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Salmonellosis in Children at a Portuguese Hospital: A Retrospective Study

Salmoneloses em Crianças num Hospital Português: Um Estudo Retrospetivo

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ABSTRACT

Introduction: Salmonellosis represents a considerable health, social and economic burden in both high- and low-income countries. Recently, in Portugal, most cases of *Salmonella* infections have been reported in children under 15 years of age. The main aim of this study was to characterize, from an epidemiological, microbiological, and clinical perspective, cases of *Salmonella* isolation among children.

Material and Methods: The authors performed a descriptive study using retrospective analysis of cases of salmonellosis, in pediatric age, at a Portuguese Level II Hospital, between January 2015 and July 2020.

Results: The population included a total of 63 children, of which 81% were Portuguese. Ethnicity was identified in 13 children, most of whom were African. The median age at diagnosis was four years old (3.5 - 9 years old). Despite the small number of cases per year in our study (11), one-third were severe enough to require hospitalization. Overall, 13% of patients were treated with antibiotics. In 63% of the isolates, serotype was identified: *Salmonella enteriditis* (38%), *Salmonella typhimurium* (22%), and *Salmonella typhi* (3%). Antibiotic resistance rates were 19% for ampicillin and 6.4% for amoxicillin-clavulanic acid and cotrimoxazole. No resistance to third-generation cephalosporins was found.

Conclusion: Given the obtained results, we intend to improve knowledge on salmonellosis in Portugal and, consequently improve prevention strategies, treatment and its notification. Although the incidence of salmonellosis has been decreasing in recent years it is the second most frequent gastrointestinal infection in the European Union, contributing to significant rates of hospitalizations and use of antibiotics in Portugal.

Keywords: Child; Portugal; Salmonella enterica; Salmonella Infections; Typhoid Fever

RESUMO

Introdução: As salmoneloses representam um desafio do ponto de vista sanitário, social e económico, tanto nos países em desenvolvimento como nos desenvolvidos. Nos últimos anos, em Portugal, a maioria das infeções por *Salmonella* foi reportada em crianças com menos de 15 anos. O objetivo principal deste estudo foi caracterizar, do ponto de vista epidemiológico, microbiológico e clínico, os casos de isolamento de *Salmonella* em crianças.

Material e Métodos: Estudo descritivo com análise retrospetiva dos casos de salmonelose em idade pediátrica, no período compreendido entre janeiro de 2015 e julho de 2020, num Hospital Português de nível II.

Resultados: A população incluiu 63 doentes, dos quais 81% eram portugueses. A origem étnica foi identificada em 13 crianças, sendo a maioria africana. A idade média de diagnóstico foi quatro anos (3,5 - 9 anos). Apesar do reduzido número de casos por ano no nosso estudo (11), um terço destes foi suficientemente grave para necessitar de hospitalização e 13% dos pacientes foram tratados com antibióticos. Em 63% dos isolamentos identificou-se o serotipo: *Salmonella enteriditis* (38%), *Salmonella typhimurium* (22%) e *Salmonella typhi* (3%). As taxas de resistência aos antibióticos foram de 19% para ampicilina e 6,4% para amoxicilina-ácido clavulânico e cotrimoxazol. Não se identificaram resistências às cefalosporinas de terceira geração.

Conclusão: Com os resultados obtidos pretendemos melhorar o conhecimento sobre as salmoneloses em Portugal e consequentemente as estratégias de prevenção, tratamento e notificação. Embora a incidência de salmoneloses tenha vindo a diminuir nos últimos anos, estas são a segunda causa mais frequente de infeção gastrointestinal na União Europeia, contribuindo para uma importante taxa de hospitalizações e de uso de antibióticos em Portugal.

Palavras-chave: Criança; Febre Tifoide; Infecções por Salmonella/tratamento farmacológico; Portugal; Salmonella entérica

INTRODUCTION

Salmonella, a member of the *Enterobacteriaceae* family, includes two species - *Salmonella enterica* and *Salmonella bongori* (rarely opportunistic in humans). Based on biochemical and antigenic characteristics, *Salmonella enterica* is further divided into subspecies (I – VI) and serotypes.[1] The serotypes *Salmonella* ser. *typhi* and *paratyphi* are categorized as typhoidal *Salmonella* whereas other serotypes are grouped as Non-typhoidal *Salmonella,* and include *Salmonella* ser. *typhimurium* and *Salmonella* ser. *enteritidis*.[1] Typhoidal *Salmonella* and non-typhoidal *Salmonella* cause different clinical syndromes.

Salmonella infections represent a considerable health, social and economic burden in both high- and low-income countries, with their incidence being much higher than the cases reported.[1] In Portugal, from 2011 to 2014, 785 cases of *Salmonella* infection have been reported, 83% of which in children under 15 years of age.[2]

The main mode of transmission of non-typhoidal *Salmonella* is by ingestion of contaminated animal food products, contact with colonized animals, consumption of contaminated water and non-animal food products, and fecal-oral spread; incubation is typically six to 12 hours.[3] In high-income countries, in immunocompetent individuals, Non-typhoidal *Salmonella* usually causes bacterial acute gastroenteritis, with secondary bacteremia occurring in up to 5% of patients.[4,5] They typically experience self-limited enterocolitis with nausea, emesis, abdominal pain, fever, and watery non-bloody diarrhea (some patients may have painless

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bloody diarrhea) lasting less than 10 days.[3] However, young infants and people with immunosuppressive conditions, hemoglobinopathies (including sickle cell disease), malignant neoplasms and human immunodeficiency virus infection are at a higher risk of invasive disease, including bacteremia, meningitis, osteomyelitis, septic arthritis, pneumonia, and cholangitis.[1,6] Also, in low-income countries, there is increased recognition of non-typhoidal *Salmonella* as a major cause of severe febrile illness.[4] Bacterial fecal shedding can continue for up to 12 weeks, especially in children younger than five years and in those treated with antibiotics.[3]

Enteric fever caused by typhoidal *Salmonella* is a severe illness with an estimated 31 million cases leading to more than 215 000 deaths worldwide annually.[3] Typhoidal *Salmonella* is predominantly transmitted through contaminated water or food with human feces. In contrast to non-typhoidal *Salmonella,* that has a worldwide distribution, typhoidal *Salmonella* infection is endemic in low-income countries, where poor sanitation and lack of access to safe food and water is an unfortunate reality.[4,5]

With exclusive human reservoirs and fecal-oral transmission, the average incubation period of typhoidal *Salmonella* is seven to fourteen days. Clinical manifestations include fever, chills, slow heart rate (more common in adults), headache, malaise, anorexia, lethargy, myalgia, cough, abdominal pain and tenderness, jaundice, hepatosplenomegaly, constipation or diarrhea, “rose spots” (faint salmon-colored macules on the trunk and abdomen), dactylitis and, in severe cases, altered mental status and shock.[3,6] These symptoms typically last two to four weeks without treatment.[3] Typhoid fever can also be associated with the following complications: terminal ileal perforation, splenic abscesses, disseminated intravascular coagulation, rhabdomyolysis with acute renal failure and neurologic manifestations due to brain abscesses or Guillain-Barré syndrome.[3] Stool bacterial shedding continues for more than three months in approximately 10% of cases.[3] In 4% of the infected individuals, excretion lasts longer than one year.[3] When this occurs, it is assumed they are chronic carriers of typhoidal *Salmonella*. The predisposition to be a chronic carrier increases with age, being rare in children, and correlates with the prevalence of cholelithiasis, which might require a cholecystectomy, due to gallbladder biofilm formation.[3,6]

While in non-typhoidal *Salmonella* infections, stool culture is typically sufficient for the diagnosis of gastroenteritis, extraintestinal involvement must be confirmed by cultures from the affected organs. Blood cultures (or bone marrow cultures) are required for diagnosing typhoidal *Salmonella* infection, with repeated cultures often needed due to low sensitivity.

Non-typhoidal *Salmonella* gastroenteritis is treated with supportive measures in immunocompetent individuals.[5] However, gastroenteritis in infants younger than three to six months, immunocompromised patients, suspected or confirmed non-typhoidal *Salmonella* invasive disease, should receive antibiotic treatment.[1,5,6] Third-generation cephalosporins (ceftriaxone or cefotaxime) are indicated for extraintestinal non-typhoidal *Salmonella* disease and typhoidal *Salmonella* (five to seven days for gastroenteritis, two weeks for bacteremia, and four to six weeks for extraintestinal infections).[3] Because antibiotic resistance against *Salmonella* is increasing, local susceptibility patterns should be taken into account. Chronic typhoidal *Salmonella* carriers should be treated with four weeks of an oral fluoroquinolone after the initial treatment.[3]

The main aim of this study was to characterize, from an epidemiological, microbiological, and clinical perspective, cases of *Salmonella* isolation among children.

MATERIAL AND METHODS

This study sample encompasses the cases of isolation of *Salmonella* in fecal and/or blood cultures in the pediatric population of this hospital from 2015 to 2020. This was a descriptive, retrospective study, developed in the pediatric department of a level II hospital, in the Lisbon metropolitan area. Data on socio-demographics, epidemiological, and clinical characteristics, diagnostic tests, treatment, evolution and preventive measures were collected from the observation of the clinical records of patients.

Microbiological methods used for the diagnosis of *Salmonella* infection included culture of clinical specimens on selective culture media, colony identification and antimicrobial susceptibility testing using the automated VITEK® 2 System (bioMerieux) with interpretation according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) clinical breakpoints and further classification with serological tests. Descriptive and statistical analysis was performed with SPSS® v.23.0 (SPSS Inc, Chicago, IL, USA).

The study was approved by the hospital ethics committee. All the data collected was aggregated and thus anonymous and confidential.

RESULTS

The population included a total of 63 children, of whom 36 (57.1%) were boys (Table 1). The majority was Portuguese (n = 51; 81%). Ethnicity was assessed in 13 children, most of whom African (n = 9; 69.3%), followed by Caucasian (n = 3; 4.8%) and Indian (n = 1; 1.6%). It was only possible to determine the parents’ origin in 14 children, with Guinea-Bissau being the most frequent place of birth (n = 4; 6.4%), followed by Brazil (n = 3; 4.8%), Cape Verde and Angola (n = 2; 3.2%, each) and finally Portugal, Belgium, and India (n = 1; 1.6%, each).

Table 1 – Results obtained for the variables analyzed in the study (part 1 of 2)

Socio-demographic characteristics				
Gender	**Male**	**Female**		
Absolute number (relative frequency)	36/63 (57.1%)	27/63 (42.9%)		
Epidemiologic context				
Age of diagnosis	**Median**	**Interquartile range**	**Average**	
Years old	4	3.5 – 9	5.9	
Cases per year	**Median**	**2015**	**2017**	**2018**
Absolute number (relative frequency)	11	14 (22.2%)	14 (22.2%)	6 (9.5%)
Nursery/school	**Contact with suspected cases**	**No know contacts**	**No data**	
Absolute number (relative frequency)	3 (10.3%)	5 (17.2%)	21 (72.5%)	
Intra-family contacts	**Siblings**	**Several family members**	**Parents**	**Cousins/ Grandparents** (each)
Absolute number (relative frequency)	8 (25.8%)	6 (19.4%)	4 (12.9%)	2 (6.5%)
Contaminated food	**Milk and eggs**	**Unidentified food**	**Unknown**	
Absolute number (relative frequency)	2 (3%)	10 (16%)	51 (81%)	
Recent trips	**Guinea-Bissau / Angola** (each)	**São Tomé and Príncipe / Brazil / India / Morocco** (each)	**Unidentified destination**	
Absolute number (relative frequency)	2 (11.8%)	1 (5.9%)	9 (52.9%)	
Clinical features				
Diagnosis	**Acute gastroenteritis**	**Occult bacteremia**		
Absolute number (relative frequency)	55 (87.3%)	6 (9.5%)		
Risk factors	**None**	**HIV infection / Prematurity / Unspecified cardiac disease / Down syndrome** (each)		
Absolute number (relative frequency)	59 (93.7%)	1 (1.6%)		
Hospitalization	**Total**	**Median age of hospitalized group**	**Median age of non-hospitalized group**	
Absolute number (relative frequency)	37 (58.7%)	7 years old (interquartile range 3 – 10.5 years)	3 years old (interquartile range 1.6 – 5.5 years)	
Analytical evaluation				
Leukocytes	**Leukopenia** (< 4500/L)	**Leukocytosis** (> 15 000/L)		
Absolute number	4	7		
C-reactive protein	**< 5 mg/dL**	**5 – 15 mg/dL**	**15 – 25 mg/dL**	**> 25 mg/dL**
Absolute number	20	23	9	2
Electrolyte imbalance	**Hyponatremia** (< 135 mmol/L)	**Hypernatremia** (> 145 mmol/L)	**Hypokalemia** (< 3.5 mmol/L)	**Hyperkalemia** (< 4.5 mmol/L)
Absolute number	3	1	6	9
Complications	**Total**	**Dehydration**	**Metabolic alkalosis**	
Absolute number (relative frequency)	24 (38.1%)	22/24 (91.7%)	2/24 (8.3%)	

(Table 1 ends on next page)

Table 1 – Results obtained for the variables analyzed in the study (part 2 of 2)

Microbiological characteristics				
Samples with *Salmonella* isolation	**Stool culture**	**Blood culture**	**Stool + blood culture**	**Blood + urine culture**
Absolute number (relative frequency)	57 (90.5%)	2 (3.2%)	3 (4.8%)	1 (1.6%)
***Salmonella* enterica serotypes**	***Salmonella typhi***	***Salmonella typhimurium***	***Salmonella enteritidis***	
Absolute number (relative frequency)	2 (3%)	14 (22%)	24 (38%)	
Resistance rates by serotype (relative frequency)	***Salmonella typhi***	***Salmonella typhimurium***	**Non-typhoidal *Salmonella***	
Ampicillin		14.3%	5%	
Amoxicillin-clavulanic acid	None	5%	< 5%	
Cotrimoxazole		< 5%	< 5%	
Treatment and follow-up				
Treatment	**Symptomatic**	**Antibiotic therapy**		
Absolute number (relative frequency)	55 (87.3%)	8 (12.7%)		
Collected new stool samples	**Total**	**Negative**	**Time after diagnosis** (on average)	
Absolute number	12	12	3.5 months	

The median age at diagnosis was four years old (interquartile range 3.5 – 9 years old) and the average was 5.9 years old (Table 1). The age group under five years was the one with most cases (n = 32; 50.8%; Fig. 1).

There was a median of 11 cases per year in the study period, with a variation from 14 cases per year (22.2%) in 2015 and 2017 and only six in 2018 (9.5%). The months of May (n = 9), June (n = 8) and September (n = 7) had the highest number of cases (Fig. 2).

Regarding the epidemiological context of the 29 children attending nursery or school, only three (10.3%) had contact with suspected cases, five (17.2%) had no known contacts and it was not possible to obtain data for the remaining children (Table 1). Concerning intra-family contacts, we obtained data from 31 children, of whom eight (25.8%) had contact with sick siblings, six (19.4%) had contact with several symptomatic family members (degree of kinship not specified); four (12.9%) had contact with parents; and two (6.5%) had contact with cousins or grandparents, respectively (Table 1). Combining data obtained from contacts with suspected cases in school and within their families, 39 children (64.1%) had contact with symptomatic patients, the majority in a family context (n = 22). Regarding potentially contaminated food, we obtained data from 12 children and in two (3%) the ingestion of contaminated milk and eggs was confirmed (Table 1). Concerning recent trips, data was retrieved from 17 children: two (11.8%) had been to Guinea-Bissau, two (11.8%) to Angola and the others had traveled to São Tomé and Príncipe, Brazil, India, and Morocco (5.9% each) – Table 1.

The most frequent initial diagnosis was acute gastroenteritis (n = 55, 87.3%) followed by occult bacteremia (n = 6, 9.5%) – Table 1. The remaining children presented with another diagnosis (urinary tract infection and fever with petechial rash). Most children had no risk factors (n = 59, 93.7%) while the others had HIV infection, prematurity, unspecified cardiac disease, and Down syndrome (n = 1, 1.6% each) – Table 1. No child had hemoglobinopathy.

Hospitalization was required in 37 children (58.7%), of whom 83.8% had acute gastroenteritis with signs of dehydration. The median age between the hospitalized *versus* the non-hospitalized groups, was seven years old (interquartile range 3 – 10.5) *versus* three years old (interquartile range 1.6 – 5.5), respectively (Table 1).

Regarding the analytical evaluation, the authors highlight: leukopenia (< 4500/L) in four patients and leukocytosis (> 15 000/L) in seven; C-reactive protein < 5 mg/dL in 20 children, between 5 – 15 mg/dL in 23, 15 – 25 mg/dL in nine and > 25 mg/dL in two children (with a maximum of 30.9 mg/dL); hyponatremia (< 135 mmol/L) in three and hypernatremia (> 145 mmol/L) in one; hypokalemia (< 3.5 mmol/L) in six and hyperkalemia (> 4.5 mmol/L) in nine patients (Table 1).

There were complications in 24 children (38.1%),

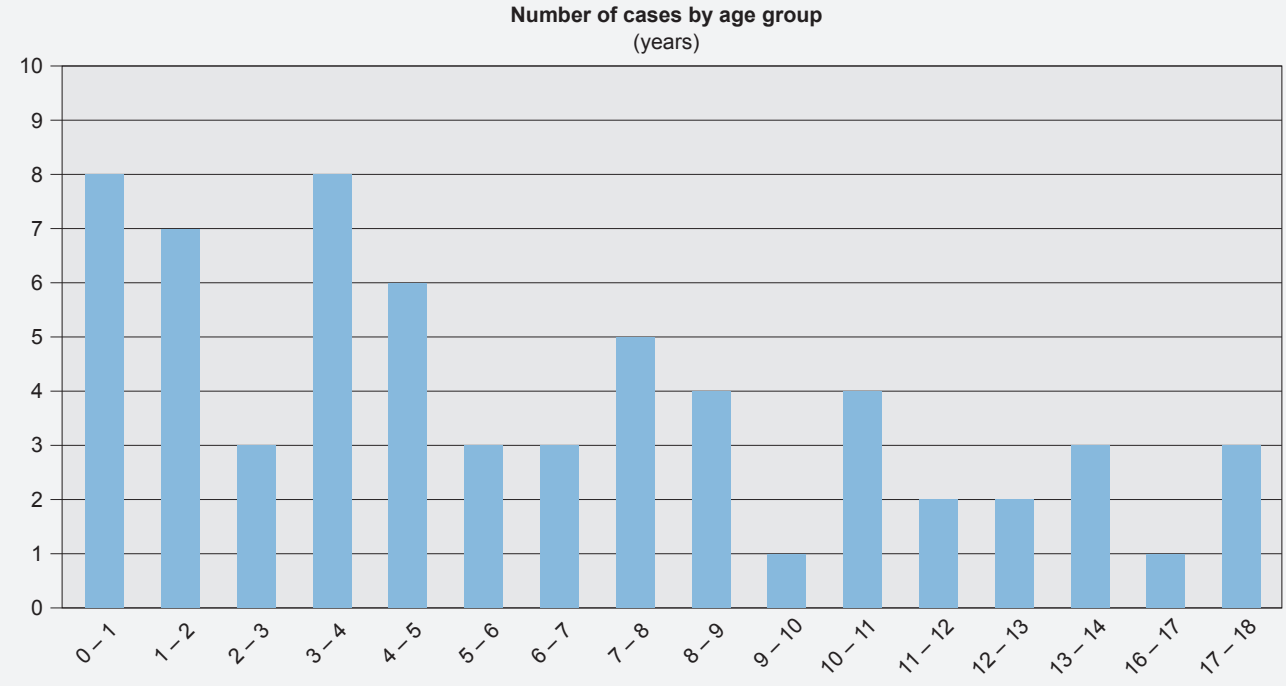

Figure 1 – Distribution of salmonellosis cases by age group, in years

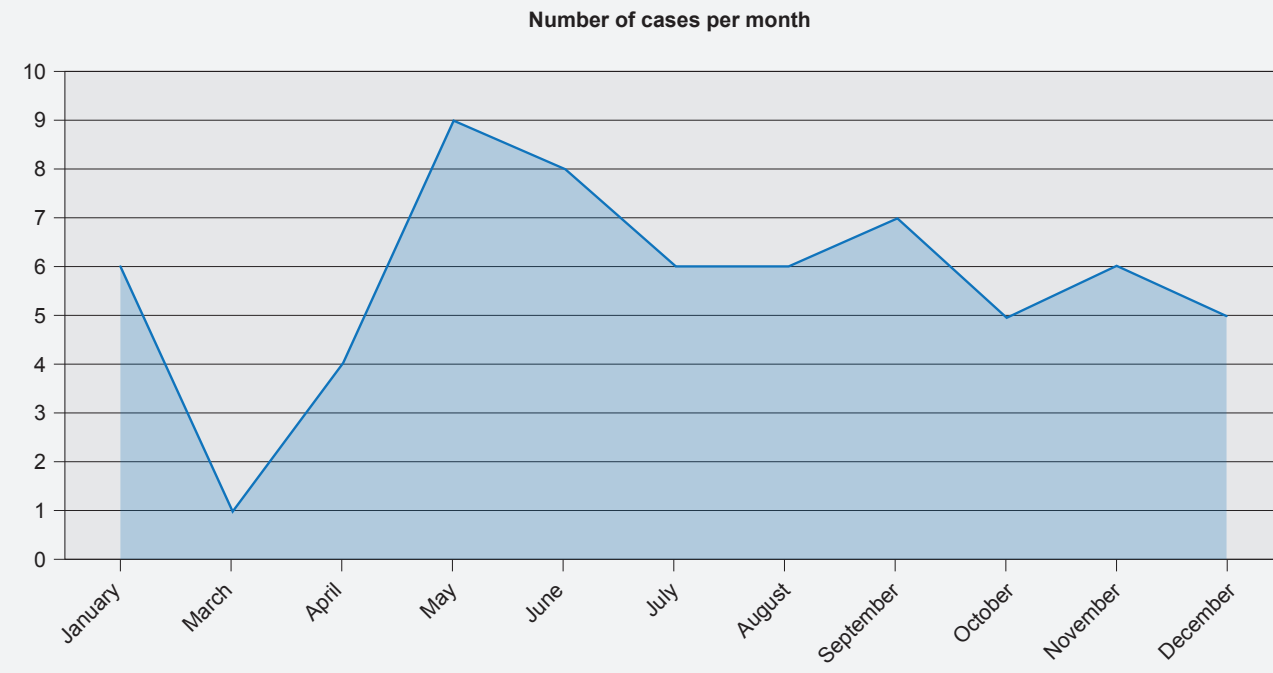

Figure 2 – Distribution of salmonellosis cases per month

with dehydration being the most frequent (n = 22; 91.7% of total complications), followed by metabolic alkalosis (n = 2; 8.3%); none of them had acute kidney injury (Table 1). Therefore, dehydration had a prevalence of 35% in the population studied.

Salmonella was isolated more frequently from stool culture (n = 57; 90.5%) but also from blood culture in two children (3.2%); in three it was isolated in both fecal and blood cultures (4.8%) and in one child it was isolated in both blood and urine cultures (1.6%) – Table 1. The identified *Salmonella* enterica serotypes were *Salmonella typhi* in two children (3%), *Salmonella typhimurium* in 14 children (22%) and *Salmonella enteritidis* in 24 children (38%) – Table 1. In 14 isolates (23%) it was only possible to determine the serogroup: group A in one, B in eight, C in three and D in two. In 23 cases (37%) it was only possible to identify the genus (*Salmonella* spp). Antimicrobial susceptibility testing was performed in 100% of the isolates and revealed 19% resistance rates to ampicillin, 6.4% to amoxicillin-clavulanate and to cotrimoxazole. The susceptibility rate to cefotaxime, from which susceptibility to ceftriaxone is inferred, was 100%. Regarding resistance by serotype, *Salmonella typhi* showed no resistance to any of the tested antibiotics. The highest rates of antibiotic resistance were identified in *Salmonella typhimurium*, namely to ampicillin in 14.3%. Still in relation to ampicillin, other non-typhoidal *Salmonella* had 5% resistance rates. Amoxicillin-clavulanic acid resistance rates were 5% for *Salmonella typhimurium* and less than 5% for other non-typhoidal *Salmonella*; lastly, for cotrimoxazole, resistance rates were less than 5% in both groups (Table 1).

Most of the children were treated only symptomatically (n = 55; 87.3%) and eight (12.7%) received antibiotic therapy due to the presence of risk factors or positive blood cultures, being ceftriaxone the most widely used (Table 1). The mean duration of antibiotic therapy was 9.5 days.

Out of the children whose medical follow-up was known (n = 18), 12 collected new stool samples, that turned out to be negative, on average, 3.5 months after the diagnosis (Table 1). All of them evolved to cure, with no chronic carriers identified. Only 27% of these *Salmonella* infections were notified.

DISCUSSION

In this study, salmonellosis was slightly more frequent in males, as observed in other Portuguese retrospective studies.[7,8]

Even though the majority of observed patients were Portuguese (81%), our hospital has a very high incidence of migrants, specially from African countries, some of them living with poor hygiene conditions, a known risk factor for this disease. We found no reference to birthplace in other Portuguese studies.

The highest incidence of salmonellosis was noted in children younger than five years old,[3,9] as observed in our study, in which this age group corresponded to about 50.8% of cases. The median age in our study was four years old, similar to the median age described in other Portuguese studies.[7,8]

There has been a significant decrease in the number of salmonellosis in recent years worldwide[2] and our results concur with this data. Comparing our results with a previous retrospective descriptive study carried out in our pediatric department, between January 1999 and August 2003 (Jacinto *et al*, unpublished, presented as a poster in "*IX Jornadas Nacionais de Infecciologia Pediátrica*", Évora, 8 – 11 October 2003) we saw an important reduction in the number of cases. Between 1999 and 2003 there were 82 cases per year, in contrast with our findings of 11 cases per year. The reduction in incidence was also observed in the retrospective study by Almeida *et al*,[7] with an annual average of 43.6 cases (between 2005 – 2009). In the Pignatelli *et al* study, the annual median was 21 cases of non-typhoidal *Salmonella* (between 1999 – 2008).[8] This reduction may be related to better hygiene conditions and better food and water control.

As noted in previous studies, this kind of infection predominates in warmer seasons, namely between May and October reaching a peak in July.[10] We achieved a higher number of cases in May, June and September as well, and the most frequent serotype in these months was *Salmonella enteritidis* (Fig. 2). As mentioned in other Portuguese studies[7,8,10] and in the Jacinto *et al* study mentioned previously, the most isolated serotype was *Salmonella enteritidis*, followed by *Salmonella typhimurium*. The INSA Epidemiological Bulletin (2014 – 2017) identified as the most frequent serotypes: *Salmonella* 4,5:i:-, followed by *Salmonella enteritidis* and *Salmonella typhimurium*, and reported an increase in *Salmonella enteritidis* serotype between 2014 and 2017.[11]

From the obtained data, 64.1% of children had contact with symptomatic patients, the majority in a familial context. Regarding the ingestion of potentially contaminated food, data was scarce, and it was only confirmed in two cases. In the Jacinto *et al* study (1999 – 2003), 27% of cases had a potential contaminated food intake of chicken and eggs while 27% had a familial or school-related context. In the Pignatelli *et al* study[8] it was assumed that eggs were a source of infection in 10% of patients and that 16% of cases occurred in the context of small outbreaks. In the Almeida *et al* study, the ingestion of a potentially contaminated food was considered in 45.3% of cases.[7]

Salmonellosis hospitalization was required in 58.7% of children, compared to 67.7% in the Jacinto *et al* study and

83.5% in the Almeida *et al* study.[7] The most frequent diagnosis was acute gastroenteritis (in 83.9%), as observed in 1999 – 2003 (in 90%). Dehydration was the most frequent complication, as seen in other studies.[7,8] These results may indicate that complications associated with salmonellosis infection are lower nowadays, corresponding to a decrease in the number of hospitalizations. These complications depend on serotype and patient, and vary from hemolytic-uremic syndrome to peritonitis, intestinal perforation, acute idiopathic pancreatitis, acalculous cholecystitis, acute liver failure, chronic diarrhea, irritable bowel syndrome, dyspepsia, manifestations of ulcerative colitis, Reiter's syndrome, or septicemia.

In the literature, a higher hospitalization rate has a predominance in lower age groups, who are more vulnerable to dehydration, and in whom it is often necessary to anticipate care in order to avoid complications.[7] Our study did not corroborate this data, as the median age from the hospitalized group was seven years, whereas from the non-hospitalized group was three years. This may be explained by the fact that the population from our study was older than usual for this disease.

Urinary tract involvement is rare, and it can occur by hematogenous spread or by ascension from the urethra to the upper urinary tract. It can occur in immunocompromised patients, patients with structural malformations of the urinary tract, nephrolithiasis, urinary catheter (or other foreign body), chronic kidney disease or very frequent sexual activity.[12] In one of the patients from our study, *Salmonella* was isolated in both blood and urine samples, but since there was no urinary tract disease, a hematogenous spread was assumed. *Salmonella* groups C and E are most frequently isolated in urine,[12] identification was not possible in this case. The other reported cases of non-typhoidal *Salmonella* bacteremia from our study were in children under two years of age, with similar distribution from groups A, B and D.

In high income countries, typhoidal *Salmonella* is usually associated with travelling to endemic areas. In this period, there were two cases of *Salmonella typhi* infection. They were siblings, both from Portugal (parents from Guinea-Bissau) and with complete Portuguese National Immunization schedules, and had not received the typhoid vaccine. They had no recent travels or suspected contacts. No pathogens were isolated in stool samples of their cohabitants (father not tested due to absence from the country).

According to literature, treatment of immunocompetent children is supportive since antibiotics do not shorten the illness course and may cause prolonged fecal bacterial shedding.[6,13] However, antibiotics (typically third-generation cephalosporins) are indicated for all individuals with typhoidal *Salmonella* and with non-typhoidal *Salmonella* extraintestinal disease.[3] In non-typhoidal *Salmonella* disease, antibiotics are also recommended for newborns; organ transplantation or AIDS; corticosteroids or others immunosuppressors; hemoglobinopathies, namely sickle cell disease; disorders of the reticuloendothelial system; asplenia; ongoing or recent chemotherapy; age under three months and over 12 years (except if apyrexia and clinical improvement).[3,13] We observed a high rate of antibiotic use (12.7%), justified by the severity of the clinical presentation, namely bacteriemia cases and presence of risk factors (HIV infection). These high rates were also observed in the Pignatelli *et al* study[8] (38% cases) and in the Almeida *et al* study[7] (11.5% cases).

Ampicillin was the most frequent antibiotic resistance in our study (19%), as in the Pignatelli *et al* study (27%).[8] In both studies, all strains were susceptible to third generation cephalosporins.[8]

When compared to the Jacinto *et al* study, it was evident that there was an important decrease of resistance rates to antibiotics for each serotype. As for ampicillin resistance rates, they decreased from 60% to 15% for *Salmonella typhimurium* and from 35% to 5% for other non-typhoidal *Salmonella.* For amoxicillin-clavulanic acid, it decreased from 50% to 5% for *Salmonella typhimurium* and 30% to less than 5% for other non-typhoidal *Salmonella*. Finally, resistance rates for cotrimoxazole decreased from around 10% to less than 5%, in both previously mentioned groups.

Salmonella resistance to antibiotics is increasing worldwide and represents a global public health concern, with increased treatment failure and risk of invasive disease.[13] As such, susceptibility patterns should be considered in patients treated with antibiotics.

It is presumed that the prevalence of salmonellosis is much higher than the one presented. One of the reasons is that, although notification in our country for all *Salmonella* infections is mandatory, many cases are not recognized during the initial assessment and therefore, are not notified. Moreover, not everyone who has a *Salmonella* infection seeks medical care, and healthcare providers may not obtain a specimen for laboratory diagnosis, or the clinical diagnostic laboratory may not be able to perform the necessary diagnostic tests.[14] In our study, from all the cases hospitalized (n = 37), 20 were not notified, probably due to early hospital discharge or late culture results. The ECDC Annual Epidemiological Report for 2017 mentions that in Portugal, the proportion of hospitalized cases was very high (72% – 85%), while salmonellosis notification rates were low.[9]

Despite the improvement in hygiene conditions, public sanitation and food safety, *Salmonella* infection persists in both high- and low-income countries. Better transmission control is possible with appropriate measures outside the medical field. Preventing contamination implies control at all stages of the food chain: specific measurements in primary

production, namely the control of animal feed and compliance with good hygiene practices in animal production and processing (to avoid cross-contamination); storage temperature control, so that growth is prevented; and particular attention to products that undergo reformulation, since it favors growth of *Salmonella*.[15] Also the treatment of municipal waters with chlorination and adequate public sanitation are basic and necessary measures to avoid cases; the elimination of the bacteria is possible through heat, at 54.4ºC for one hour or 60ºC for 15 minutes; regarding contact with animals, hand washing is imperative immediately after the collection of animal feces and/or use of gloves.[16] In the medical field, typhoid vaccine is an important way of preventing disease, despite the licensed vaccines not providing complete protection.[6] The Portuguese Society of Travel Medicine recommends vaccination of travelers over two years of age, who travel to endemic areas and whose stay lasts for more than one month, or whose travel, even if lasting less than one month, may confer an increased risk of contracting the disease.[17] There are currently no vaccines for non-typhoidal neither paratyphoid *Salmonella*.[3]

The main limitations of the study were the small sample size and the retrospective analysis. The consultation of clinical records was totally dependent on the notes made, with the omission, in some cases, of important data for epidemiological and clinical characterization of salmonellosis.

The authors consider that it would be important to carry out a prospective national study, in order to ascertain the real incidence of salmonellosis in Portugal, as well as the most frequent strains and antibiotic resistance. The notification rate, although mandatory, remains below expectations. Another area that needs further investigation is the indications for treatment of non-typhoidal *Salmonella*, namely the most effective antibiotic and optimal duration, through randomized controlled trials, in order to standardize recommendations and reduce resistance to antibiotics.

CONCLUSION

The present study obtained recent data regarding the epidemiology, microbiology (identifying serotypes and respective antibiotic resistance rates) and clinical presentation (symptomatology, risk factors, complications, hospitalization rates, treatment performed and follow-up after discharge) of pediatric salmonellosis cases in a Portuguese level II Hospital, between 2015 and 2020.

With these results we intend to improve knowledge on salmonellosis and its prevention strategies, treatment and notification in Portugal. Although the incidence of salmonellosis has been decreasing in recent years, it is the second most frequent gastrointestinal infection and an important cause of foodborne outbreaks in the European Union,[9] contributing to a high rate of hospitalizations and use of antibiotics in Portugal.

PREVIOUS PRESENTATIONS

This study was partially presented as a poster at European Society for Paediatric Infectious Diseases (ESPID) 2021, which took place online between the 24th and 29th of May.

AUTHOR CONTRIBUTIONS

IFM, SC, RVC: Design of the data collection instruments; data collection and analysis; draft of the manuscript; revision and approval of the manuscript.

SJ, PC, MJB, AF: Conceptualization of the study; revision and approval of the manuscript.

SS: Revision and approval of the manuscript.

PROTECTION OF HUMANS AND ANIMALS

The authors declare that the procedures were followed according to the regulations established by the Clinical Research and Ethics Committee and to the Helsinki Declaration of the World Medical Association updated in 2013.

DATA CONFIDENTIALITY

The authors declare having followed the protocols in use at their working center regarding patients' data publication.

COMPETING INTERESTS

The authors have declared that no competing interests exist.

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