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Isolation and identification of bacterial causes of diarrhea in children less than 2 years old in Al-Muthanna Governorate

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Abstract

Diarrhea is the second leading cause of children's death under five years of age worldwide. The most common microbiological causes of gastroenteritis vary by age group, geographic region, and type of diarrhea. This study designed to identify the bacterial causes of children's diarrhea under two years of age in Samawah /Al-Muthanna Governorate. Out of 84 patients, 21 sterile stool swabs were collected from children suffering from diarrhea in the Maternity and Children Hospital in Samawah for the period from 1 to 31 December 2021. Samples were cultured on selective diagnostic culture media. Antibiotic sensitivity test was performed for bacterial isolates using discs diffusion test. Out of 84 total hospitalized child, there were 61 (72.61%) children with diarrhoea. Whereas, the percentages of other diseases were 2% (2.38), 16 (19.04), 3 (3.57%), 1% (1.19%), 1 (1.19%) and 1% (1.19%) for jaundice, bronchitis, dyspnea and asthma, suckling interruption, hypoglycemia and pneumonia, respectively. According to age, the highest percentage of diarrhea 24(39.344%) was recorded in children less than one month. Moreover, the number and percentages of diarrhea at ages 2, 3, 4, 6, 5 7, 8, 9, 10, 11, 12 and 24 months were as follows: 5 (8.196%), 3 (4.918%), 4 (6.557%), 4 (6.557%), 2 (3.278%), 4 (6.557 %), 0.%, 3 (4.918%), 3 (4.918 %), 0%, 6 (9.83%) and 3 (4.918%) respectively. The total percentage of bacterial isolation was 100%, that comprised 38.10%, 14.29%, 4.76 %, 9.52 %, 23.81%, and 9.52% for *E. coli*, *Salmonella typhi*, *Salmonella typhimurium*, *Klebsiella*, *Shigella sp.*, and *Proteous sp.* respectively. In conclusion, this study approved that multiple-drug resistant, gram-negative enterobacteria were the causes of diarrhea in children under-age of two years.

Keywords: Multiple-drug resistant, Children, Diarrhea, *E. coli*, Iraq, *Shigella*

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Introduction

Diarrheal diseases are the primary cause of death in children under five years of age worldwide, with estimated deaths reaching 2.5 million yearly [1]. The most important clinical signs of diarrhoea are acute watery stool that begins acutely and lasts for less than 7 or 14 days. It also involves the passage of frequent loose or watery stool with or without visible blood. Vomiting and fever are presented in most cases.

Dehydration is the crucial complication of acute watery diarrhoea that may lead to blood and tissue acidosis and death. *Rotavirus*, *enterotoxigenic Escherichia coli*, *Shigella*, *Campylobacter jejuni*, *Cryptosporidia*, *Vibrio cholerae*, and *Salmonella* are the important causes of acute watery diarrhoea in children [2]. *Shigella*, *Campylobacter jejuni*, *Salmonella*, and very infrequently *Entamoeba histolytica* cause dysentery, which is another form of diarrhoea accompanied by visible blood in stool, anorexia, and rapid weight loss due to damage to the intestinal mucosa by invasive bacteria [3].

World widely, diarrhoea is the most critical health problem in the paediatric clinic and is the first illness in children under 5 years of age, especially in developing countries and accounts for approximately 63% of the global burden [4]. Environmental factors and changes in the season are predisposed to the distribution of diarrhoea and contribute to the multiplication and survival of causative agents and its mode of transmission [5]. However, bottle feeding for infants (aged 1-6 months) is closely associated with malnutrition, lowering immunity, and lead to diarrhoea [6]. Nonetheless, the rates of diarrhoea in fully breastfed children are lowest than in partially breastfed and highest in fully weaned children [7].

Antimicrobial agents treat most diarrheal cases to prevent disease progression and reduce complications. The public health implication of diarrhoeal disease cannot be overemphasized, and epidemiological studies of diarrhoea have been reported from Iraq [8]. The cases of diarrhoea in children less than 5 years old were reported in Ibn Al-Atheer Children's Hospital in Mosul/ Iraq, and 105 isolates of *E. coli* were diagnosed in hospitalized children suffering from diarrhoea. Moreover, bacterial sensitivity to antibiotics was also studied.

The study showed a high incidence of male infection, 51.61%, compared to females, 33.06%. The study also showed high resistance of *Escherichia coli* to many antibiotics such as Trimethoprim, Chloramphenicol, Amikacin, Sulfonamide, and Carbenicillin (Amera Ali Ahmed., 2009). In Al-Muthanna Governorate / Al-Samawa city/ Iraq seasonal epidemics of acute diarrhoea are usually reported during different seasons, especially winter and summer. Many cases were diagnosed in Al Hussien hospital and private clinics. A literature review revealed scarce published articles regarding diarrhoea and its causes in Al-Muthanna Governorate. Consequently, this study intends to detect the bacterial causes of diarrhoea in infants and children under two years of age at the Maternity and Children Hospital in

Samawah / Al Muthanna Governorate. Moreover, to diagnose and classify the isolates using routine microbiological methods and to determine their sensitivity or resistance to antibiotics.

Materials and Methods

Twenty-one diarrhea stool samples were collected from patients aged 0 to 5 years old attending Maternity and Children Hospital / Samawah/ Al-Muthanna Governorate from 1 to 31 December 2021 out of 84 hospitalized children during the research period. Daily visits were made to the children's wards to follow up on the sick cases with diarrhea. The diarrheal stool samples were collected into sterile fecal swabs with transfer media. The patient's details were labeled correctly on the universal swabs, including name, age, and sex.

This study was approved by the Research Ethical Committee / Al Muthanna university. All samples were immediately sent to the microbiology laboratory in a cool box. The specimens were processed according to the guidelines described previously [9] for the laboratory diagnosis of enteric pathogens. All fecal samples were inoculated on sterile selective & diagnostic media (nutrient broth, blood agar, MacConkey agar, Eosin Methylene Blue (EMB), Salmonella-shigella agar) and incubated aerobically at 37 °C for 24 hours.

Consequently, the isolated pure bacterial cultures were subjected to various morphological and routine biochemical tests. The bacterial isolates were identified using Bergey's Manual of Systematic bacteriology. The following tests were done on bacterial isolates including Gram stain, Motility, Oxidase, Urease, Indole test, Methyl red test, Voges Proskauer test, Catalase test, Coagulase test, Simmons Citrate Agar, Triple Sugar Iron, Fermentation of glucose, lactose and sucrose.

The antimicrobial sensitivity test was done for bacterial isolates using commercial antimicrobial discs according to the disc diffusion method described previously [10]. The following different concentrations of antibiotic discs were used in this study:

- TE -10 µg: Tetracycline
- AMC-30 µg: Amoxicillin/ Clavulanic
- LEV-5 µg: Levofloxacin
- GN-10 µg Gentamicin,
- CAZ- 30 µg: ceftazidime
- SXT-25 µg: Trimethoprim/ Sulfamethhazol

All isolates were cultured on Muller Hinton broth for 4-6 hours. Then the turbidity of the bacterial suspensions was compared with 0.5 Macfarland's barium sulfate standard solution. The standardized bacterial suspension was streaked on Muller Hinton. Agar using a sterile cotton swab and left to dry for 5-10 minutes before placing the antimicrobial discs. Later, the mentioned antimicrobial discs were placed on the surface agar and incubated aerobically at 37 °C for 24 hours. The diameters of the inhibition zones around each disc were recorded using the caliber, and the results were compared with the standard tables (CLSI, 2017).

Results

During the study period, there were 61 children with diarrhea out of a total 84 hospitalized children with a percentage of (72.61%). While the numbers and percentages of other pathological cases were 2 (2.38), 16 (19.04%), 3 (3.57), 1 (1.19), 1 (1.19) and 1 (1.19) for jaundice, Bronchitis, dyspnoea, suckling interruption, hypoglycaemia and pneumonia, respectively (Table.1).

Table 1.

Shows the number and percentages of children with diarrhoea out of the total number of hospitalized children during the study period

Case diagnosis	Number	Percentage%
Fever, Vomiting, enteritis, Diarrhea & dehydration	61	72.619
Jaundice	2	2.38
Bronchitis	16	19.04
Respiratory Dyspnea	3	3.57
suckling interruption	1	1.19
Hypoglycemia	1	1.19
Pneumonia	1	1.19
Total	84	

The results of this study showed a higher percentage of females 34 (55.73%) with diarrhoea than males, 27(44.26%) (Figure. 1).

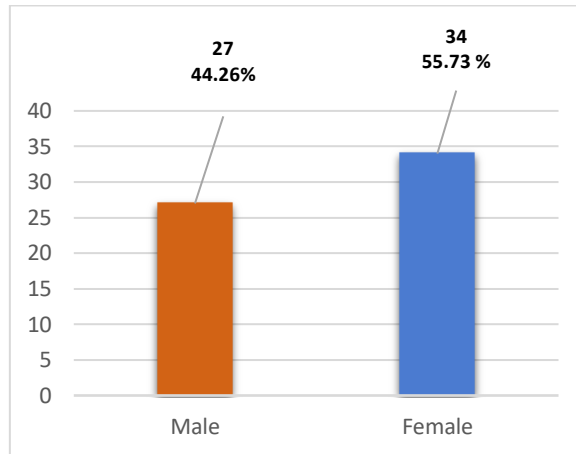


Figure 1.

Shows the number and percentages of female and male children with diarrhoea.

This study also showed a variation in the number of children with diarrhoea according to age. The highest incidence was recorded at the age one month or less (24 (39.344%), while the numbers and percentages of diarrhoea at 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12 and 24 months were 5 (8.196%), 3 (4.918%), 4 (6.557%), 4 (6.557%), 4 (6.557%), 2 (3.278%), 4 (6.557%), 0(0%), 3 (4.918%), 3 (4.918%), 3 (4.918%), 0 (0 %), 6 (9.83%) and 3 (4.918%) respectively (Figure.2).

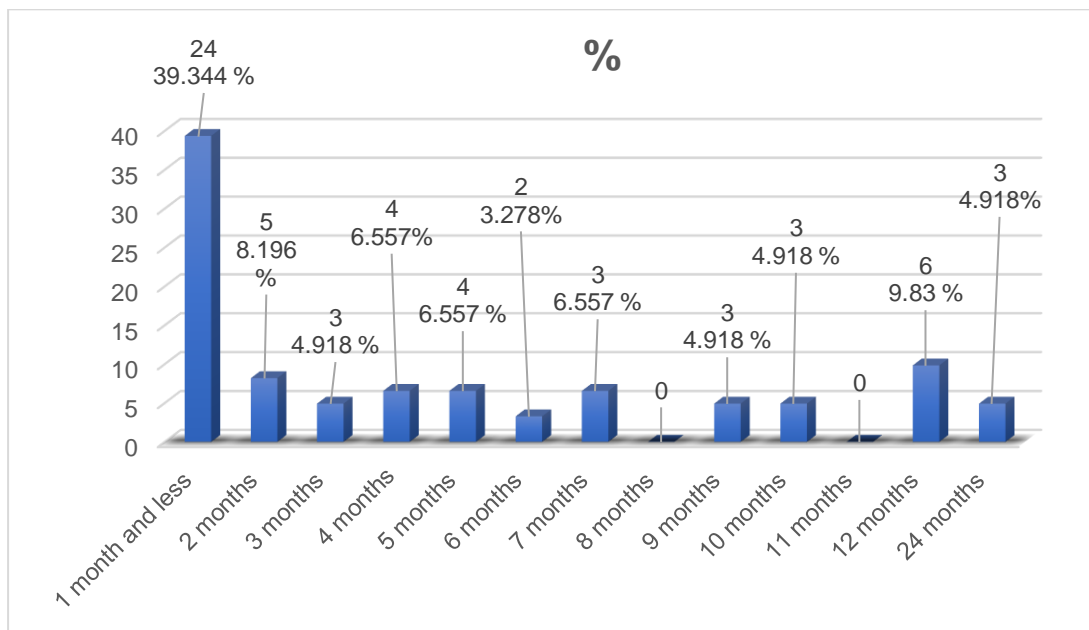


Figure 2.

Shows the number of diarrhoea in children according to the age of patients

Out of 21 cultured faecal samples, 21 bacterial isolates were investigated with 100% of isolation. According to the results of the routine biochemical tests, *E. coli* were reported the highest percentage 8 (38.10%) of bacterial isolates, while the number of other bacterial isolates were: 5 (23.81%), 3 (14.29%), 1 (4.76%), 2 (9.52) %, and 2 (9.52) for *Salmonella typhi*, *Salmonella typhimurium*, *Klebsiella sp*, *Shigella sp.* and *Proteus sp.* respectively (Figure. 3& 4).

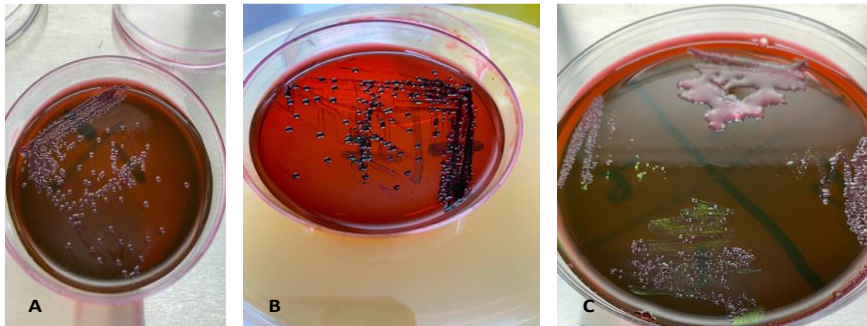


Figure 3.

Shows the different colonies of isolated bacteria

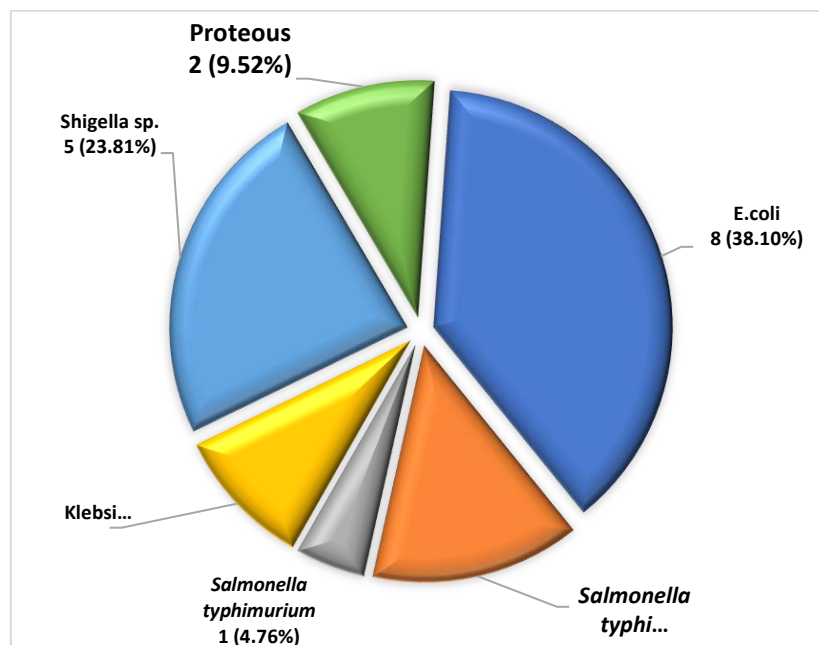


Figure 4.

Shows the number and percentages occurrence of isolated bacteria from stool samples of children with diarrhoea

The results of antibiotic sensitivity test showed multiple-drug resistance isolates to various antimicrobial discs including: Tetracycline (TE -10 µg), Amoxicillin/ Clavulanic (AMC-30 µg), Levofloxacin (LEV-5 µg), Gentamicin (GN-10 µg), ceftazidime (CAZ- 30 µg) and Trimethoprim/ Sulfamethazol (SXT-25 µg). The percentages of resistant and sensitive bacteria were 19 (90.477 %) and 2 (9.523 %), respectively (Figure. 5. & Table. 2).

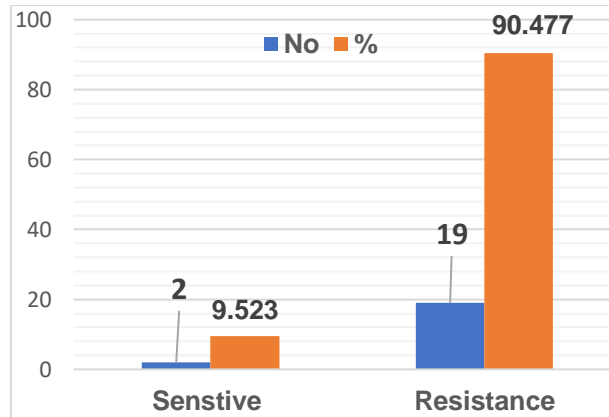


Figure 5.

Shows the number and percentages of sensitive and resistance bacterial isolates

Table 2.

In vitro susceptibility patterns of the bacterial isolates from diarrhoea stool samples.

Bacterial isolates	CA- 30 µg	SXT-25 µg	CN-10 µg	LEV-5 µg	TE -10 µg	AMC-30 µg	Interpretation
Porteous vulgaris	0	0	0.08	0	0	0	Resistance
Shigella sp.	0	0	0	0	0	0	Resistance
E. coli	0	0	0.01	0.03	0	0	Resistance
Salmonella typhimurium	0	0	0.13	0.19	0.1 2	0	Resistance
E. coli	0	0	0.04	0.17	0	0	Resistance
Shigella sp.	0	0.15	0.1	0.14	0.1 4	0.24	Sensitive
E. coli	0	0	0	0	0	0	Resistance
E. coli	0	0	0	0	0	0	Resistance
Salmonella typhi	0.18	0	0.03	0.17	0.0 8	0.1	Resistance
Klebsiella sp	0.04	0	0.03	0.19	0.0 8	0	Resistance
Salmonella typhi	0	0	0	0	0.1 1	0	Resistance
E. coli	0	0	0	0	0.1 7	0	Resistance
Shigella flexner	0	0	0	0	0	0	Resistance
E. coli	0	0	0	0	0.0 5	0	Resistance
Shigella flexner	0	0	0.05	0.01	0	0.1	Resistance
E. coli	0	0	0	0.22	0	0	Resistance
Salmonella typhi	0	0	0	0	0	0	Resistance
Porteous vulgaris	0	0	0	0	0	0	Resistance
Klebsiella sp	0	0.24	0.08	0.23	0	0	Resistance
Shigella flexneri	0	0	0	0	0	0	Resistance
E. coli	0	0	0.02	0.00 3	0	0	Resistance

Discussion

Diarrhea is the most common disease among children, which collectively indicates the serious public health challenge globally, particularly as the leading cause of death for children after respiratory diseases. Childhood diarrhea under five years constitutes about 63% of global health problems. The accurate and timely diagnosis of the pathogens of diarrhoea is a critical issue. The conventional methods are laborious and time-consuming in diagnosing the bacterial causes of diarrhea; however, they fail to identify pathogens that are difficult to culture in the laboratory. Previous studies showed difficulties in determining the causative agent in about 40% of cases of diarrhea [10].

Diarrhea is a severe disease for children under five years of age because of the high percentage of water in their bodies. Diarrhea affects the body's electrolytes and water, causing dehydration, that considered an emergency requiring medical intervention in newborns, especially if diarrhea is accompanied by fever and lasts for more than 24 hours [11]. The results of the current study found that the incidence percentage of diarrhea among hospitalized children in Maternity and Children Hospital / Samawah/ Al-Muthanna Governorate was 61 (72.619 %), which is higher than the results reported previously by other researchers [12].

However, the current incidence percentage of diarrhea is approaching the global records. It also found that diarrhea is the first major cause of child mortality in different countries due to predisposing factors such as bottle feeding, contaminated water and food, and poor hygiene and sanitation procedures [13]. This study also showed that the number and rate of infection in females were higher than in males, 27 (44.26%) and 34 (55.73%) for males and females, respectively. These results disagree with the previous study in the Iraqi city of Mosul, where the incidence rate of males was higher than females, 51.61% and 33.06%, respectively [14]. The current study also showed that the highest incidence of diarrhea was recorded at one month or less (22 (39.344%). At the same time, there were variations in the number and incidence of diarrhea in other age groups. These results are compatible with previously reported studies [15].

Globally, a higher prevalence of diarrhea has been recorded in children than adults, especially among children under five years of age [16]. These studies approved that the incidence of diarrhea among children is highest in the age group less than 24 months and decreases with increasing age [17]. The immunological status and the lack of antibodies to the enterotoxin-producing bacteria like *Escherichia coli* play an essential role in the pathogenesis of childhood diarrhea. However, the level of these antibodies increases with the frequency of children's exposure to these bacteria with age [18].

The results of the current study also showed that the total percentage of bacterial isolation from stool was 100%. Moreover, *E. coli* constituted the highest number of bacterial isolates,

8 (38.10%), while the percentages of other bacterial isolates were: 5 (23.81 %). 3 (14.29%), 1 (4.76%), 2 (9.52%), and 2 (9.52%) for *Salmonella typhi*, *Salmonella typhimurium*, *Klebsiella sp.*, *Shigella sp.*, and *Proteus sp.* respectively.

This result is compatible with the results of previous studies [19]. A previous study approved that the high percentage of bacterial isolation might be due to contamination of food and milk, climatic factors, socio-economic status, and environmental conditions [20].

The current study also showed a multiple-drug resistance of the bacterial isolates to antibacterial discs in the in vitro antibiotic sensitivity test. The percentages of sensitive and resistant isolates were 3 (9.523%) and 19 (90.477%), respectively. These results agree with previous studies that proved the isolation of antibiotic-resistant bacteria from stool swabs of children with diarrhea [21]. A previous study revealed isolation of resistant *Escherichia coli* at a very high rate (97.5%) [22]. Another study approved the isolation of multiple-drug resistance enteric coliform bacteria from persistent diarrhea in children [23].

Moreover, another previous study reported the highest rates of resistance (100%) against Amc, Cm, Tc, and Cip, with 38.7% for the most common resistance pattern for Amc, Gm, Cip, Tc, and Cm (Roy et al., 2006). Another researcher recorded similar results for multiple antibiotic resistance, especially for *E. coli* and the percentage of multi-resistance isolates was 34.67% for Trimethoprim, which acts as a protein competitive [24].

The presence of antibiotic resistance phenomena in the isolated pathogenic bacteria is a serious indication of the spread of antibiotic resistance in the community due to the excessive use and misuse of antibiotics to treat many cases without a medical prescription. The emergence and spreading of antibiotic resistance in bacteria are complex, very effective, and responsible for the emergence of many clinical problems, especially in treating bacterial infections. There are many factors responsible for the development of resistance to antibiotics. These include the inappropriate and random use of antibiotics, failure to adhere to the treatment course prescribed by the specialist doctor, and possession of multiple mechanisms, including antibiotic impermeability, mutations, and changes in the structure of the outer membrane of the pathogenic bacteria. Additionally, the widespread of highly resistant *Escherichia coli* to antibiotics can effectively or efficiently replace genetic material with other bacteria such as *Salmonella*, *Shigella*, *Yersinia*, and *Vibrio*. Moreover, a chromosomal mutation reduces the permeability of the cell membrane and reduces the accumulation of the drug or a change in its absorption characteristic [25-26]. It may also increase the resistance of *Escherichia coli* to antibiotics due to its possession of plasmids capable of conjugation and transmission between bacteria.

Conclusion

This study approved the high occurrence of diarrhea in children less than 2 years old in Al Muthanna province compared to other diseases such as bronchitis, jaundice, suckling interruption, dyspnea, hypoglycemia, and pneumonia. Moreover, diarrhea was reported in a high percentage of females than in males. On the other side, a high percentage of diarrhea was reported in one month or less compared to other ages. *E. coli* was revealed the highest percentage of bacterial isolates compared to *Salmonella typhi*, *Salmonella typhimurium*, *Klebsiella sp*, *Shigella sp*, and *Proteus sp*. All bacterial isolates showed multi-drug resistance in the in vitro antibiotic sensitivity test. More efforts are required from authorities to reduce diarrhea in children under the age of five years because diarrhea is among the significant causes of morbidity and mortality in children.

Recommendation

The authors recommend restricting the selling of antibiotics without prescription in pharmacies to prevent development of multi-drug resistance microorganisms. More future study needs to be done to investigate the epidemiology of diarrhea and its important causes in children in Al Muthanna province in Iraq.

Ethics approval and consent to participate

The study had been approved by the local ethics committee of Al Muthanna University.

Human and animal rights

No Animals were used in this research. All human research procedures followed were in accordance with the ethical standards of the committee responsible for human experimentation (institutional and national), and with the Helsinki Declaration of 1975, as revised in 2013.

Consent for publication

We obtained the written informed consent from each subject or subject's parent.

Conflict of interest

The authors declare no conflict of interest, financial or otherwise.

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