected after 72 hours of hospitalization.

Besides, in 33 of the hospitalized patients, regardless of the number of days of hospitalization, in addition to stool culture, an etiologic investigation was performed using the FilmArray® molecular panel in stool samples. Of these, 25 were in the general ward, 5 in intermediate care, and 3 in intensive care. Of the 33 FilmArray® panels processed, 20 were positive, 8 of them with identification of viral agents only, and 3 with detection of *Campylobacter*. In addition, 3 of these patients were reported to have positive stool cultures for a bacterial agent (2 cases of *Salmonella* and 1 of *Yersinia*), agents also detected in the molecular panel but not as the sole etiological agents.

Cost associated with stool culture requests

Over the five years of stool culture requests, the cost according to FONASA values reached \$4,798,610. Table 4 shows the distribution by year and cost. 80.1% of these cultures were requested in the PED, in addition

Table 3. Stool cultures collected from hospitalized pediatric patients according to the day of sample request (3 days = 72 hours), positivity, and hospitalization unit (n = 247)

	Total	Sample collection unit			
	(n = 247)	General ward (n = 156)	Intermediate Care Unit (n = 71)	Intensive Care Unit (n = 20)	p
Stool cultures requested, n (%)					
Before 72 hours	140	101	33	6	0,002*
After 72 hours	107	55	38	14	
Positive stool cultures, n (%)					
Before 72 hours	3	3	0	0	1,0
After 72 hours	1	1	0	0	1,0
Days hospitalized after 72 hours, median (IQR)	10 (5-28)	9 (6-32)	12 (7-23)	9,5 (7-24)	0,09

*Statistically significant

Table 4. Total pediatric stool cultures processed per year according to requesting service (unit) and annual direct cost of the test based on FONASA rates, 2018 to 2022

	2018	2019	2020	2021	2022	Total
Unit cost according to FONASA (\$)	3.490	3.590	3.680	3.760	3.930	
Number of stool cultures processed and requested in the Pediatric Emergency Department	375	319	236	11	113	1.054
Number of stool cultures processed and requested in inpatients unit	53	56	32	29	77	247
Total stool cultures processed	428	375	268	40	190	1.301
Total costs (\$)	1.493.720	1.346.250	986.240	150.400	746.700	4.723.310

\$: chilean pesos (CLP)

to a trend towards a progressive decrease in the overall number of these tests processed since 2018 (except for 2021). However, in 2022, there was an increase in the total number of stool cultures requested for hospitalized patients.

Discussion

This study highlights the low overall positivity rate of stool cultures in pediatrics, reaching 2.2%, a figure consistent with previously available information^{11,12} and similar to older published experiences in adults, such as that of Koplan et al.⁷, who reported a 2.4% positivity rate in 2,468 patients evaluated with stool culture, and the retrospective experience of Meropol et al. in 1989¹⁶, who evaluated the results of 250 stool cultures from pediatric patients under 18 years of age performed upon hospital admission finding a 2.8% positivity rate in one year; however, unlike our experience, the seven positive tests were in children under two years of age.

The information obtained supports the poor overall performance of stool culture for the microbiological diagnosis of AGE in the pediatric population, particularly in infants, where a lower positivity rate of 0.8% was observed, which seems to be secondary to the predominance of viral etiology of this infection in this age group²⁻⁴. However, a statistically significant difference was found in the positivity of stool cultures from schoolchildren (5.9%) and adolescents (5.8%) compared to infants.

One of the limitations of this study is that the clinical picture (fever, duration of diarrhea, presence of dysentery, reason for requesting the test) was not evaluated, in accordance with the study design and the Ethics Committee's approval. Additionally, in many patients, the samples were collected in the Emergency Department, and the patient was discharged ($n = 841$). Of these, 16 tests were positive (1.9%), compared to 4.2% in those who were hospitalized (positivity 4.2%), a significant difference probably caused by some clinical or laboratory condition unknown to us.

Regarding the etiologies detected, *Salmonella* spp and *Shigella* predominated, which is in line with other published experiences. Furthermore, recognizing the low sensitivity and, in our experience, the non-existent detection of *Campylobacter* by Hucker staining, we believe that, despite the higher cost, it is necessary to routinely incorporate specific media with greater sensitivity for this agent¹⁷.

The decrease in the number of pediatric stool cultures requested and processed during 2021 was due to a reduction in pediatric beds due to the COVID-19 pandemic and the closure of the emergency depart-

ment following a serious fire that affected the hospital.

The evaluation of stool cultures from patients already hospitalized showed that in 57% of cases, the procedure was performed within 72 hours of admission. However, there were requests for tests performed after this hospitalization period, pointing to a probable diagnosis of hospital-acquired AGE. These requests were made at a median of 10 days and a 75th percentile of 28 days, with a significant difference between requests in critical care units versus patients in general wards. There was only one positive test performed more than 3 days after hospitalization, from a sample collected 4 days after admission, with a positive result for *Yersinia enterocolitica*. This reinforces the long-standing and well-known concept of the "3-day exclusion rule," a strategy that promotes not testing patients with diarrhea with this test after 3 days of hospitalization, a statement consistent with what has been described for adult patients in Chile¹³ and Japan¹⁸, among others. Also noteworthy is the experience of Fan et al.¹⁹, who applied a 20-month prospective design, in which they collected stool cultures from adults and children hospitalized for more than 72 hours during the first 10 months and in the following 10 months they suspended this practice, concluding from their results that stool cultures should not be collected after 3 days unless there are major epidemiological reasons, a concept also supported by Le Guern et al., who also refer to the concept of savings in healthcare costs²⁰.

Considering the contribution of new diagnostic techniques, different etiologies, and types of patients, including immunocompromised patients, the 3-day rule remains in force, and diagnostic techniques such as molecular panels should be used in these situations²¹. Therefore, in addition to re-educating clinicians regarding this behavior, there should be continuous reinforcement from the microbiology laboratory, applying a rejection policy for receiving and processing these samples, except in specific cases, such as a possible hospital-acquired outbreak secondary to a common food source⁸.

Although stool culture is a representative sample of the source of bacterial infection, it contains a high load of commensal flora; hence, the importance of culturing in selective media. Early sampling is recommended in the progression of the clinical picture, with adequate transport to the laboratory in non-refrigerated transport media, with only one sample being sufficient^{22,23}.

Given the work involved and its low yield, stool culture is also not very cost-effective. In our experience and based on the costs associated with the FONASA value (with a debatable real market price), it points to a perhaps unnecessary expense, particularly in the

Emergency Department, where 81% of requests originate, including more than a third of breastfeeding patients. In fact, four children under the age of 2 who had positive stool cultures were not hospitalized after their medical evaluation, meaning that this test did not influence decision-making.

Since the objective of stool culture is to isolate bacteria in order to determine the need for antimicrobial therapy, several markers have been proposed over the years to guide the etiologic diagnosis, such as the detection of fecal leukocytes, lactoferrin, or occult blood in stools; however, these have shown poor sensitivity or specificity²⁴. Currently, etiological diagnosis is achieved quickly with the application of molecular panels that identify bacteria, viruses, and parasites. Although significantly more expensive, these panels are widely supported in various publications focused mainly on patients who require hospitalization²⁵, reporting a positivity rate of over 29% with secondary savings in isolations, tests, and antimicrobials²⁶. In addition, they have a sensitivity of 94% to 100% depending on the pathogen and a specificity of 97%²⁷. A national study evaluating the use of molecular panels in pediatric patients attending a private emergency department reported a positivity rate of 78.8% in 198 patients, with 35.5% of samples being polymicrobial, detecting 72.9% of bacterial etiologies among the 229 microorganisms identified²⁸.

Although it was not the objective of this study, the use of molecular panels limited to certain conditions in hospitalized patients also showed high positivity. In some patients, depending on their clinical behavior, it should be considered to expand the study by collecting blood cultures for etiologic identification.

We recommend avoiding stool cultures in infants and suggest collecting stool cultures exclusively from patients with diarrhea and risk factors such as immunosuppression, presence of dysentery, possibly in neonates, or those with a history of recent travel²⁹, as well as outbreaks where the source is suspected to originate from food or water³⁰ and in epidemiological studies or surveillance programs. Stool samples should not be collected after 72 hours of hospitalization.

Conclusions

Given the low overall positivity rate, performing stool cultures on all patients with AGE seems debatable. Recognizing that few circumstances justify the empirical use of antimicrobials, clinical efforts should be directed toward symptomatic management, especially when there is no indication for hospitalization of infants with AGE, rationalizing its use to specific host conditions, clinical presentation, or epidemiological aspects, avoiding its use after 72 hours of hospitalization, and considering new routine diagnostic tools specific to *Campylobacter*, and molecular tools for selected cases where the identification of a microorganism can guide management.

Ethical Responsibilities

Human Beings and animals protection: Disclosure the authors state that the procedures were followed according to the Declaration of Helsinki and the World Medical Association regarding human experimentation developed for the medical community.

Data confidentiality: The authors state that they have followed the protocols of their Center and Local regulations on the publication of patient data.

Rights to privacy and informed consent: This study was approved by the respective Research Ethics Committee, which, according to the study's characteristics, has accepted the non-use of Informed Consent.

Conflicts of Interest

Authors declare no conflict of interest regarding the present study.

Financial Disclosure

Authors state that no economic support has been associated with the present study.

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