

Enteric and Infectious Diarrheal Diseases in Yemeni Children Admitted with Complicated severe Malnutrition

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□ ABSTRACT □

The aim of this study was to identify the microbial causes of malnutrition in children less than five years with focusing on the most common pathogenic microorganism that cause diarrhea, and the association between diarrhea and malnutrition.

One hundred thirty seven stool samples were collected from malnourished children who are suffering from diarrhea and attending Alsabeen Governmental Hospital specialist for Pediatric in Sana'a city. Most children 24% were (36-27 weeks) age group followed by (9-16 weeks) in rate of 21% and males were higher than females in all age groups. All samples were examined for the presence of Bacteria, Rotavirus, *Candida albicans*, and Parasites.

Results yield that there is association between the risk contracting bacterial, viral and protozoa infection with different sex and age groups. *Candida albicans* were the most dominant (35.8%) and *Escherichia coli* O157 was a majority cause of diarrhoeal illness (22.6%) followed by *Rotavirus* (13%). Amikacine has shown the highest sensitivity against *E.coli* O157 (96%) followed by levofloxacin (70%) then Nitrofurantion (66.7%), while Ampicillin, Cefixime shown the highest resistance to *E.coli* O157. Malnourished children kwashiorkor have shown the highest rate of mortality (42.6%), while mortality associated with marasmus in 22(17.9%).

Keywords: Malnourished, diarrhea, children, Enteropathogenic, Mortality.

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أمراض الإسهالات المعوية والإنتانية في الأطفال اليمنيين المقبولين باختلاط سوء التغذية الحاد

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□ ملخص □

كان الهدف من هذه الدراسة الكشف عن الكائنات الحية المسببة لسوء التغذية عند الأطفال أقل من 5 سنوات مع التركيز على الأحياء الدقيقة الأكثر شيوعاً المسببة للإسهالات والتراشق بين الإسهالات وسوء التغذية. تم جمع مئة وسبعة وثلاثون عينة براز لأطفال راجعوا مستشفى السبعين الحكومي و المتخصص بأمراض الطفولة في العاصمة صنعاء. تم فحص العينات لتحري وجود الجراثيم، فيروس الروتا، المبيضات البيض، الطفيليات.

كان معظم الاطفال من الفئة العمرية (27-36) اسبوع 24% يتبعها الفئة العمرية (9-16) اسبوع 21% وكان عدد الذكور أعلى من الاناث في كل الفئات العمرية.

أظهرت النتائج اختلاف شدة الإصابة باختلاف الجنس والفئات العمرية حيث كانت فطريات المبيضات البيض هي الأكثر انتشاراً (35.8%) كما أن بكتيريا *Escherichia coli* O157 حققت أعلى نسبة للعدوى (22.6%) تلاها فيروس الروتا (13%). وقد أظهرت نتائج اختبار الحساسية للمضادات الحيوية لـ *Escherichia coli* O157 المعزولة بأن Amikacin كان الدواء المفضل بنسبة حساسية تساوي 96%، Nitrofurantion 70%، levofloxacin 66.7% بينما Ampicillin و Cefixime أبدى معدلات حساسية منخفضة وبنسبة مقاومه 100%. وكانت أعلى نسبة وفيات للأطفال الذين عانوا من الكواشيوركور (42.6%) بينما حالات الهزال بنسبة (17.9%).

الكلمات المفتاحية: سيئ التغذية، الاسهالات، الاطفال، المسببات المرضية المعوية، الموت.

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Introduction:

Diarrhea and Malnutrition diseases are a leading cause of childhood morbidity and mortality, particularly in developing countries. It is considered to be a public health problem and the second leading cause of death among children under five years old (1,2,3). Protein energy malnutrition is defined by two standards, under the normal weight for age (underweight), height for age (stunting) and weight for height (wasting). Wasting indicates recent weight loss, whereas stunting usually results from chronic weight loss (4,5,6).

Yemen has the third highest rate of malnutrition in the world and the Child malnutrition is a serious problem. Almost half of children under the age five, about two million children are chronically malnourished and another one million are suffering from acute malnourishment. Malnutrition is measured by anthropometric status (weight for age, weight for height, and mid-upper arm circumference). Diarrhea is an epidemiological problem in Yemen but hasn't been studied suitably. However, data about this problem's has been gotten through available data. As recorded in the last census 2010. According to the world meter information which reported that; the total Yemeni population about 29,825,964 and 39% are children (United Nations Statistics Division 2020).

This study aimed to find the relationship between the diarrhea in malnourished children and the causative microorganisms including bacteria, viruses, yeast and parasites and to focusing on some health and epidemiological risk-factors that give good knowledge about infectivity and transmission of diseases.

Material and Methods:

One hundred and thirty seven stool samples were collected from malnourished children under five years old who had acute or chronic diarrhea and admitted to at Al-Sabaeen Hospital the governmental pediatric hospital in Sana'a capital city and received all cases from different area of Yemen. Samples were collected during one year in period from December 2012 to December 2013.

Patient's information were collected through questionnaire taken from patient's parent and medical files including; gender, age, anthropometrics measurements, symptoms and other risk factors which may related with causing agents of diarrhea in malnourished children.

Collection and transport stool samples

Two specimens were collected in sterile swabs and prepared for microscopic examination, culturing and rotavirus investigation.

Microscopically and staining procedures:

Each fresh sample were examined microscopically by using a saline, while *Cryptosporidium* spp and *Isosporaspp* were diagnosed by the Modified Ziehl-Neelsen Stain.

Another slide were fixed in polyvinyl alcohol (PVA) and allowed to air dry and processed according to (Wheatley's Trichrome (Modification of Gomori Trichrome) Protocol) (7,8,9,10)

Isolation and identification of pathogenic bacteria

All specimens were cultured in various selective media such as; xylose lysine deoxycholate agar (XLD), Macconkey sorbitol agar, thiosulphate citrate bile-salt sucrose agar (TCBs) and selenite broth. Plates were incubated for 18 hours at 37°C aerobically, the selenite broth then subculture onto *Salmonella-Shigella* agar.

Campylobacter Selective Medium is inoculated with loopful of Specimen and incubated in Candle Jar (microaerophilic) at 42°C overnight or 37°C for up to 48 hours (8,11).

Identification of isolated bacteria

Colonies had been identified based on morphologic characteristics and other standard Biochemical reaction, Motility Indol Urea (MIU), Kligler Iron Agar (KIA) and Oxidase tests were used to identify and different Species of bacteria (11, 12).

Detection of *E.coli* O157:

All lactose fermenting, sorbitol non-fermenting, motile positive and indole positive colonies will be examined by *E.coli* O157 for the agglutination.

When the reagent mixed with *E.oli* which has Antigens correspondent to the reagent, the antigen antibody reaction produced agglutination.

This reaction observed macroscopically (***E.coli* antiserum O157 - Denka seiken Co. Ltd., Tokyo, Japan**).

Detection of *Salmonella* Spp by Serotest *Salmonella* vi Antisera

-By using platinum wire, transfer a portion of loopful colony from nutrient agar, mix the saline.

-If agglutination is found with O polyvalent sera and non-agglutination with saline, further test with O group or O factor which present in the positive polyvalent sera (12)

Yeast detection (*Candida albicans*)

All specimens were cultured for candida by diluting 0.2 g of faeces in 1.8 ml of sterile saline solution. A 10µl aliquot was then plated on Sabouraud's dextrose agar containing 300µg/ml chloramphenicol and 10µg/ml gentamicin. Plate cultures for yeasts were incubated at 35°C for 48 hours before identifying the colonies.

C albicans was identified by the production of germ tubes and confirmed by the production of chlamydospores (13).

Rice Extract Agar is used for promotion of chlamydospore formation by *Candida albicans* as a means of differentiating them from other *Candida* species.

Chlamydospore production test:

Chlamydospore production test was performed by inoculating *Candida* isolates on corn meal agar supplemented by 8ml of tween 80. The samples were previously grown in SDA were seeded as 4 parallel streaks in rectangular piece of agar placed in between two slides and the plates were incubated in wet chamber at 30°C for 72 hour. The plates were visualized under an optical microscope. The double walled rounded spore will be observed as chlamydospore (14,15).

Antibacterial sensitivity test

Susceptibility of all pathogenic isolated bacteria to antibacterial discs was determined by the disc diffusion test on using Muller-Hinton agar according to the manufacturer's instructions (HiMedia Laboratory Ptv. Limited, Mumbai, India). The antibacterial discs were placed onto the surface of the inoculated Muller-Hinton agar plates with sterile forceps, plates incubated for 24 hrs at 37 °C.

The plates were examined, the diameters of the complete bacterial growth inhibition zones, across the diameter of the discs measured with a ruler (16).

Specimen collection for ELISA

Stool samples may be used as unpreserved or frozen, or in preservation media of 10% formalin.

Unpreserved samples should be kept at 2-4 °C and tested within 24 hours of collection. Samples that cannot be tested within this time should be frozen at 15 °C to 20 °C or lower until used. Freezing does not adversely affect the test. All dilutions of unpreserved stools must be made with diluted wash buffer.

Rotavirus (Fecal) Antigen Detection ELISA

Rotavirus Antigen Detection ELISA is an in vitro procedure for the qualitative determination of rotavirus antigen in feces.

During the first incubation, the rotavirus antigens that present in the stool supernatant are captured by antibodies attached to the wells. The second incubation adds an additional anti-rotavirus antibody that sandwiches the antigen. The third incubation attaches horseradish peroxidase to the sandwich. After washings to remove unbound enzyme, a chromogen is added which develops a blue color in the presence of the enzyme complex and peroxide. The stop solution ends the reaction and turns the blue color to yellow (17).

Anthropometric measurement

Height and weight were determined according to standard anthropometric methods (International Society for the Advancement of Kin anthropometry: ISAK) Height was measured to the nearest 0.1 centimeters (cm) in bare feet with participant standing upright against a mounted stadiometer.

Weight was measured to the nearest 0.1 kilogram (kg) with participants lightly dressed (underwear and T-shirt) using a table scale (18).

Anthropometric measures obtained as following:

- i. Checking for bilateral oedema**
Bilateral oedema is the sign of kwashiorkor children with bilateral oedema are directly identified to be acutely malnourished.
- ii. Taking the MUAC**
MUAC is used as an alternative measure of thinness to weight-for-height it is particularly used in children from one to five years.
- iii. Taking the weight.**
- iv. Taking the length / height (19).**

Results and Discussion:

Results:

A total of 137 diarrhea samples were collected from malnourished children under five years' old 83(60.5%) males and 54(39.5%) females, the number of males higher than females in almost all age groups. Most patients were in age group (27–36) weeks (24.1%) followed by age group (9–16) weeks (21.2%) (Table.1).

Acute diarrheas, abdominal pain, fever, vomiting, lack of energy and dehydration were the most clinical symptoms.

Watery stool frequency ranged from five times to 12 times per day and the duration of diarrheas ranged from three to 99 days, mean number of days was 35.

84/137 (61.3%) were positive for enteropathogenic microorganisms and *Candida albicans* were the most dominant 49 (35.8%) followed by *Escherichia coli* O157 31 (22.6%) then Rotavirus 18 (13.1%). *Salmonella* spp 11 (8%). *Shigella*, *Yersinia enterocolitica*, *Vibrio alginolyticus* presented in the same number 2 (1.5%), while *Vibrio parahaemolyticus* and *Aeromonas* spp just one (0.73%). Protozoa also found in 5(3.6%) which included (3) *Cryptosporidium*, (1) *Isospora* and (1) *Giardia* (Table.2).

Table.1: The association of infection with different sex and age groups

Sex & Age groups	Infection		OR	CI	X ²	P-value
	No	%				
Sex						
Male n= 83	47	56.6	1.21	(0.6 – 2.56)	0.3	0.58
Female n= 54	28	51.8	0.82	(0.4 – 1.7)	0.3	0.58
Age groups						
>9 Ws n=11	7	63.6	1.5	(10.37– 6.44)	0.38	0.5
9 – 16 Ws n=29	17	58.6	1.22	(0.5 – 3.04)	0.22	0.6
17 – 26 Ws n=20	11	55	1.01	(0.36 – 2.9)	0.00	0.9
27 – 36 Ws n=33	14	42.4	0.52	(0.22-1.23)	2.7	0.10
37 – 46 Ws n= 13	9	69.2	1.98	(0.52- 8.1)	1.22	0.29
47 – 56 Ws n=19	12	63.2	1.5	(0.5 – 4.6)	0.63	0.4
≥57 Ws n=12	5	41.7	0.50	(0.14- 2.11)	0.91	0.3

3/7 (42.6%) nutritional status of malnourished children kwashiorkor shown the highest rate of mortality while mortality associate with marasmus in 22(17.9%) but there was no death case associate with whom were marsmic-kwashiorkor Table.3. There were no statistical significant associate other age groups with death. Out of ten antibiotics used, Amikacin (AK) shown the highest sensitivity against or to *E.coli O157* 29 (96%) followed by Levofloxacin (LE) 21 (70%) then Nitrourantion (NIT) 20 (66.7%) while Ampicillin (AMP), cefexim (CFM) shown the highest resistance to *E.coli O157* (Table.4).

Table.2: Distribution of microorganisms that isolated from malnourished children suffering diarrhea.

Microorganisms	Male n = 83		Female n = 54		Total n = 137		X ²	P-value
	No	%	No	%	No	%		
<i>Aeromonasspp</i>	0	0	1	1.9	1	0.73	1.55	0.21
<i>Candida albicans</i>	30	36.1	19	35.2	49	35.8	0.01	0.9
<i>E. coli O157</i>	20	24.1	11	20.4	31	22.6	1.24	0.26
<i>Salmonella spp</i>	8	9.6	3	5.6	11	8	0.74	0.39
<i>Shigellaspp</i>	1	1.2	1	1.9	2	1.5	0.10	0.75
<i>Vibrio algioleticus</i>	2	2.4	0	0	2	1.5	1.32	0.25
<i>Vibrio parahemoleticus</i>	0	0	1	1.9	1	0.73	1.6	0.21
<i>Rotavirus</i>	12	14.5	6	11.1	18	13.1	0.32	0.21
<i>Protozoa</i>	2	2.4	3	5.6	5	3.6	1.7	0.19
<i>Yersinia enteroletica</i>	2	2.4	0	0	2	1.5	1.32	0.25

Protozoa: Cryptosporidium =3, Gardialamblia=1, Isospora=1

Table.3: The association between the type of nutritional status with mortality rate.

Nutritional status	Death n= 25		OR	CI	X ²	P-value
	No	%				
Marasmus n = 123	22	17.9	0.8	(0.18- 3.96)	0.11	0.74
Kwashiorkor n = 7	3	42.6	3.6	(0.6- 21.5)	2.99	0.083
Marasmic-kwashiorkor n = 7	0	0	0	(0-3.56)	1.65	0.19

Table.4: Antibiotics sensitivity of *Ecoli O157* isolated from malnourished children suffering diarrhea

Antibiotics	Sensitivity		Resistant	
	N	%	N	%
Amikacin	29	96.7	1	3.3
Ampicillin	0	0	30	100
Cefixime	0	0	30	100
Cefotaxime	3	10	27	90
Levofloxacin	21	70	9	30
Nalidixic acid	10	33.3	20	66.7
Nitrofurantoin	20	66.7	10	33.3
Piperacillin	0	0	30	100
trimethoprim	4	13.3	26	86.7

Discussion

Diarrhoea is a major public health problem in Yemen. Differences in the prevalence, etiology, clinical presentation, complications, and outcome between malnourished individuals have been well described in other countries (20,21). However, there was no information describing these differences in those who are malnutrition in low-income settings such as Yemen, addressing this knowledge gap are an important contribution to the study of diarrhoeal disease surveillance.

When the infectious causes of diarrhoea were investigated (Bacteria, Rotavirus, Protozoa and yeast) the most common associated microorganisms of diarrhoea among our malnourished children was *Candida albicans* which considered pathogenic among immune-compromised patients, *Candida albicans* don't cause diarrhoea among well-nourished children. This finding is similar to that reported by Gracey (22, 23).

On the other hand this result is in contrast to previous study by Forbes *et al.*, (2001) which there was no association between fecal *Candida albicans* and diarrhoea and they put several explanations for this discrepancy; firstly, earlier studies did not include control groups. Secondly, it is possible that, *Candida* may have a different effect in malnourished children. Finally, malnutrition may encourage proliferation of yeast species, and the association with diarrhoea may be co-incidentally (13).

The majority bacterial cause of diarrhoeal illness in our malnourished children was *E.coli* O157 (22.6%). This result is different from that reported in developing countries in which the most common cause of gastroenteritis is rotavirus and only a smaller proportion of diarrhoeal disease were attributed to bacterial pathogens such as *E. coli*, *Salmonella*, *Shigella* species, *vibrio species*, and *Clostridiumdifficile* (24), (25).

Result is different from previous Yemeni study (**Banajeh et al., 2001**) in which bacterial agents, as Enteropathogenic *E.coli* was the most common followed by *Shigella* then *Salmonella* (26). Furthermore, our result is different from that reported in Oman in which *Shigellaspp* was the most common followed by *Salmonella spp* and *E.coli* and that reported in Bahrain in which *Campylobacterspp* is the most common, while *E.coli* and *Shigella* presented in similar to our percentage (**Elhaget al., 2009**) (27). The high rate of *E.coli* O157 among our cases might be effected by the animal sources, in which most of the our patients' mothers have direct contact with animals (cattle and sheep) which considered host reservoir of *E.coli* O157, and bad hygiene of mothers help to transmitted *E.coli* O157 to children.

Rotavirus as a cause of diarrhoea was low in our malnourished children in which it count only 13.1%, lower than that reported in well-nourished children elsewhere as in Bangladesh (40%) (28). However our result is similar to that reported in Bangladesh in other study by Checkley which this study found that the rate of Rotavirus infections are higher among normal and overweight children than among malnourished children (24). The observation of lower rate of rotavirus infections among malnourished children under 5 years, can be explained by the fact that higher rates of rotavirus among well-nourished individuals may be related to healthy epithelial cells that are required for attachment of this pathogen as well as for the development of rotavirus diarrhea (29). It is likely that well-nourished children are not suffering from other diseases of the digestive tract that may present in underweight children. Another explanation could be differences in the hygienic practices of families of children who are well nourished. In Yemen, it may be that well-nourished children are from families with a better socio-economic status, and this population may have different eating behaviors, which may be in turn responsible for differed likelihood of developing food-borne diseases. A propensity for consuming food away from home may lead to increased consumption of food prepared with poor food hygiene practices (30, 31).

In regarding to the role of vaccination against Rotavirus results yields that; there were no difference between a result with vaccinated and un-vaccinated malnourished children with (13.7%) and (12.9%) positive to Rotavirus respectively, this result can be explained by that, our patients had defect immune system due to malnutrition (32).

Conclusions

This study has provided useful information about the causative agents of diarrhoea among malnutrition children in Yemen. The majority of diarrhoeal illness in Yemeni malnourished children was caused by *E.coli*-O157; different from that reported in developing countries in which Rotavirus is the most common cause of gastroenteritis. The rate of associated diarrhoea with positive culture of *C. albicans* was high. There was association between the risk contracting bacterial, viral and protozoa infection with different sexes and age groups. There was high rate of antibiotic resistant trains are increasing among isolated bacterial species as described worldwide.

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