

Selected enteropathogens and clinical course in children hospitalized with severe acute gastroenteritis in Barbados

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Abstract:

Objectives: The primary aim of this study was to determine the prevalence of selected bacterial and viral enteropathogens in children hospitalized with acute gastroenteritis and the secondary aim was to characterize the clinical course and the outcome.

Methodology: A retrospective audit of children (<15 years) admitted with acute gastroenteritis during January 2008 to October 2010. Stool samples were analyzed for bacterial pathogens and for the Rotavirus. Demographics, clinical presentations, hospital course and outcome were extracted from the admission records.

Results: There were 571 children hospitalized with acute gastroenteritis, which accounted for 11% of all medical hospitalization in children. Overall, 42.9% of these children were ≤ 12 months in age. Stool test result was documented in 46.6% of children hospitalized with gastroenteritis and an enteropathogen was isolated in 36.8% of cases with documented stool test result. Non-typhoidal Salmonella species was the most commonly isolated enteropathogen accounting for 21.1% of all the documented cases. Rotavirus was identified as an etiological agent in 9.0%. Of the 56 children who had non-typhoidal salmonella gastroenteritis, 54(96.4%) were younger than 5 years. The median duration of hospitalization was 2 days (Range 1 day to 9 days). There were no deaths.

Conclusion: Non-typhoidal salmonella was the most common enteropathogen isolated and this was followed by the Rotavirus.

Key words: Acute gastroenteritis, Etiology, Children, Barbados, Caribbean

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Introduction

Acute gastroenteritis (AGE) constitutes one of the major causes of child morbidity and mortality and is an important public health issue in both the developed and the developing countries. Children ⁽¹⁾ less than five years of age have 3.3 diarrheic episodes per year, and more than one-third of the deaths in this age group are associated with diarrhea. ⁽²⁾ Worldwide, every year there are approximately 3-5 billion cases of acute gastroenteritis and 2 million deaths in children less than five years of age (most from 6 months to 12 years) are caused by this disease. ⁽¹⁻³⁾ In the United States, gastroenteritis accounts for about 10% (220,000) of admissions to hospital, more than 1.5 million outpatient visits, and around 300 deaths in children under 5 annually. ⁽⁴⁾ The cost of gastroenteritis to the community is huge but often underestimated if costs to the family, including lost time at work, are not considered. The main etiology of the acute gastroenteritis is related to a wide range of viruses (adenovirus, astrovirus, calcivirus, enterovirus, and rotavirus), bacteria (such as *Campylobacter jejuni*, *Escherichia coli*, *salmonella spp.*, *Vibrio cholerae*, *Yersinia enterocolitica*, and *Aeromonas spp.*) and enteroparasites such as *Giardia spp.*, *Cryptosporidium spp.*, and *Entamoeba histolytica*. ⁽⁵⁻⁶⁾ The prevalence and the relative importance of the various bacteria and viruses in the etiology of acute diarrheal disease varies depending on the geographic location and the economic status of the region.

In the English speaking Caribbean, the most recent available data from Trinidad and Tobago indicated that in 1999 the mortality rate from intestinal infectious diseases among children less than 14 years old was 2.8 per 100 000 children under 14 years, while the crude death rate from all causes for all age groups was 800 per 100,000. ⁽⁷⁾ In Barbados, acute diarrheal disease accounts for 9 to 13.5% of all pediatric admissions to the hospital. ⁽⁸⁾ The only reported study on childhood diarrhea from the English Caribbean is out of the island of Trinidad. In this hospital-based study rotavirus was implicated as the most important etiologic agent for gastroenteritis. ⁽⁹⁾ In a recent study from Trinidad which analyzed stool samples for bacterial pathogen concluded that the *Shigella sonnei* was the most prevalent, and has the highest probability of being an important etiological agent of childhood diarrhea in Trinidad. ⁽⁷⁾ In

many hospitals in developing countries lacking clinical microbiology laboratories, the number of studies on the cause of diarrhea in children is limited.

Our study was conducted with the following two specific objectives: (1) To determine the prevalence of selected enteropathogens (*E. coli*, *Salmonella spp.*, *Shigella spp.*, *Campylobacter spp.*, *Yersinia spp.*, *Staphylococcus aureus* and Rotavirus) in children hospitalized with acute gastroenteritis in Barbados and (2) to characterize the epidemiology and clinical course and outcome among children with severe acute diarrheal diseases requiring hospitalization.

Methods

This report is based on a retrospective audit of children less than 15 years of age with acute gastroenteritis admitted to the Queen Elizabeth Hospital (QEH). The QEH is the only tertiary hospital in Barbados and provides over 95% of the inpatient care for children in this country. Children with acute gastroenteritis who are seen at the primary health care centers or in the private offices of the general practitioners or the private offices of the pediatricians across the island are referred to the Pediatric ward at the QEH for admission. Children may also be seen directly at the emergency department of the QEH and if they are diagnosed to have severe AGE then they too are referred to the Pediatric ward for admission.

Study population & Stool Samples

The observation period for this study extended from January 2008 through October 2010. All children (<15 years) admitted to the QEH and where one of the discharge diagnosis was acute gastroenteritis were included in this study. All nosocomial gastroenteritis and gastroenteritis cases which were known to be non-infectious were excluded. Children who had a history of the symptoms of gastroenteritis such as frequent loose stool for more than 2 weeks were excluded from this study. Children who were known to be diagnosed with any immunodeficiency state were also excluded from the study.

Children admitted for AGE routinely have blood counts, random blood glucose and serum electrolytes done. Stool culture, barring practical limitations associated with stool

sample collection in children, is also a routine practice. The most common reason for failure to collect a fecal specimen was the discharge of the patient within 24 hours of admission. Blood culture and urine cultures are done in children presenting with temperature $>38^{\circ}\text{C}$ and or when there is Leukocytosis (Total WBC count $>12,000$). Fecal specimens were transported the same day to hospital laboratories of the departments of Bacteriology, where they were stored at 4°C until processing. Specimens for bacteriological culture were inoculated into appropriate media on the day of collection. All of the children with severe AGE get intravenous rehydration initially and are then switched to oral hydration as soon as the child's dehydration is corrected (usually over 6 to 8 hours) and the child is able to drink sufficiently to compensate for the ongoing losses. Antibiotics are rarely used if ever in immunocompetent children with AGE.

Isolation and Identification of Bacterial Pathogens

Stool samples from patients with AGE are tested at a centralized public health laboratory. Culturing on differential, selective and enrichment medium was performed for isolation of *E. coli*, *Salmonella* species, *Shigella* species and *Campylobacter* spp. and their subsequent identification was performed by biochemical methods.

For the isolation of *Salmonella* and *Shigella* species and other gram negative enteric bacilli, stool sample was directly plated on Xylose-Lysine Desoxycholate (XLD) agar and McConkey agar and incubated at 37°C for 24 hours. Preliminary identification were based on colony appearance on selective agar media and confirmed using biochemical and serological testing. Biochemical tests included Oxidase test and sugar fermenting tests. For the isolation of *Campylobacter* species stool samples were enriched in Bolton broth and then streaked onto modified cefoperazone charcoal deoxycholate agar (mCCDA) (*Campylobacter* blood free agar base; Oxoid Ltd., Basingstoke, United Kingdom) supplemented with CCDA selective supplement (Oxoid Ltd.) and incubated at 42°C for 48 hours in a microaerophilic atmosphere generated using the CampyGen gas generating system (Oxoid Ltd.). Isolates were Gram stained and subjected to the oxidase test.

Detection of Rota virus

For the qualitative detection of the Rotavirus antigen, the Xpect™ Rotavirus test a rapid, membrane-based, immunogold assay for the qualitative detection of rotavirus antigens in human faecal specimens was used. An extract is first prepared by suspension of the specimen in the provided extraction buffer solution. The buffer containing the extracted specimen is then added to the device sample well. The reaction between a positive sample and the coloured particle-conjugated antibody will form a complex that migrates along the membrane. An immobilized capture antibody will form a coloured line at the S (specimen) area upon reacting with the coloured complex. An internal control line C (control) area is built-in to ensure that the test has been performed correctly.

Data collection and analysis

Patient demography, date of admission, other additional diagnoses at the time of discharge, outcome and the date of discharge or death were recorded from the ward admission register. Individual case records were reviewed for all the cases of AGE admitted at QEH. Record of stool culture done on these cases and the results, course of hospitalization, any complications, any pre-existing co-morbid conditions and the duration of hospitalization were extracted using a standardized data collection sheet. Data on the total number of admissions to the pediatric ward over the study period was also collected from the ward admission register.

Data extracted from the admission register and the case notes were entered into a specially designed Microsoft Access data base and analyzed using the Microsoft Excel program. A 95% confidence interval was calculated for all proportional data and a Chi-square was used to detect significance in variation. A P value of <0.05 was considered significant. Since the study was simply a clinical audit carried out by the clinician who was the direct care provider for all the children audited and did not involve any direct or indirect intervention of any kind, no ethical approval was sought.

Results

Study population and the stool samples

Over the 34 month period from January 2008 through October 2010, there were a total of 571

cases of AGE admitted to the QEH. This accounted for 11% of all medical hospitalization among the children in Barbados. From among the 571 children with AGE admitted to the QEH, stool culture results were recorded for 266 (46.6%) cases. The age and gender distribution of the all the children with AGE admitted to the QEH and those that had a documented stool investigation results are shown in Table 1. Overall 245 (42.9%, 95% Confidence Interval – 38.8%, 47.1%) children were 12 months or younger and 370 (64.8%, 95% CI – 60.8%, 68.8%) children were 24 months or younger at the time of admission for the AGE. Female to male ratio was 1:1.4. The overall annual incidence rate of severe AGE requiring hospitalization was 4.1 (95% CI – 3.6, 4.7) per 1000 under 15 children per year. The annual incidence rate for severe AGE for children under 5 years was 26.8 (95% CI – 24.4, 29.4) per 1000 under 5 children. An analysis of the seasonality in the occurrence of severe form AGE in children requiring hospitalization revealed multiple peaks (Figure 1). Cases of AGE peaked during the month of June with two other smaller peaks in the months of February and October. October November is the hot and wet season in this country.

Isolation and Identification of Pathogens

Of the 266 children with documented stool test results, stool tests were positive for an enteropathogen in 98(36.8%, 95 Confidence Interval – 31%, 43%). Among the 218 children <5 years who had documented stool test result, 92 (42.2%, 95%CI – 35.6%, 49.1%) children had a positive test for an enteropathogen. Among the 48 children 5 years and older, 8 (16.7%, 95 CI – 8%, 30%) had stool tests positive for an enteropathogen. Non-typhoidal Salmonella species was the most commonly isolated enteropathogen accounting for 21.1% (95% CI – 16.4%, 26.5%) of all the cases where a stool test result was documented (Table 2). Rotavirus was identified as an etiological agent in 24 cases (9.0%, 95% CI – 6.0%, 13.3%). Of the 56 children who had non-typhoidal salmonella gastroenteritis, 54(96.4%) were younger than 5 years. Figure 2 shows the age distribution of salmonella and rotavirus enteritis in children less than 5 years. Non-typhoidal salmonella species peaked in the first year of life while Rotavirus enteritis peaked in the second year of life (Figure 2). Of the 34

staphylococcal isolates, 30 were isolated from the samples where salmonella species were also isolated and 2 were isolated from samples where campylobacter was isolated.

Clinical course

Clinical presentations and the hospital course of the children admitted with AGE is shown in Table 3. Children presented to the hospital for admission on the second or third day of the symptoms. The majority of the children with AGE from Salmonella species presented with bloody stool -31 cases (55.4%), vomiting -37 cases (66.1%) and fever of >38°C-39 cases (69.6%). Among children with Rota virus AGE, 18 (75%) vomiting, 4 (16.7%) had bloody stool and 5(20.8%) had fever. The median duration of hospitalization for the 571 cases of AGE admitted during the observation period was 2.8 days (Range 1 day to 13 days). The median duration of stay for the non-typhoidal salmonella AGE was 4.3 days (Range 2 to 13 days) where for the Rotavirus AGE was 2.2 days (Range 1 to 4 days). The median duration of stay for the 8 cases of Campylobacter jejuni was 4.5 days (Range 3 to 9 days). Of note, among the 56 children with confirmed non-typhoidal salmonella 39 (66.9%; 95% CI 55, 80) had fever (>38° C) and 42 (75%; 95% CI 61, 85) had leukocytosis (>12,000/cubic ml). Two children with non-typhoidal Salmonella had associated bacteremia, they had both had associated fever and leukocytosis. Both of them recovered after a course of intravenous antibiotics. There were 3 children who required brief Pediatric Intensive care for less than 48 hours for closer monitoring and treatment of sepsis (2 cases) and electrolyte imbalance (1 case). There were no deaths noted among the 571 children admitted with the AGE during the study period.

Discussion

In this preliminary report, the first such report of community-acquired infectious AGE in children necessitating hospitalization from the English Caribbean, we describe the frequency of selected etiological enteropathogens and their epidemiological and clinical characteristics. Barbados, one of the English speaking Caribbean countries has a total population of 250, 010 (2011) including 54, 599 (22%) under 15 children and a population density of 581/sq km. Its Gross National Income

per capita is US\$ 18, 240 (2009) and its total expenditure on health as a percentage of GDP stands at 6.8% (2009). The Infant Mortality Rate (2005) is 11 per 1000 live births and the life expectancy at birth m/f (2009) is 73/80 years. It has a well-organized state run health care infrastructure with free health care for its citizens at the point of delivery. There are 8 polyclinics which serve as the primary health care delivery points and a single tertiary health care institution which is the only institution with facility for inpatient care for children. Of note, rotavirus vaccination is not a routine practice in Barbados and very few children get immunized for rotavirus AGE (personal communications).

AGE accounted for 11% all medical admissions in children and it was the third most common cause for hospitalization among children under 15 years. A previous study from this country in 1999, had made a similar observation.⁽⁸⁾ The majority (80%) of the children admitted with AGE was under 5 years (Table 1). It is well established that the AGE affects younger children much more commonly than the older children.⁽¹⁰⁻¹²⁾ Male preponderance in AGE as noted in this study has been reported in numerous published studies.⁽¹⁰⁻¹⁴⁾ An interesting epidemiological finding from this study was that three different peaks were observed during the year in the months of February, June and September and three troughs in the months of March, July and December. The troughs coincided more or less with the times when schools are closed and many day care nurseries are also likely to be closed. It would be interesting to study all the cases of AGE in this country to see if it follows a similar pattern of peaks and troughs in its occurrence rate.

Non-typhoidal salmonella species were the most common (21%) pathogen isolated from the cases of severe AGE admitted to the hospital followed by the Rotavirus (11%). This is in contrast to our knowledge. There is no published report of enteropathogen studies of severe AGE among children that required hospitalization from the English Caribbean. There are numerous population based studies of etiology and disease burden of infectious AGE from the region including Barbados.^(7, 9, 15, 16) One study from the Trinidad and Tobago in 1982 reported Rotavirus as the most common enteropathogen for AGE in children.⁽⁹⁾ Subsequently another study from the same

country reported shigella as the commonest bacterial enteropathogen for the childhood AGE.⁽⁷⁾ Of zoonotic importance in Trinidad was the reported association between human salmonellosis and isolation of salmonella in livestock and in pet dogs.^(15, 16) There has been a phenomenal rise in the number of *Salmonella enteric* serovar enteritidis (*S. enteritidis*) strains isolated from various sources (humans, foods, and animals) in Trinidad and elsewhere in the Caribbean from 1992 to date (Caribbean Epidemiology Center, personal communication).

Worldwide, Rotavirus is reported to be the most common cause of community acquired severe infectious AGE.⁽¹⁹⁻²³⁾ This report clearly raises the question about the preponderance of Rotavirus as an enteropathogen in severe form of infectious AGE in the Caribbean. The findings in the study are supported by the findings from a recently published population based study by of the AGE in Barbados.⁽¹⁵⁾ In this report, which was part of multi-Caribbean country study carried out by CAREC (the Caribbean Epidemiology Center, based in Port-of-Spain in the republic of Trinidad and Tobago), non-typhoidal Salmonella species was the commonest isolate, although the study projected that Norovirus may be the most common cause of AGE in Barbados based on the under testing for Norovirus. In a recent study from Morocco, Norovirus was reported to be the eteopathogens in nearly a sixth of all cases of AGE in children.⁽²⁴⁾ This study failed to demonstrate that Rotavirus was the most common cause of AGE in children younger than 5 years. It is noteworthy, that Rotavirus vaccine is not a part of the government's expanded immunization program in any of the English speaking Caribbean countries including Barbados.⁽²⁵⁾ Over 85% of children in Barbados receive their immunizations through the state run primary health care centers under the Government's expanded immunization program free of cost.⁽²⁶⁾ A very small percentage of children (<15%) receive their immunization receive their immunization at the private care settings and a smaller proportion of these children may have had Rotavirus immunization.

The course of severe AGE among children in this population was relatively short (median duration of hospitalization was 2.8 days) and largely uncomplicated. Among the notable complications were the two cases of bacteremia

in children with Salmonella AGE. Both these children recovered after a course of intravenous antibiotics. Cases of bacteremia in children with non-typhoidal Salmonella AGE have been reported in literature. ⁽²⁷⁾ Notably, among children hospitalized with non-typhoidal salmonella 70% (39/56, P=0.002) had temperature >38° C and 75% (42/56, P=0.014) had Leukocytosis (Total WBC Counts

>12000/cubic ml). Also, of note 16.7% (4/24) of children hospitalized with Rotavirus AGE had blood in their stool.

The major limitations of this report includes short observation period, low positivity rate of stool samples for an etiological agent and low rate of stool testing in the cases of severe AGE in children. Non-availability of sufficient clinical information on the cases was another limitation.

Table1. Basic Demographic profile for children hospitalized with AGE.

Age Group	All AGE (n = 571)	AGE with documented stool results (n = 266)
0<5yrs	443 (77.5%)	218 (81.9%)
5-<10yrs	84 (15%)	29 (10.9%)
10-<15yrs	30 (5.4%)	11 (4.1%)
Missing data	14 (2.5%)	8 (3.0%)
Gender		
Female	238 (41.6%)	104 (39.1%)
Male	326 (57.0%)	156 (58.6%)
Missing data	7 (1.4%)	6 (2.3%)

Table 2. Etiopathogens of severe AGE that required hospitalization among 266 children in Barbados.

Etiology	<5 Years	5-15 Years	Numbers positive
Salmonella	54 (22%)	2 (6%)	56 (21.1%)
Rotavirus	24 (9.6%)	0(0%)	24 (9.0%)
Campylobacter	12 (5.6%)	4(12%)	16 (6.0%)
Staphylococcus	32(13.2%)	2(6%)	34 (12.8%)
None	144 (54.1%)	24 (75%)	168 (52.1%)

Table 3. Presentation and hospital course characterization of severe AGE among children based on etiopathogens.

Characteristics	Salmonella species (n = 56)	Rotavirus (n = 24)	No isolate (n = 168)
Duration of illness at the time of hospitalization	2.6 Days	1.4 Days	1.8 Days
Bloody stool	31 (55.4%)	4 (16.7%)	5 (2.9%)
Vomiting	37 (66.1%)	18 (75%)	56 (33.3%)
Fever (Temperature >38°C)	39 (69.6%)	5 (20.8%)	72 (42.8%)
Respiratory symptoms	3 (5.4%)	2 (8.3)	88 (52.4%)
Leukocytosis (>12000 ml ³)	42 (75%)	3 (12.5%)	55 (32.8%)
Hyponatremia	11 (19.6%)	3 (12.5%)	18(10.1%)
Hypernatremia	2 (3.6%)	0 (0%)	5 (3.0%)
Hypokalemia	3 (5.4%)	1 (4.1%)	5 (3.0%)
Complications	12 (21.4)	4 (16.7%)	15 (9.0%)
Duration of hospitalization	4.3 Days	2.2 Days	2.4 Days

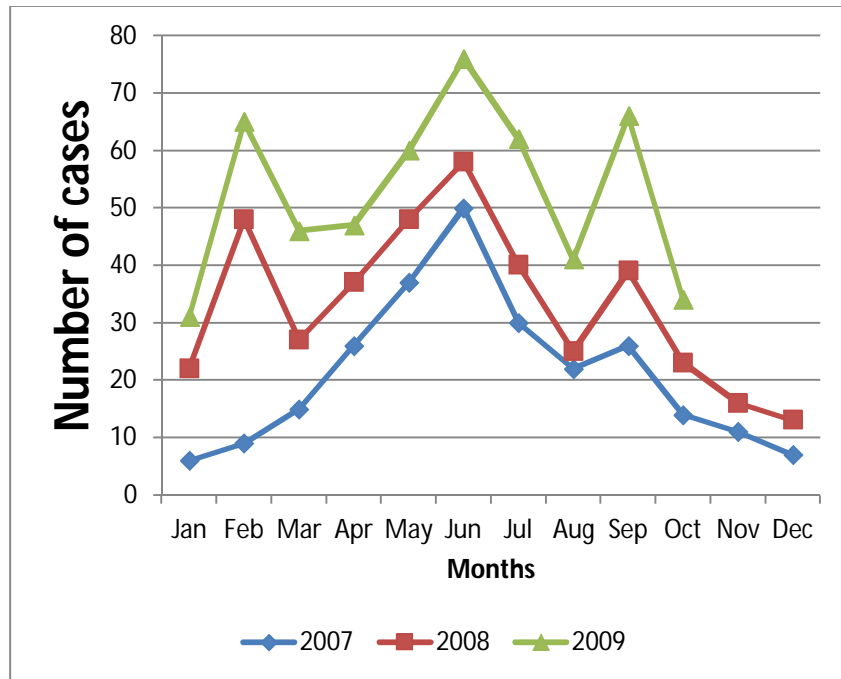


Figure 1. Seasonal distribution of all the cases of childhood AGE hospitalized in Barbados.

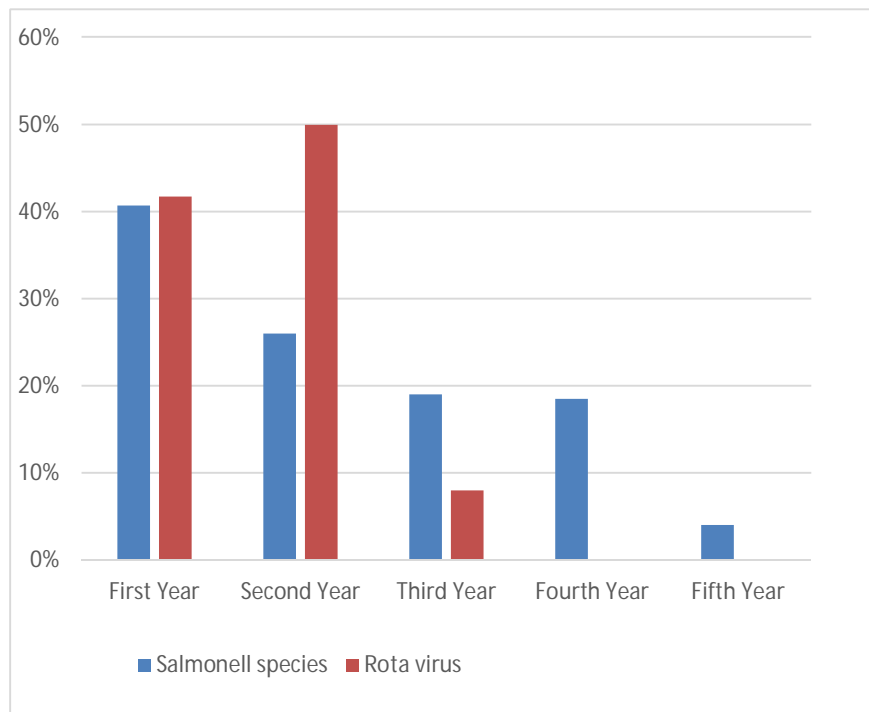


Figure 2. Age distribution of cases of salmonella and rotavirus acute gastroenteritis among children <5 years in Barbados.

Conclusion

This preliminary report demonstrates that the non-typhoidal *Salmonella* species is the most common cause of severe forms of community acquired AGE necessitating hospitalization among children in this country. Severe non-typhoidal acute gastroenteritis in children often presents with high fever and leukocytosis. Rotavirus was isolated in just over a tenth of all severe cases of AGE in children requiring hospitalization and where a documented stool test result was available. There is a need of a more comprehensive, well designed study of the etiology of AGE in this country and in the wider Caribbean as this may have implications for the need for Rotavirus vaccination of children.

Acknowledgment

The authors are thankful to Mr. Husain Patel of the Government Public Health Laboratory at Sir Winston Scott Polyclinic for his assistance in this study.

Conflict of Interest Declarations

Funding: None

Conflicts of interest: None declared.

Ethical approval

Ethical approval was sought from the Institutional Ethics Committee at the Queen Elizabeth Hospital, Barbados. However, ethical approval was deemed not necessary as the study was an audit of case records by the authors providing medical care to these children at the hospital.

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