



Original article

Metronidazole-sensitive organisms in children with severe acute malnutrition: an evaluation of the indication for empiric metronidazole treatment

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ABSTRACT

Objectives: Children with severe acute malnutrition (SAM) are treated with empiric amoxicillin or penicillin and gentamicin because of the high risk of severe infections. Experts have suggested, based on available evidence, adding metronidazole to cover anaerobic bacteraemia and diarrhoea caused by *Giardia duodenalis* or *Clostridium difficile*. The objective of this study was to assess the importance of these infections in children with SAM.

Methods: Children from 6 months to 15 years with SAM were enrolled and followed clinically. Aerobic and, when patient weight permitted, anaerobic blood cultures were done using Bactec® system, and isolates identified with matrix-assisted laser desorption ionization–time of flight mass spectrometry. Stool samples were tested for *C. difficile*, *G. duodenalis* and *Entamoeba histolytica* by PCR.

Results: A total of 334 children were enrolled and 174 out of 331 (53%) for which data on this was available had diarrhoea. Of 273 patients tested by blood culture, 11 had bacteraemia (4.0%, 95% CI 2.3–7.1%) but none with strict anaerobic bacteria (0/153, 95% CI 0–2.4%). There was no difference in the prevalence of *C. difficile* between children with (5/128, 4%) and without (7/87, 8%) diarrhoea (OR 0.47, 95% CI 0.14–1.53), and no difference in the prevalence of *Giardia* between these groups (78/138, 60% vs. 46/87, 53%; OR 1.34, 95% CI 0.77–2.32). Children with *C. difficile* had higher mortality than those without this infection (3/11, 27%, vs. 7/186, 4%; OR 43, 95% CI 3.9–483).

Conclusion: Our results do not provide support for empiric metronidazole to cover for anaerobic bacteraemia. Trials evaluating the effect of empiric treatment and its effect on *G. duodenalis* and *C. difficile* are warranted. **M. Zangenberg, Clin Microbiol Infect 2020;26:255.e7–255.e11**

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Introduction

Infections are a common cause of death in children with severe acute malnutrition (SAM). According to one review, the average prevalence of bacteraemia among children with SAM is 17% [1], but

more recent studies found a lower prevalence of around 5% [2,3]. The most common causes of bacteraemia among children with SAM are *Salmonella*, *Escherichia coli*, *Staphylococcus aureus* and *Streptococcus pneumoniae* [4,5]. Children with SAM are treated with antibiotics empirically: hospitalized cases with parenteral penicillin and gentamicin and outpatients with oral amoxicillin [6]. The rationale for empiric systemic antibiotics is the high risk of infections and non-specific symptoms of sepsis in SAM, and the limited microbiology laboratory capacity in places where SAM is common [7]. Even with the current empiric antibiotic regimens, the

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case fatality rate of SAM remains high, in some settings up to 16% [8]. Experts have suggested adding empiric metronidazole to cover for possible anaerobic bacteraemia [9–11], although its prevalence has not been assessed in this patient group.

Some authors also argue that the risk of gastro-intestinal infection with *C. difficile* or *G. duodenalis* justifies the use of empiric metronidazole treatment in children with SAM [10,12]. However, the prevalence of *C. difficile* and its relevance in SAM, particularly among children below the age of 1 year [13], is unknown. While *G. duodenalis* is commonly found in stool samples from children with SAM, its contribution to morbidity and mortality is controversial [14–16].

The objective of this study was to determine the aetiology of bacteraemia, including anaerobes, and to assess the prevalence of the metronidazole-sensitive enteropathogens *G. duodenalis*, *C. difficile* and *Entamoeba histolytica* in children with SAM. Furthermore, we wished to relate these findings to severity of disease and clinical outcome.

Methods

Study design and participants

This was a prospective observational study, implemented in three sites in Ethiopia: Jimma University Specialized Teaching Hospital (JUSTH), Serbo Health Centre (SHC) and the Missionary of Charity Clinic (MiCC). The parent study was designed to evaluate the usefulness of clinical algorithms for determining bacteraemia and treatment outcome. Enrolment started at JUSTH in June 2016, at SHC in February 2017 and MiCC in June 2017 and was completed in May 2018. Children with SAM between 6 months and 15 years of age, seeking healthcare at one of the three sites were enrolled after seeing the clinical staff and giving consent, but before any treatment was started.

The definition of SAM was one or more of the following: weight for height <70% of the median of the National Center for Health Statistics (NCHS) reference curve, mid-upper arm circumference (MUAC) < 115 mm and the presence of bilateral oedema. Besides nutritional therapy, including essential vitamins and supportive care, the children received empiric antibiotic treatment with amoxicillin (50–100 mg/kg/day) and if hospitalized gentamicin (5 mg/kg/day) was added.

Data collection

Demographic and clinical data were collected by research nurses using standardized case report forms. We collected details on treatment and outcomes from follow-up visits during admission and from the hospital medical records.

Specimen collection

Before treatment was started, we collected a venous blood sample aseptically. The amount of blood was based on the child's weight and total blood volume [17]. First, an EDTA plasma tube (Vacurette, Becton Dickinson, Vienna, Austria), a Serum Sep Clot tube (Vacurette) and one aerobic blood culture vial (BACTEC Peds Plus/F medium, Becton Dickinson) were obtained. For patient safety, we limited the number of blood samples from the smallest children and prioritized blood samples for blood count, biochemistry and an aerobic blood culture. From children above 7 kg we collected one additional anaerobic blood culture vial (BACTEC Lytic/10 Anaerobic/F). We supplemented with one aerobic vial from children weighing more than 9 kg and two aerobic vials from children above 11 kg. The bottles were weighed before and after blood collection (Sartorius TE412, JUSTH and EatSmart ESKS, MiCC and SHC).

Stool samples were collected using plastic-lined diapers and transferred to a collection tube or directly in the tube. When feasible, stool samples were collected before treatment was started, but treatment was not delayed if a stool sample could not be obtained immediately.

Samples were immediately brought to the research laboratories in JUSTH whereas in SHC and MiCC tubes for biochemistry and stool samples were kept at 4°C and blood cultures were kept at ambient temperature (<8 hr) until transported to JUSTH, once daily.

Laboratory methods

We used an automated blood culture system (BACTEC FX40, Becton Dickinson, Baltimore, MD, USA) to incubate the blood cultures and a few drops were aspirated from bottles flagged as positive for subculture on agar plates. From aerobic bottles subculture was done on chocolate agar in a humidified atmosphere with 5% CO₂, from anaerobic bottles in addition on chocolate agar in O₂-depleted atmosphere using incubation containers with sachets creating an anaerobic environment (GasPak EZ anaerobe container system sachets, Becton Dickinson). The positive samples were analysed in the local microbiology laboratory and the results of the identification and susceptibility testing were immediately reported to the clinical staff. Isolates were frozen at –80° in a skimmed milk, tryptone, glucose, glycerol transport medium and sent to Department of Clinical Microbiology, Rigshospitalet, Denmark. After re-culture on agar plates the isolates were identified using matrix-assisted laser desorption ionization–time of flight (MALDI-TOF) mass spectrometry (Microflex, Bruker, Bremen, Germany). If identification was not possible we performed real-time PCR (LightCycler 480, Roche, Basel, Switzerland), sequenced the PCR products (GATC, Eurofins, Hamburg, Germany) and aligned to standard databases (SepsiTTest BLAST and NCBI BLAST). Antimicrobial susceptibility testing (AST) was conducted by disc diffusion (Oxoid, Basingstoke, UK) and with gradient minimum inhibitory concentration (MIC) strips (Etest, bioMérieux SA, Paris, France). AST procedures and interpretation was done according to EUCAST guidelines [18].

The Department of Clinical Immunology in the Rigshospitalet, Copenhagen, screened the serum samples for HIV using Vitros anti-HIV1+2 Enzyme Immunoassay (Ortho Clinical Diagnostics, Pencoed, Bridgend, UK) and confirmatory testing using INNO-LIA HIV I/II Score (Fujirebio Europe, Gent, Belgium).

Stool samples were frozen at –80°C, shipped to Denmark and tested with the *C. difficile* assay and 'Enteric Parasite Panel' (EPP) on the BD MAX platform (Becton Dickinson). The tests detect nucleic acids for the *C. difficile* toxin B gene and from *G. duodenalis* and *E. histolytica*, respectively (BD MAX Cdiff assay and BD MAX EPP, Becton Dickinson, Canada). If results were unresolved due to inhibitory specimen or reagent failure, analyses were repeated, if necessary, after diluting the stool samples in S.T.A.R. buffer (Roche) to counteract PCR inhibition.

We analysed the complete blood count using an automated haematology analyser (KX-21 N, Sysmex Corporation, Bellport, NY, USA).

Definitions

We defined growth of an organism known to cause bacteraemia in children as a 'pathogen'. Gram-positive rods (with specific exceptions that were not encountered), coagulase negative staphylococci (CoNS) or non-beta haemolytic streptococci were defined as 'likely contaminants' if they grew in a single blood culture bottle, and as 'possible contaminants' if they grew in two or more bottles obtained from the same venepuncture from the same patient. We used a standard definition of medical complications [6,19].

If children died, were transferred to another health facility, still had SAM or were undergoing treatment with no further follow-up data available after 4 weeks of treatment, they were categorized as having 'likely treatment failure'.

Statistical methods

We used EpiData 3.1 (EpiData, Odense, Denmark) to enter and validate data and SAS Enterprise Guide, Version 7.11 of the SAS System for Windows (SAS Institute Inc., Cary, NC, USA) for data analysis. A p -value < 0.05 was considered statistically significant and 95% confidence intervals (CI) were used. While the NCHS reference curve were used to identify children with SAM, the weight for height/length z-score (WHZ) based on WHO standard curves for children 6 months to 5 years and body mass index for age for children 5–15 years were used to present the data [20]. We used mixed linear or logistic regression models, both with random effects for site. We assessed weight gain by measuring the first oedema-free weight, and repeated weight measures during treatment and on discharge from the health facility. To assess the effect of relevant variables on weight gain, we used a mixed model linear regression analysis, with a Gaussian covariance structure for repeated measurements and random effects for site.

Ethical issues

The study was approved by Jimma University Institutional Review Board (Reference HRP/239/2015) and the Ethiopian National Research Ethics Review Committee (Reference 310/285/2017) and received consultative approval by the Danish National Committee on Health Research Ethics (Reference 1800407). After obtaining written informed consent from caregivers the children were considered eligible.

Results

Of 343 children screened, 334 (97%) were enrolled in the study. The majority of children (92%, 306/334) were treated as inpatients, although 46% (154/333) did not have any medical complications and could potentially have been treated as outpatients. One child died before clinical data were collected. Fifty-three per cent (174 of 331 for whom data was available) had diarrhoea. Sixteen per cent (50/310) had received antibiotics in the week prior to enrolment but none reported having received metronidazole (Table 1). Data on

Table 1
Background characteristics of the enrolled children

	n^a	Value
Female sex, % (n)	333	48 (160)
Age, months	333	33.0 (14.0; 60.0) ^b
>59, % (n)	333	27 (90)
Mid-upper arm circumference	332	117 (19) ^c
In children without oedema	118	106 (16) ^c
Weight-for-length/height z-score (6–59 months) ^d	261	−2.5 (1.8) ^c
In children without oedema	87	−3.8 (1.1) ^c
BMI for age (5–14 years) ^d	29	−4.0 (1.9) ^c
Bilateral oedema	333	63% (215)
Medical complications	333	54% (179)
Diarrhoea	331	53% (174)
Caregiver-reported fever	330	28% (94)
Treatment for malnutrition during the last week	282	9% (24)
Treatment with antibiotics during the last week	310	16% (50)

^a Number of children for whom data were available.

^b Median (25th; 75th percentiles).

^c Mean (standard deviation).

^d WHO z-scores.

treatment outcome were available for 283 out of 334 (85%) of whom 17 (6%) died and another 57 (20%) had other kinds of treatment failure. None of the children had HIV.

Blood cultures

Of the 334 children enrolled, 273 (82%) children had at least one aerobic blood culture drawn and 153 (46%) an anaerobic blood culture. Of the 120 children for whom an anaerobic blood culture was missing, 103 (86%) weighed less than 7 kg and were therefore not eligible for more than an aerobic blood culture.

We did not find any strict anaerobic bacteria (95% CI 0–2.4%). More specifically, we did not find any metronidazole-sensitive organisms that are commonly insensitive to routine treatment with penicillin such as *Bacteroides* spp. Ten per cent (27/273) of the patients had a positive blood culture with any organism and a pathogenic bacterium was detected in 4% (11/273, 95% CI 2.3–7.1%) (Table 2). Twenty-three had growth of a single organism and four had growth of two organisms. Of the 17 patients with suspected contaminants (one had both a contaminant and a pathogen), 15 were likely contaminants and the remaining two possible contaminants. The median blood volume cultured was 1.17 mL (IQR 1.00–2.00) for the first aerobic bottle and 1.95 mL (IQR 1.00–3.00) for the anaerobic bottle.

We did not find a high level of resistance among the bacterial isolates (Table S1).

Follow-up data were available for 233 of the 273 children with a blood culture (85%). Of the children with a pathogen in their blood three out of ten (30%) died, while seven out of 207 (3%) of the children with negative blood cultures died (OR 12, 95% CI 2.6–58). Two of 12 (17%) children with growth of a suspected contaminant died as opposed to the seven out of 207 (3%) with a negative blood culture, (OR 7.5, 95% CI 1.3–45). Adjusting for medical complications, oedema and age had no significant effect on this mortality estimate (Table S2). Bacteraemia was not associated with axillary temperature, leucocyte count, pulse rate or respiratory rate (Table S2).

Table 2
Bacteria isolated from the 27 children with growth in blood culture^a

	n
Pathogens	12
Gram positive	
<i>Staphylococcus aureus</i>	3
<i>Streptococcus pneumoniae</i>	3
Gram negative	
<i>Escherichia coli</i>	2
<i>Acinetobacter</i> sp.	2
<i>Salmonella typhimurium</i>	1
<i>Neisseria meningitidis</i>	1
Contaminants	19
Gram positive	
<i>Staphylococcus epidermidis</i>	2
<i>Staphylococcus hominis</i>	3
<i>Staphylococcus simulans</i>	1
<i>Streptococcus cristatus</i>	1
<i>Bacillus</i> sp.	5
<i>Microbacterium</i> sp.	1
<i>Brevibacterium</i> sp.	1
<i>Micrococcus</i> sp.	2
<i>Arcanobacterium</i> sp.	1
<i>Arthrobacter</i> sp.	1
Gram variable	
<i>Paenibacillus</i> sp.	1

^a Four children had growth of two different bacteria. One child had both *Streptococcus pneumoniae* and *Escherichia coli*, one child had a pathogen and a likely contaminant, and two children had two different contaminants.

Stool analysis

G. duodenalis

A stool sample was collected from 218 (65%) of the children and follow-up data were available from 198 (91%) of these. Presence of *G. duodenalis* was common (124/218, 57%) and we did not find an association between *G. duodenalis* and diarrhoea; 60% (78/138) of children with diarrhoea and 53% (46/87) without diarrhoea had *G. duodenalis* in their stool (OR 1.34, 95% CI 0.77–2.32) (Table 3). The children with *G. duodenalis* were younger, weighed less at enrolment and had bilateral oedema more frequently (Table S3). There was no difference in weight gain between children with and without presence of *G. duodenalis* and it was not associated with treatment failure or mortality (Table S3).

C. difficile

Of the 216 children with a stool sample tested for *C. difficile*, 27 (13%) were below 12 months of age. We detected DNA from toxigenic *C. difficile* in 12 out of 216 (6%) children, and we did not find any difference in the prevalence between children with (5/128, 4%) and children without (7/87, 8%) diarrhoea (OR 0.47, 95% CI 0.14–1.53). Presence of *C. difficile* was associated with mortality as 27% (3/11) with *C. difficile* died compared with 4% (7/186) of the children without this infection (OR 4.3, 95% CI 3.9–48.3, adjusted for age, diarrhoea, admission weight, bilateral oedema, history of antibiotics and medical complications (Table 3 and Table S4). Excluding children below 12 months from the analysis gave similar results (Table S4). *C. difficile* was not associated with admission weight or weight gain during follow-up (Table S4).

E. histolytica

None of the samples contained DNA of *E. histolytica* (0%, 95% CI 0–1.7%) (Table 3).

Discussion

We did not find any cases with bacteraemia with strict anaerobic bacteria. While *G. duodenalis* was common in the stool samples, the presence of *G. duodenalis* was not associated with diarrhoea. Presence of *C. difficile* was not associated with diarrhoea, but children with *C. difficile* in their stool had a higher mortality than children without this infection.

Our study has several limitations. First, our sample size was relatively small, since we did not collect an anaerobic blood culture from children below 7 kg due to patient safety. Furthermore, the median blood volume for the anaerobic bottle was lower than the recommended 3 mL and we cannot rule out false-negative tests. Furthermore, the history of metronidazole use prior to admission was based on caregiver recall and could have been underestimated.

Table 3
Results of the stool analysis

Pathogen	DNA detected		Positive test result, children with diarrhoea ^b		Positive test result, children without diarrhoea		Children with positive test result who died	
	n total/n	%	n total/n	%	n total/n	%	n total/n	%
<i>G. duodenalis</i>	124/218	57	78/130	60	46/87	53	3/114	3
<i>C. difficile</i>	12/216 ^a	6	5/128	4	7/87	8	3/11	27 ^c
<i>E. histolytica</i>	0/217 ¹	0	0/129	0	0/87	0	N/A	N/A

^a Analysis for *C. difficile* was not possible for two cases and for *E. histolytica* for one case.

^b Data on diarrhoea missing for one case.

^c Presence of *C. difficile* was associated with mortality.

Thus, larger studies are needed to formally exclude a major role of anaerobic bacteraemia in patients with SAM. Small intestinal bacterial overgrowth (SIBO) has been proposed as an argument for metronidazole in SAM. We did not examine this possibility, since the connection between SIBO and SAM is speculative, and the evidence for treating SIBO with metronidazole is limited [21]. Lastly, our findings might be less relevant for settings with high HIV prevalence, although even among HIV-positive children with SAM the clinical evidence for empiric metronidazole use is uncertain [10].

We found no cases with strict anaerobic bacteraemia. While there are no studies on children with SAM for comparison, previous studies among paediatric patients without acute malnutrition have given similar results. A study among paediatric oncology patients did not find any clinically significant infections with anaerobes [22] and a study reviewing data from a paediatric emergency department found strict anaerobes in only 0.05% of the samples [23]. We found bacteraemia with a known pathogen in 4% of the children which tallies with recent studies in children with SAM [2,24]. Some previous studies that found a considerably higher prevalence of bacteraemia either only included children with medical complications in addition to SAM [25] or were conducted in settings with high HIV prevalence [26]. We tried to ensure a strict aseptic blood collection procedure and had lower rate of cultures with suspected contaminants than similar studies [27,28]. The children with suspected contaminants had significantly increased mortality. Thus, it is possible that some of the suspected contaminants were true infections and could play a pathogenic role in children with SAM. This has also been suggested in studies of neonatal sepsis [29]. Bacteraemia was not associated with axillary temperature, leucocyte count, pulse rate or respiratory rate, confirming that diagnosis of invasive infection in children with SAM is challenging without blood cultures.

Two studies among children with SAM, using PCR, reported a prevalence of *Giardia* of 33% and 38%, respectively [14,15], which is in line with our findings. The lack of association between *Giardia* and diarrhoea in our study might be due to lack of power, but our finding is in line with several studies, some of which reported higher detection rates of *Giardia* among non-diarrhoeal controls than among children with diarrhoea [16,30]. Previous studies also failed to find an association between *G. duodenalis* and clinical outcome among children with SAM [14,15]. This questions the relevance of diagnosing children with SAM for *G. duodenalis*.

An association between *C. difficile* and mortality has previously been reported in children without SAM [31]. We are, however, not able to conclude anything on causality and our finding might be confounded by more frequent use of antibiotics among the severely ill patients. One previous study assessed the rate *C. difficile* infection in children with SAM but failed to find any cases [14]. While it is currently not recommended to test children below 12 months because asymptomatic carriage is thought to be common, a study found that 26% of all children hospitalized with symptomatic *C. difficile* infection were below 1 year of age [13]. More research is needed in this area, especially among children with SAM, in whom the relevance of infection with *C. difficile* among children below the age of 1 year is unknown.

Infection with *E. histolytica* was previously thought to be a common cause of childhood diarrhoea [32]. Several recent studies also found no or very few infections by PCR, suggesting that microscopy, which was used in earlier studies, might have overestimated its incidence [16,33].

Conclusion

Taken together, our data do not provide support for the general use of metronidazole in children with SAM. *G. duodenalis* was

common among children without diarrhoea and treatment of asymptomatic *C. difficile* and *G. duodenalis* infections remain controversial, particularly in young children [34]. We suggest that future clinical trials of metronidazole use in children with SAM should include molecular testing for *G. duodenalis* and *C. difficile* to stratify for treatment effects on these organisms. Anaerobic blood culture may be redundant for diagnosing bacteraemia in children with SAM.

Access to data

The datasets generated and analysed during the current study are not publicly available due to regulations by the Danish Data Protection Agency.

Transparency declaration

We declare no competing interests. This study was funded by University of Copenhagen, Denmark, Aase and Ejnar Danielsen Fond, Denmark (grant ID 10-001539), the Augustinus Foundation, Denmark (grant ID 15-1923) and Rigshospitalet, Denmark. The funders of the study did not have a role in the design, data collection, analysis, interpretation, or report writing.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.cmi.2019.05.022>.

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