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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 0.23 – 0.96), 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 C. 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
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 (JUTSH), Serbo Health Centre (SHC) and the Missionary of Charity Clinic (MicCC). The parent study was designed to evaluate the usefulness of clinical algorithms for determining bacteraemia and treatment outcome. Enrolment started at JUTSH in June 2016, at SHC in February 2017 and MicCC 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 (Vacuette, Becton Dickinson, Vienna, Austria), a Serum Sep Clot tube (Vacuette) 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 Aerobic/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, JUTSH and EatSmart ESKS, MicCC 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 JUTSH whereas in SHC and MicCC tubes for biochemistry and stool samples were kept at 4°C and blood cultures were kept at ambient temperature (<8 hr) until transported to JUTSH, 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°C 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, Conshohocken, USA) and aligned to standard databases (SepsiTBLAST 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 HRPGC/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 333) died as opposed to the seven out of 207 (3%) with a negative blood culture. 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 (85%). Of the children with a pathogen in their blood culture (58%), 53% (174) 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.

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).

**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.

**Table 1**

<table>
<thead>
<tr>
<th>Background characteristics of the enrolled children</th>
<th>n</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female sex, % (n)</td>
<td>333</td>
<td>48 (160)</td>
</tr>
<tr>
<td>Age, months</td>
<td>333</td>
<td>330 (140; 60.0)*</td>
</tr>
<tr>
<td>&gt;59, % (n)</td>
<td>333</td>
<td>27 (90)</td>
</tr>
<tr>
<td>Mid-upper arm circumference</td>
<td>332</td>
<td>117 (19)*</td>
</tr>
<tr>
<td>In children without oedema</td>
<td>118</td>
<td>106 (16)*</td>
</tr>
<tr>
<td>Weight-for-length/height z-score (6–59 months)*</td>
<td>261</td>
<td>2.5 (1.8)*</td>
</tr>
<tr>
<td>In children without oedema</td>
<td>87</td>
<td>3.8 (1.1)*</td>
</tr>
<tr>
<td>BMI for age (5–14 years)*</td>
<td>29</td>
<td>4.0 (1.9)*</td>
</tr>
<tr>
<td>Bilateral oedema</td>
<td>333</td>
<td>63% (215)</td>
</tr>
<tr>
<td>Medical complications</td>
<td>333</td>
<td>54% (179)</td>
</tr>
<tr>
<td>Diarrhoea</td>
<td>331</td>
<td>53% (174)</td>
</tr>
<tr>
<td>Caregiver-reported fever</td>
<td>330</td>
<td>28% (94)</td>
</tr>
<tr>
<td>Treatment for malnutrition during the last week</td>
<td>282</td>
<td>9% (24)</td>
</tr>
<tr>
<td>Treatment with antibiotics during the last week</td>
<td>310</td>
<td>16% (50)</td>
</tr>
</tbody>
</table>

a Number of children for whom data were available.

b Median (25th; 75th percentiles).

c Mean (standard deviation).

d WHZ z-scores.
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 0.34–0.69). 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 43, 95% CI 3.9–483, 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 is based on caregiver recall and could have been underestimated.

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 diarrhea 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 bacteremia 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 Righospitalet, 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.

### References


