Short-duration selective decontamination of the digestive tract infection control does not contribute to increased antimicrobial resistance burden in a pilot cluster randomised trial (the ARCTIC Study)

Iain Robert Louis Kean,1 John A Clark,1 Zhenguang Zhang,1 Esther Daubney,2 Deborah White,2 Paloma Ferrando-Vivas,3 Gema Milla,3 Brian Cuthbertson,4 John Pappachan,5 Nigel Klein,6 Paul Mouncey,3 Kathy Rowan,3 John Myburgh,7 Theodore Gouloumis,2 Stephen Baker,8 Julian Parkhill,9 Nazima Pathan1,1 ARCTIC research team10

ABSTRACT
Objective Selective decontamination of the digestive tract (SDD) is a well-studied but hotly contested medical intervention of enhanced infection control. Here, we aim to characterise the changes to the microbiome and antimicrobial resistance (AMR) gene profiles in critically ill children treated with SDD-enhanced infection control compared with conventional infection control.

Design We conducted shotgun metagenomic microbiome and resistome analysis on serial oropharyngeal and faecal samples collected from critically ill, mechanically ventilated patients in a pilot multicentre cluster randomised trial of SDD. The microbiome and AMR profiles were compared for longitudinal and intergroup changes. Of consented patients, faecal microbiome baseline samples were obtained in 89 critically ill children. Additionally, samples collected during and after critical illness were collected in 17 children treated with SDD-enhanced infection control and 19 children who received standard care.

Results SDD affected the alpha and beta diversity of critically ill children to a greater degree than standard care. At cessation of treatment, the microbiome of SDD patients was dominated by Actinomyces, specifically Bilophobacterium, at the end of mechanical ventilation. Altered gut microbiota was evident in a subset of SDD-treated children who returned late longitudinal samples compared with children receiving standard care. Clinically relevant AMR gene burden was unaffected by the administration of SDD-enhanced infection control compared with standard care. SDD did not affect the composition of the oral microbiome compared with standard treatment.

Conclusion Short interventions of SDD caused a shift in the microbiome but not of the AMR gene pool in critically ill children at the end mechanical ventilation, compared with standard antimicrobial therapy.

WHAT IS ALREADY KNOWN ON THIS TOPIC
⇒ Selective decontamination of the digestive tract (SDD)-enhanced infection control has a significant impact on the reduction of ventilator-associated pneumonia during hospitalisation for critical illness.
⇒ SDD reduces morbidity and mortality in adult intensive care units.
⇒ Large studies examining the changes in antimicrobial resistance attributable to SDD have relied on microbial culture or quantitative PCR.

WHAT THIS STUDY ADDS
⇒ This study observed no significant change in clinically relevant antimicrobial resistance gene abundance when patients were treated with SDD-enhanced infection control compared with standard care.
⇒ Changes were observed in the gut microbiome of children treated with SDD, with perturbation continuing up to 2–3 months post-treatment.
⇒ This six-site cluster randomised trial is the largest study to examine longitudinal changes to antimicrobial resistance genes using shotgun metagenomic sequencing.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY
⇒ This study prepares the groundwork for a larger multicentre trial which can examine longer exposures to SDD-enhanced infection control in children.
⇒ This study suggests that microbiome recovery may be delayed after SDD treatment.

INTRODUCTION
The reduction of healthcare-associated infections (HCAIs) and improved antimicrobial stewardship are important for patient outcomes and improving global health.

The majority of HCAIs are caused by a small subset of opportunistic pathogens, generally Staphylococcus aureus, Candida albicans and
Gram-negative aerobic rods (GNARs). Ventilator-associated pneumonia (VAP) was previously reported as the second most common form of HCAI after bloodstream infection, with VAP affecting between 10% and 20% of children admitted to paediatric intensive care unit (PICU) with a 30-day mortality of 30%. However, recent studies indicate that VAP is overtaking bloodstream infections in PICU settings contributing to 56% of all PICU HCAIs, leading to lengthened hospitalisation.

Microbiological diagnosis of VAP is suboptimal with a high proportion of culture-negative samples, but in many cases, intestinal organisms are identified. Selective digestive tract decontamination (SDD, also described as selective decontamination of the digestive tract) aims to reduce the ingress of intestinal microorganisms, including GNAR and Candida spp into the lower respiratory tract of mechanically ventilated patients and reduce the risk of VAP.

Multiple reviews of clinical trials of adult patients have concluded that a full protocol of oral paste and gastric suspension SDD combined with intravenous antimicrobials was effective in reducing VAP compared with standard care (SC). The recent SuDDICU trial reported that SDD treatment reduced the incidence of phenotypical antimicrobial resistance (AMR) from cultured organisms and new bacteria compared with conventional treatment, but did not significantly affect overall mortality in adult intensive care units (ICUs).

Paediatric trials of SDD have generally been small, single-unit trials. Three out of four trials reported a reduction of infections from Gram-negative bacteria in the SDD group, while Barret et al. observed no difference in infection rate. To address the paucity of SDD trial data available in a paediatric clinical setting, the Paediatric Intensive Care and Infection Control (PiCnIC) pilot clinical trial examined the feasibility for a future definitive clinical trial to examine the safety and efficacy of SDD-enhanced infection control to prevent HCAIs in critically ill children requiring at least 48 hours of mechanical ventilation.

In this pilot study, we outlined the baseline changes of AMR in critically ill children who had received mechanical ventilation and antimicrobial therapy and leveraged the wider PiCnIC Study by examining the temporal changes on the microbiome composition and antimicrobial gene carriage in faecal and oropharyngeal compartments in a subset of critically ill children receiving either standard or SDD-enhanced infection control.

Having previously reported on PICU-derived changes in paediatric microbiomes through 16S rRNA gene sequencing, this study is the first to examine the proportional composition of microbiota linked to the pool of AMR genes and the effect of SDD-enhanced infection control both acutely and during recovery in critically ill children.

To study the effects of both standard and SDD-enhanced infection control on oropharyngeal and intestinal microbiota and the temporal effects on AMR gene carriage, paired oropharyngeal and rectal swabs were taken from a subset of critically ill children enrolled to a National Institute for Health and Care Research-funded pilot trial of SDD (the PiCnIC trial, IRAS 239234, ISRCTN40310490).

MATERIALS AND METHODS

An extended method section is available in the online supplemental materials and methods.

Trial design

The protocol for the pilot SDD trial was previously reported by Brown et al. (ISRCTN40310490) with further details available from Pathan et al. The study consisted of five periods: pre-study ecology (1 week), baseline phase (phase one) (8 weeks), pre-intervention ecology (1 week), intervention phase (phase two) (9 weeks) and post-study ecology (1 week). The intervention phase was divided into SC and SDD, with three hospitals randomly assigned to each arm. All patients were administered standard antimicrobial therapy as needed. SDD was administered in addition to SC at 6-hour intervals. The SDD treatment consisted of an oral paste consisting of tobramycin (2% weight per volume (w/v)), colistin (2% w/v) and nystatin (2% w/v), and a suspension containing tobramycin (10 mg/mL), colistin (8 mg/mL) and nystatin (2×10^5 IU/mL). SC in UK PICUs is the administration of a third-generation cephalosporin upon arrival in the PICU unless otherwise indicated.

Enrolment and consent

In order to establish the immediate impact of critical illness on the microbiome, faecal samples were taken at admission in a cohort of critically ill children requiring mechanical ventilation for suspected lower respiratory tract infection. A delayed consent model was used, and informed consent was obtained within 48 hours of sample collection.

To evaluate the impact of SC and SDD-enhanced infection control on the paediatric faecal microbiome during and after critical illness, paired oropharyngeal and faecal samples were collected from a subset of children enrolled to the PiCnIC pilot trial following informed consent, within 6 hours of admission (prior to SDD treatment), peri-extubation when SDD treatment was completed and in convalescence (6–8 weeks post-discharge).

Age-matched healthy control microbiomes were selected from previously published data from the USA (n=63).

Patient cohort

The PiCnIC Study enrolled 368 children. Phase one recruited 207 children to SC, with phase two recruiting 161 children. Children recruited during phase two were divided by intervention with 94 recruited at SC units and 67 recruited at units administering SDD. This substudy enrolled 36 children across both trial phases. Phase one recruitment comprised three children to whom SC was administered, with the remaining 16 children recruited during phase two. All 17 SDD-treated patients were recruited during phase two.

Sample collection

Oropharyngeal and rectal swabs were taken as soon as clinically feasible using a sterile cotton swab (MWE Medical Wire, Corsham, UK) and stored in sterile containers containing 1 mL of DNA/RNA Shield (Zymo Research, Irvine, California, USA). Oral swabs were used in place of oropharyngeal swabs for home collection kits. In children enrolled to the intervention arm of the PiCnIC trial, pretreatment oropharyngeal swabs were performed after the initiation of mechanical ventilation but prior to first application of the oropharyngeal paste. Pretreatment rectal swabs from SDD patients were taken as soon as possible but within 6 hours of SDD treatment. Pretreatment swabs from SC patients were taken as soon as possible after mechanical ventilation. For all patients, when a clinical decision was made to end ventilation, treatment swabs were collected peri-extubation; oropharyngeal and rectal swabs were taken as soon as clinically possible once extubation was decided. Collected swabs were stored in 1 mL of DNA/RNA Shield. Where facilities permitted, swabs were stored at −80°C as soon as possible and shipped on dry ice, otherwise swabs were returned to Cambridge University.
Hospital at ambient temperature via priority mail. Post-PICU recovery samples were collected between 2 and 3 months after PICU discharge by families and returned directly to the University of Cambridge. Oral swabs were stored in 1 mL of DNA/RNA Shield, and faecal samples were collected using OmniGene Gut tubes (DNA Genotek, Ottawa, Ontario, Canada). Samples were returned to Cambridge University Hospital at ambient temperature by priority mail. Samples were received at the University of Cambridge and stored at −80°C until extraction.

Sample processing and sequencing library preparation
Samples were extracted using the PowerSoil Pro DNA extraction kit (Qiagen, Hilden, Germany). For oropharyngeal, oral and rectal swabs, 400 µL of DNA/RNA Shield preservation medium containing sample was extracted with 400 µL of extraction buffer. For OmniGene Gut tubes, 250 µL of medium-containing sample was extracted with 550 µL of extraction buffer.

DNA quantification was performed using a Qubit4 (ThermoFisher, Waltham, Massachusetts, USA) high-sensitivity double-stranded DNA assay. Sequencing libraries were prepared using the NEBNext Ultra II DNA library preparation kit (NEB, Ipswich, Massachusetts, USA) following the standard protocol and sequenced with a NovaSeq 6000 (llumina, San Diego, California, USA).

Data processing
Sequences were quality trimmed using trim_galore V.0.5.0 and host sequences were removed using Bowtie2 V.2.3.5.1 and the GRCh38.p14 human reference genome. Metagenomic composition was determined from cleaned reads using Kraken2 V.2.0.9-beta and the nt database (05/05/2023). AMR genes were identified from paired-end reads using ARIBA V.2.14.6 and the CARD database V.3.2.7. When comparing intercohort datasets, we corrected for batch effect using Gibbons et al’s percentile normalisation method.

Statistical analysis was performed using R V.4.2.0. Read counts were normalised per million reads. Phylogenetic analysis consisting of non-metric dimensional scaling (nMDS) clustering of Bray-Curtis distances and alpha diversity was performed using vegan V.2.6-2. Clustering analysis was performed with the Adonis2 function, controlling for multiple measures where appropriate. Multiple comparisons of non-parametric variables were performed using repeated Wilcoxon tests with the paired test used as required for paired data. Multiple comparison adjustment was performed using the Benjamini-Hochberg method, and probability is reported as the q value (adjusted p value) in cases of multiple comparisons. Data transformation and plotting were performed using the tidyverse suite. MaAsLin2 V.1.10.0 was used for multivariate analysis. We chose to set alpha at a rate of 1 in 20 for p and q values.

Analysis of resistance genes
To focus the analysis of AMR, genes involved in resistance to non-clinically relevant compounds, such as heavy metals, were excluded. Tetracyclines are not indicated for use in children due to the effects on bones and teeth. Genes detected with ARIBA were compiled for each patient and measured in reads per kilobase gene per megabase of sequencing (RPKM).

Trial reporting
This report used the Strengthening the Reporting of Observational Studies in Epidemiology cohort reporting guidelines.

An extended materials and methods section is available in the online supplemental materials and methods.

RESULTS
Patient information
A baseline PICU population that comprised of 86 children admitted to the PICU between 2020 and 2022, for whom faecal samples were collected within the first 48 hours of PICU admission was examined for faecal microbiome composition and AMR gene carriage. Further patient demographics were reported by Clark et al. Samples from these patients were compared with the microbiomes of 63 age-matched healthy children (table 1).

The study population comparing SDD with SC was formed of 36 children from whom serial samples were collected (figure 1). Of these, 17 received SDD and 19 received SC. Primary admission diagnoses are shown in table 1. Antimicrobial use is summarised in online supplemental figure 1A. The PincNC trial was a pilot trial to assess the feasibility of administering SDD on a unit-wide basis in PICUs in England and was not powered for clinical significance. This substudy was also a pilot project to identify the feasibility of monitoring the microbiome and AMR of critically ill children receiving SDD-enhanced infection control. The strict criteria required for serial sample collection reduced our enrolment to 36 patients (figure 1).

Faecal microbiome composition but not AMR gene carriage at PICU admission is impacted by antimicrobial administration
Despite the high level of antimicrobials administered to critically ill children, alpha diversity (Shannon’s Index) in admission samples remained comparable with healthy controls (figure 2A). This trend was also observed with Chao1 (figure 2B). Critically ill children had increased representation of opportunistic pathogens such as Enterococcus, Klebsiella and Escherichia, and reduced abundance of complex carbohydrate fermenters such as Bacteroides, Phocaeicola, Faecalibacterium and members of the Lachnospiraceae (figure 2C). When comparing beta diversity calculated as Bray-Curtis distance using nMDS plotting, we observed distinct separation of study groups after applying the correction by Gibbons et al to correct for technical factors (permutational analysis of variance (PERMANOVA) p=0.002; figure 2D). MaAsLin2 analysis indicated that many opportunistic pathogens including those with intrinsic resistance to colistin were significantly enriched in our baseline critically ill children, and secondary fermenters were decreased (figure 2E, all values of q<0.05).

When comparing the AMR profiles of US healthy control children and UK critically ill children, we observe difference in their composition with significant separation of AMR profiles based on gene family (figure 2F; PERMANOVA p<0.001). When comparing the class of AMR target with MaAsLin2, we observed that the gut microbiota of critically ill children had increased levels of resistance genes to drugs in the classes phosphonic acids, elfamycins, peptide antimicrobials, gysre and topoisomerase inhibitors, and disinfecting agents along with multidrug efflux pumps. The control microbiomes were enriched for genes conferring resistance to tetracyclines (figure 2G, all q values of <0.05). We observed no significant difference in the median total AMR gene burden (RPKM) between antimicrobial-exposed admission samples of critically ill children and age-matched healthy children (p=0.545; figure 2I). From these results, we endeavoured to understand the changes manifested by the administration of standard antimicrobial therapy and SDD-enhanced infection control longitudinally on the microbiome and resistome.
SDD is associated with distinct compositional alterations in the lower GI microbiome

No statistically meaningful fluctuation was observed in the alpha diversity of children receiving SC measured by Shannon’s Index (figure 3A). In contrast, the median alpha diversity of SDD patients declined over treatment and remained lower at 2–3 months post-treatment than controls (figure 3A). No significant difference was observed for any alpha diversity index or pairwise comparison therein after false discovery rate (FDR) correction (figure 3A,B). The beta diversity of SC patients diverges between admission and extubation samples, but the recovery samples cluster tightly with the admission samples (figure 3B). The beta diversity of children receiving SDD-enhanced infection control clustered closely at admission and extubation; however, there was a large divergence upon recovery from admission samples. After accounting for pairwise observations, no clusters were statistically separated by multivariate PERMANOVA after FDR adjustment. Relevant beta diversity comparisons are illustrated in online supplemental figure 2.

Children who received SDD-enhanced infection control (n=17) had 10 dominant taxa whose median composition accounted for the majority of gut microbial composition (figure 3D–F). At the genus taxonomic level, these 10 taxa based on sequencing read abundance were: *Bifidobacterium, Bacteroides, Enterococcus, Escherichia, Finegoldia, Klebsiella, Veillonella, Anaerococcus, Mediterraneibacter* and *Streptococcus*. As a comparator of our clinical cohort, we selected the 10 taxa with the largest median abundance in the SC admission group not represented in the top 10 control bacteria: *Eggerthella, Prevotella, Phocaeicola, Erysypheicolosistradium, Collinsella, Blautia, Flavonifractor, Rhabdibacterium, Akkermansia* and *Citrobacter*. At admission, a high degree of similarity between the median profiles of SC patients and SDD patients was observed (figure 3C and online supplemental figure 3). Analysis of admission microbiomes using MaAsLin2 identified significantly increased *Acinetobacter* (q=7.356×10⁻⁴, coefficient=3.17), *Finegoldia* (q=5.579×10⁻⁶, coefficient=2.52), *Peptoniphilus* (q=3.696×10⁻¹, coefficient=1.74) and *Rothia* (0.0045, coefficient=0.63) in the SDD cohort. At extubation, the median relative abundance of *Bifidobacterium* was highly divergent between treatments; however, none of the 20 most abundant taxa from admission samples were significantly different after correction for multiple tests (figure 3D and online supplemental figure 4). MaAsLin2 analysis of the extubation microbiome identified enrichment of *Dialister* (q=1.23×10⁻⁵, coefficient=2.54), *Lactasebacillus* (q=5.27×10⁻⁵, coefficient=2.62), *Selenimonas* (q=8.518×10⁻¹, coefficient=2.22) and *Clostridium* (q=0.0373, coefficient=0.96) in SC patients, while SDD patients had increased *Stutzerimonas* (q=0.01811, coefficient=4.27), *Acinetobacter* (q=0.0249, coefficient=4.61), *Paracoccus* (q=0.0249, coefficient=4.07) and *Rhabdibacterium* (q=0.0322, coefficient=0.99); however, median levels of *Acinetobacter, Stutzerimonas* and *Paracoccus* in both groups were below 0.01% by relative proportion. The compositional difference between SDD and SC patients at phylum, family, genus and species level can be observed in online supplemental figure 5.

### Table 1 Patient information

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Healthy controls (n=63)</th>
<th>PICU Baseline (n=86)</th>
<th>Standard care (n=19)</th>
<th>SDD (n=17)</th>
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<tr>
<td>Age (years), med (IQR)</td>
<td>1.2 (0.4–4.3)</td>
<td>1.2 (0.4–5.2)</td>
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<td>Sex (male)</td>
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<td>56 (65.1%)</td>
<td>11 (57.9%)</td>
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<td>Weight (kg), med (IQR)</td>
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<td>Hospital stay</td>
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<td>PIM3 score, med (IQR)</td>
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<td>Ventilation time (hours), med (IQR)</td>
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<td>87.0 (60.5–125.0)</td>
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<td>Ventilation-free hours, med (IQR)</td>
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<td>6 (35.3%)</td>
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<td>Survival</td>
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<td>Hospital discharge (%)</td>
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<td>30 days post-PICU (%)</td>
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Data presented as median values with 25th and 75th centile.

*Days free from PICU or ventilation at 30 days post-admission.

**SDD**, not recorded; **PICU**, paediatric intensive care unit; **PIM3**, Paediatric Index of Mortality 3; **SDD**, selective decontamination of the digestive tract.
At recovery, the SDD microbiomes were dominated by *Bifidobacterium* (figure 3F); however, this difference was not significant from SC (online supplemental figure 6). No significant difference in bacterial composition was identified in the top 20 bacteria (corrected for multiple comparisons). The changes in microbiome of patients receiving SDD-enhanced infection control who returned a recovery sample can be seen in online supplemental figure 7.

**SDD does not affect overall composition in the oropharyngeal microbiome**

The oropharyngeal microbiome can be considered a low-biomass biome, as such the majority (80%, IQR 66–86%) of shotgun metagenomic sequencing reads were from host DNA. After correcting for host reads and normalisation, we examined the alpha and beta diversity of the oropharyngeal...
microbiome. No significant difference in alpha diversity was observed in either the SC or SDD group at all time points for Shannon’s Index or Chao1 (figure 4A,B). We observed very little deviation in the beta diversity of oropharyngeal microbiota of patients in either treatment group or between sampling time points (figure 4C and online supplemental figure 8). In the SC group at admission, a dominance of Streptococcus and Rothia with high amounts of Neisseria was observed (figure 4D). In the SDD patients, the 10 most abundant taxa were Streptococcus, Rothia, Veillonella, Actinomyces, Schaadia, Prevotella, Haemophilus, Neisseria, Staphylococcus and Granullicatella (figure 4D). MaAsLin2 identified higher carriage rates of Pseudomonas in SDD patients ($q=3.998 \times 10^{-5}$, coefficient=2.15) and Corynebacterium in SC patients ($q=1.259 \times 10^{-4}$, coefficient=1.65). At extubation, SC patients had expanded representation of Prevotella, Actinomyces and Corynebacterium. This was combined with a reduction in the median proportion of Neisseria, Haemophilus and Schaadia (figure 4D). An expansion of Streptococcus and Prevotella was observed.
Figure 3  Changes in the lower GI microbiome during SDD-enhanced infection control in critically ill children. (A) Alpha diversity of patients calculated at species level using Shannon’s Index. No significant difference was observed after correcting for multiple comparisons. (B) Chao1 index of microbial richness. No significant difference was observed after correcting for multiple comparisons. (C) nMDS plot of beta diversity clustering of faecal microbiomes based on Bray-Curtis distances. No significant difference was observed in the clustering of any group after multiple corrections. Diamond=SDD admission, inverted triangle=SDD extubation, asterisk=SDD recovery, circle=SC admission, square=SC extubation, triangle=SC recovery. (D) Composition of the lower GI microbiome of patients at admission compared with healthy controls. The 10 most abundant families of microbiota by median proportion in the GI microbiome of standard care admission patients, and the 10 most abundant microbiota families in SDD admission patients by median proportion not represented in the first list were selected with the remaining taxa combined as ‘other’. (E) Lower GI composition of patients at extubation. Taxa are identified as in D. (F) Microbiome composition as median proportions at recovery. Taxa identified as in D. nMDS, non-metric dimensional scaling; SC, standard care; SDD, selective decontamination of the digestive tract.

Figure 4  Changes in the oral microbiome during SDD-enhanced infection control in critically ill children. (A) Alpha diversity in SC patients measured as Shannon’s Index at species level at admission, extubation and recovery 2–3 months post-admission. (B) Chao1 index of microbial richness. (C) Microbiome beta diversity of PICU patients receiving SC. No statistical difference was calculated by PERMANOVA. Diamond=SDD admission, inverted triangle=SDD extubation, asterisk=SDD recovery, circle=SC admission, square=SC extubation, triangle=SC recovery. (D) Composition of the oral microbiome of patients at admission compared with healthy controls. The 10 most abundant families of microbiota by median proportion in the oropharynx of SDD patients at admission, and the 10 most abundant microbiota families in SC admission patients by median proportion not represented in the first list were selected with the remaining taxa combined as ‘other’. (E) Oral microbiome composition of patients and controls at extubation. Taxa are identified as in D. (F) Microbiome composition as median proportions at recovery. Taxa identified as in D. MDS, metric dimensional scaling; PERMANOVA, permutational analysis of variance; PICU, paediatric intensive care unit; SC, standard care; SDD, selective decontamination of the digestive tract.
in the SDD group at extubation, paired with a reduction in the median proportion of *Actinomyces* and *Veillonella* (figure 4E). MaAsLin2 analysis of the oral microbiome indicated significantly increased *Gemella* \(q=4.263 \times 10^{-10}\), \(c=2.99\) and *Haemophilus* \(q=5.20 \times 10^{-7}\), \(c=2.38\) in SDD patients. The microbiome of SC children at recovery was similar to their admission microbiome (figure 4F). SDD oral microbiomes at recovery had increased median *Rothia* (figure 4F). No significant differences were observed for any of the top 20 oral bacterial families between SC and SDD patients at each time point (online supplemental figures 9–11). MaAsLin2 did not identify any bacterial taxa with significant difference between treatment groups at recovery.

**SDD does not increase GI AMR gene carriage burden compared with standard infection control in critically ill children**

We found no significant difference in the median total RPKM across time points and treatment groups (figure 5). Resistance genes against 25 different drug classes and pathways of multidrug resistance were identified in our study (figure 5). The major component of most AMR profiles was macrolide–lincosamide–streptogramin (MLS) resistance (figure 5B and online

Figure 5 AMR gene proportion and composition from faecal and oral samples. AMR genes were assembled by ARIBA to the MEGARes V.3.0 library and normalised as reads per kilobase per megabase of sequencing (RPKM). (A) Total AMR gene burden in the lower GI microbiome. The sum of all AMR gene RPKM was compared across all groups. (B) RPKM of each AMR class detected in the faecal microbiome of individuals with SC. (C) RPKM of each AMR class detected in the faecal microbiome of individuals with SDD-enhanced infection control. (D) nMDS of AMR genes in faecal samples identified by ARIBA using the CARD V.3.2.7 database. Diamond=SDD admission, inverted triangle=SDD extubation, asterisk=SDD recovery, circle=SC admission, square=SC extubation, triangle=SC recovery. (E) Total AMR gene burden in the oropharyngeal microbiome. The sum of all AMR gene RPKM was compared across all groups. (F) RPKM of each AMR class detected in the oropharyngeal microbiome of individuals with SC. (G) RPKM of each AMR class detected in the oropharyngeal microbiome of individuals with SDD-enhanced infection control. (H) nMDS of AMR genes in oral samples identified by ARIBA using the CARD V.3.2.7 database. Diamond=SDD admission, inverted triangle=SDD extubation, asterisk=SDD recovery, circle=SC admission, square=SC extubation, triangle=SC recovery. AMR, antimicrobial resistance; MLS, macrolide, lincosamide and streptogramin B; nMDS, non-metric dimensional scaling; SC, standard care; SDD, selective decontamination of the digestive tract.
supplemental figure 14). The second most abundant resistance class was ribosomal subunits with mutations conferring resistance against two or more drug classes followed by non-ribosomal tetracycline resistance, multidrug efflux pumps, beta-lactams and peptide antibiotic resistance (figure 5, online supplemental figure 12B,C and online supplemental figure 14).

No mcr colistin resistance genes were detectable in the microbiome of our patients in this study and we observed no major change in the proportion of colistin-resistant organisms across treatment groups or time points.

At admission, MaAsLin2 identified significantly more representation of membrane porins with reduced permeability to beta-lactams (q=8.76×10^{-3}, coefficient=1.31), triclosan-resistant gyrA (q=8.76×10^{-3}, coefficient=1.30) and daptomycin-resistant gshF (q=9.51×10^{-3}, coefficient=1.21). From extubation samples, MaAsLin2 identified significantly increased representation of tetracycline protection proteins (tetO, tetM, tetW, tet32) (q=1.06×10^{-3}, coefficient=1.63) and ctx-M beta-lactamasises (q=0.0455, coefficient=1.01) in SC patients, while SDD patients had an increase in fluoroquinolone-resistant parC (q=3.295×10^{-3}, coefficient=1.67). No significant differences in AMR genes between treatment groups were observed at recovery. The majority of AMR genes classified as resistant to peptide antimicrobials were identified as daptomycin or lysisin resistance genes in Gram-positive organisms. We identified the gene families annk, annT, basR, basS, cprB, cprS, eptA, eptB, pmrF and ugd which are involved in 4-amino-4-deoxy-L-arabinose biosynthesis and polyoxin resistance in the faecal microbiomes of our patients. No genes were significantly different between groups after correcting for multiple comparisons (online supplemental figure 15). Of the 35 beta-lactam resistance genes identified, 23 were beta-lactamasises. Examining the gene families ace, act, ampc, blaz, cdd, cmy, ctx-M, cbiA, cepA, cfxA, dha, ec, en, len, mal, mdr, orn, oxa oxy, pdc, pla, slv and tem, we observed no significant difference between gene family presence after correction for multiple comparisons (online supplemental figure 16). The aminoglycoside resistance genes identified from sequencing were aac(3)-IId, aac(6’)-Ib7, aac(6’)-If, aac(6’)-Ii, aac(6’)-Im, aac(6’)-Ie-aph(2)-la, aac(6’)-IB-Su, ant(2”)-Ia, ant(3’’)-Ia, ant(4’)-la, ant(6’)-la, ant(6’’)-Ia, ant(9’)-Ia, aph(2”)-Ia, aph(2”)-Ig, aph(3’)-Ib, aph(3’)-Ila, aph(3’)-Iia, aph(3’)-Iib, aph(3’)-la, aph(6’)-Ia, cbrC, cfr, emrE, aadA, aadA13, aadA2, aadA24, aadA5, aadS, acrD, kdpD and kdpE (genes conferring resistance to tobramycin highlighted in bold text). No significance was observed after corrections for multiple comparisons (online supplemental figure 17).

**DISCUSSION**

The use of SDD for infection control and AMR is a hotly contested topic revolving around the effect of SDD on AMR.7 14 Reduction in AMR has previously been reported in SDD trials in critically ill adults. By leveraging a pilot cluster randomised clinical trial,15 we were able to examine the microbiomes and resistomes of children treated in PICUs receiving SDD or standard infection control for the duration of mechanical ventilation.

We observed no difference in total AMR gene abundance between these groups. Of study-relevant AMR classes, only ctx-M beta-lactamasises (one of the leading extended-spectrum beta-lactamasises) were differentially increased at extubation in SC patients. This could suggest an important role for SDD in controlling the spread of this critically important class of antibiotic resistance genes.

Changes to the microbiome of critically ill children have previously been examined using 16S rRNA gene sequencing,37 38 but this is the first study to use metagenomic shotgun sequencing on longitudinal samples in the paediatric population in order to examine the AMR pool of the upper and lower GI microbiome.

Buelow et al38 reported microbiome and resistome changes in a single adult patient receiving SDD-enhanced infection control using shotgun metagenomics. They reported a significant increase in tobramycin-modifying enzyme after 14 and 16 days of SDD38; this result was not observed in our study which had much shorter per-patient intervention duration. The highly individual effect of aminoglycoside resistance observed previously38 and the lack of significant change in AMR gene burden observed in our study would suggest that SDD is no worse than SC in driving AMR in PICUs; however, a sufficiently powered study is required to confirm this.

While many clinical trials and systematic reviews have reported the efficacy of SDD,6 9 11 14 38–43 the argument against the use of SDD in clinical practice persists.38 44–46 However, care must be taken when comparing the findings of our study with data from adult patient cohorts given the differences in microbiome composition and underlying morbidity between children and adults.

Certainly, our work highlights the importance of assessing microbiome recovery in any future definitive studies of SDD-enhanced infection control in critically ill children.

We found the microbiome richness of critically ill children to decrease with increased duration of stay in the PICU.37 A small decrease in alpha diversity was noted in the SDD-treated cohort at the time of extubation and in samples taken in convalescence. This is most likely due to the targeting of metabolic keystone species by both routinely administered antimicrobials as well as SDD.47 48 It would be remiss to assume that because SDD was designed to control the abundance of GNAR, that they are the only affected taxa.49

Previous studies of SDD in adults have reported reduced VAP and are variably associated with the reduction in HCAIs.9 14 40 Of the Gammaproteobacteria, the *Enterobacteriaceae* represented the largest proportion of gut-resident GNAR in our study. The proportion of *Enterobacteriaceae* in faecal samples was reduced after treatment with SDD compared with admission samples and post-treatment SC samples. From these data, we can conclude that SDD was effective in reducing susceptible GNARs in our...
patients. Previous work has reported the eradication of *E. coli* by days 9–10 of full SDD administration with intravenous third-generation cephalosporins.\(^{31}\) Our median time to extubation was 4–5 days at which point SDD was discontinued. In the study by Buelow et al.,\(^{31}\) an SDD administration time between 1 and 5 days coincided with relative proportions of *E. coli* 16S rRNA gene signal in critically ill adult patients that was comparable with healthy controls. This delayed reduction of bacterial burden may explain the presence of moderate levels of *Escherichia* in our extubation samples. The reduction in the median proportion of the *Enterobacteriaceae* at extubation was expected, but there was an unexpected increase in their median proportion in recovery samples, combined with an expansion in *Bifidobacterium* and a decrease in observed alpha diversity. The *Pseudomonadaceae* were not significantly affected by SDD treatment; however, a larger study is needed to confirm this effect.

It is well established that antimicrobials affect the microbiome.\(^{52-55}\) Chng et al.\(^{56}\) reported a sustained decrease in alpha diversity after the cessation of antimicrobial therapy and identified the absence of early and midpoint fermenter organisms\(^{56}\) generating metabolites used as an energy source by *Enterobacteriaceae*.\(^{31}\) This change may be driven by SDD treatment coincided with reduced AMR in bacteria,\(^{9,42,60}\) which is to administer a third-generation cephalosporin or a broad-spectrum penicillin in combination with a beta-lactamase inhibitor. Our healthy controls were selected from a large cohort study conducted in the USA due to lack of available shotgun metagenomic data for UK and European children. The technical factors between our children and the US control children metagenomic sequencing may differ.

**CONCLUSION**

SDD did not increase the AMR burden of patients at the end of their treatment but may extend the microbiota recovery period of patients. Our study observed that patient microbiomes were different during extubation and recovery. SDD patients had a greater abundance of *Actinomycetota* at extubation and reduced *Enterobacteriaceae*, while recovery SDD samples appeared high in Gram-negative organisms. More change was observed in the microbiome of SDD-treated patients than the SC group and is most likely due to the effects of SDD.

**Author affiliations**

1Department of Paediatrics, University of Cambridge, Cambridge, UK
2Cambridge University Hospitals NHS Foundation Trust, Cambridge, UK
3ICNARC, London, UK
4Sunnybrook Hospital, Toronto, Ontario, Canada
5University of Southampton, Southampton, UK
6University College London, London, UK
7The George Institute for Global Health, Newtown, New South Wales, Australia
8Department of Medicine, University of Cambridge, Cambridge, UK
9Department of Veterinary Medicine, University of Cambridge, Cambridge, UK
10Department of Veterinary Medicine, University of Cambridge, Cambridge, UK
11ARCTIC research team, Cambridge, UK

**Twitter** John A Clark @doctorjclark and Nazima Pathan @drnazimapathan

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**Methods section for further details.**

**Patient consent for publication** Not required.

**Ethics approval** An ethics application was made to the West Midlands-Black Country Research Ethics Committee (20/WM/0061) and received a favourable opinion on 3 November 2020, with approval granted by the HRA on 20 November 2020. The PICnIC pilot RCT was registered on the ClinicalTrials.gov database. Registration was confirmed on 30 October 2020 (reference number ISRCTN40310490\(^{19}\)). Deferred written informed consent was obtained from the guardians of all patients in this trial. This substudy has been approved by the South West–Cornwall and Plymouth Research Ethics Committee (20/SW/0057, IRAS)

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**Patient and public involvement** Patients and/or the public were involved in the design, or conduct, or reporting, or dissemination plans of this research. Refer to the Methods section for further details.

**Patient consent for publication** Not required.

**Ethics approval** An ethics application was made to the West Midlands-Black Country Research Ethics Committee (20/WM/0061) and received a favourable opinion on 3 November 2020, with approval granted by the HRA on 20 November 2020. The PICnIC pilot RCT was registered on the ClinicalTrials.gov database. Registration was confirmed on 30 October 2020 (reference number ISRCTN40310490\(^{19}\)). Deferred written informed consent was obtained from the guardians of all patients in this trial. This substudy has been approved by the South West–Cornwall and Plymouth Research Ethics Committee (20/SW/0057, IRAS)
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Gut microbiota


Figure S1
Figure S1: Antimicrobial use in patient cohort, and comparison of healthy UK children to healthy US children. A) Antimicrobial use for children admitted to PICU. Antimicrobial use was scaled to doses (administered)/doses/day (age adjusted based on BNFC guidelines).
Figure S2
Figure S2: Pair- and treatment group-wise comparisons of nMDS clustering. Q values represent p values adjusted for multiple comparisons using the FDR correction.
Figure S3
Figure S3: Admission lower gastrointestinal tract microbiota. A comparison of the 20 microbiota identified in Figure 2. Total statistical difference calculated by Kruskal-Wallis test. Multiple tests corrected by FDR.
Figure S4
Figure S4: Extubation lower gastrointestinal tract microbiota. A comparison of the 20 microbiota identified in Figure 2. Total statistical difference calculated by Kruskal-Wallis test. Multiple tests corrected by FDR.
Figure S5
Figure S5: Comparison of extubation microbiomes at multiple taxonomic levels.
Figure S6
Figure S6: Recovery lower gastrointestinal tract microbiota. A comparison of the 20 microbiota identified in Figure 2. Total statistical difference calculated by Kruskal-Wallis test. Multiple tests corrected by FDR.
Figure S7
Figure S7: Compositional change of SDD patients. A-G) Alluvial plots identifying compositional change at Genus level in the seven SDD patients returning a recovery sample.
Figure S8
Figure S8: pair and treatment group-wise comparisons of nMDS clustering. Q values represent p values adjusted for multiple comparisons using the FDR correction.
Figure S9
Figure S9: Admission oropharyngeal microbiota. A comparison of the 20 microbiota identified in Figure 4. Total statistical difference calculated by Kruskal-Wallis test. Multiple tests corrected by FDR.
Figure S10
Figure S10: Extubation oropharyngeal microbiota. A comparison of the 20 microbiota identified in Figure 4. Total statistical difference calculated by Kruskal-Wallis test. Multiple tests corrected by FDR.
Figure S11
Figure S11: Recovery oropharyngeal microbiota. A comparison of the 20 microbiota identified in Figure 4. Total statistical difference calculated by Kruskal-Wallis test. Multiple tests corrected by FDR.
Figure S12
Figure S12: Pair and treatment group-wise comparisons of nMDS clustering for Faecal AMR. Q values represent p values adjusted for multiple comparisons using the FDR correction.
Figure S13
Figure S13: Pair and treatment group-wise comparisons of nMDS clustering for Oral AMR. Q values represent p values adjusted for multiple comparisons using the FDR correction.
Figure S14
Figure S14: Antimicrobial resistance changes in the faecal microbiome of critically ill children. Antimicrobial resistance genes assembled using ARIBA against the CARD database v 3.2.7 were examined to determine differences between standard care and SDD-enhanced infection control. Gene counts were normalised as reads per kilobase(gene) per million sequencing reads (RPKM). A) Total gene count, b) Macrolides, Lincosamides, and Streptogramins (MLS), c) Multi-compound ribosomal mutants, d) Tetracyclines, e) Multi-drug efflux, f) beta-lactams, g) gyrases and topoisomerase inhibitors, h) peptide antibiotics (including polymyxin), i) phosphonic acid and derivatives, j) inhibitors of rpoB, k) mupirocin-like compounds, l) aminoglycosides, m) elfamycins, n) disinfecting agents and detergents (triclosan), o) dianimopyrimidines, p) sulfonamides, q) genes reducing permeability to antimicrobials, r) oxazolidinone compounds, s) nitrofurans, t) nitroimidazoles, u) multi-drug acetyltransferases, v) phenicols, w) pyrazines, x) pleuromutilins, y) nucleosides, z) glycopeptides, aa) fusidane, ab) bicyclomycin-like.
Figure S15
Figure S15: Comparisons of Colistin resistance genes. Multiple comparisons performed with repeated Wilcoxon tests and p values adjusted using FDR.
Figure S16
Figure S16: Comparisons of beta-lactamase genes. Multiple comparisons performed with repeated Wilcoxon tests and p values adjusted using FDR.
Figure S17
Figure S17: Comparisons of aminoglycoside resistance genes. Multiple comparisons performed with repeated Wilcoxon tests and p values adjusted using FDR. Genes AAC(3)-IId, AAC(6')-Ib7, AAC(6')-If, AAC(6')-II, AAC(6')-Im, AAC(6')-le-APH(2)-Ia, AAC(6')-IB-Su, ANT(2'')-Ia, APH(2'')-IIa, APH(2'')-If, APH(2'')-Ig are reported in the literature to confer tobramycin resistance.
SUPPLEMENTAL MATERIALS AND METHODS

Data Processing:

Sequences were quality trimmed using trim_galore v0.5.0 with default settings [1], and host sequences were removed using Bowtie2 v2.3.5.1 [2] and the GRCh38.p14 human reference genome [3]. Metagenomic composition was determined from cleaned reads using Kraken2 v2.0.9-beta [4] and the nt database (2023/05/02) [5].

Kraken2 was run with paired input sequences and confidence set to 0.1. Kraken data was compiled by identifying reads at each taxonomic level. Taxonomic classification was compiled at species level, with reads identified only at higher taxonomic levels being designated as "_unclassified". Only taxa with > 10 reads were included in further analysis.

AMR genes were identified from paired-end reads using ARIBA v2.14.6 and the CARD database v 3.2.7 [6,7]. ARIBA was run with an assembly threshold of 0.6 and a minimum id value of 80.

Statistical analysis was performed using R v4.2.0 [8]. Read counts mapping to host DNA, spurious taxa (plants, food, contaminants in negative controls) were removed from further analysis. After the removal of confounding counts, bacterial sequence counts were normalised to reads per million reads. Using the assumption in which sequencing reads are modelled along a Gamma distribution, unclassified reads of higher taxonomic level were proportionally distributed across their subdivisions.

To perform analysis of the alpha- and beta-diversity of samples, taxonomy was aggregated at species level. Using vegan v 2.6-2 [9], and the diversity function, we calculated Shannon’s Index for each sample. Shannon’s index was visualised by
creating Tukey’s Box and Whisker plot using the Tidyverse package ggplot2 [10] with the plotting function geom_boxplot. To identify changed between groups, paired Wilcoxon tests were used for intra-treatment samples, and unpaired test for inter-treatment comparisons. Comparisons were made between the following groups SDD-1:SDD-2, SDD-1:SDD-3, SDD-1:SC-1, SDD-2:SDD-3, SDD-2:SC-2, SDD-3:SC-3, SC-1:SC-2,SC-1:SC-3, and SC-2:SC-3. The resultant p-values for the repeated Wilcoxon tests were corrected using Benjamini and Hochberg’s False Discovery Rate (FDR) correction as applied by p.adjust function of base R.

The Chao1 index was calculated by using the estimateR function of vegan using the floor function on the calculated RPMR value. Visualization and multiple comparisons were performed as for Shannon’s Index.

Beta-distances were calculated against the table of species normalised as RPRM using the vegdist function of vegan with Bray-Curtis distances. To visualise the clustering, we performed nMDS using the metaMDS function of vegan using 200 iterations, and vectors 1 and 2 were plotted using ggplot and the geom_point function. The analysis of clustering was performed using Adonis2. To identify separation between groups, Adonis2 was performed for each pair-wise comparison of the above list, and between all timepoints of treatment groups. When comparisons were from a single treatment group (paired or the entire group), Adonis2 was stratified by patient identifier. The resulting p-values from pair-wise and treatment-groupwise Adonis2 calculations were normalised using FDR.

To visualize the composition of bacterial taxonomy, taxonomic data was aggregated at a Genus level. From the RPMR normalised table, counts were aggregated using the aggregate function of the stats package of R. To identify the 10 most abundant taxa in SDD patients, the median counts of each bacterial genera were calculated
using the summarise function of dplyr (Tidyverse suite). Median was chosen as our read-count data is not normally distributed. To identify the 10 most abundant genera in the SC group, the median count for each taxa was calculated, the list of 10 taxa from SDD patients was removed from list of medians, and the next ten most abundant bacteria were selected. All other taxa were aggregated into “Other” to simplify graphing. Standard column graphs were created using ggplot2 and the geom_col function, setting the position to “fill”. For continuity and ease of interpretation, the taxa selected from admission samples was used to observe the changes across timepoints.

For the alluvial plots, we used the ggplot2 extension package ggalluvial, and taxonomy from the above boxplots. Alluvial plots were included to highlight changes in the microbiome composition throughout each treatment.

The software package MaAsLin2 v 1.10.0 [11] was used to perform further interrogations of the microbiomes. To analyse time dependent change in groups, patient ID was set as a factor and applied as a random effect, sampling number, weight and age were treated as fixed effects and a minimum prevalence of 0.4 was applied. To compare treatment dependent changes, treatment group, weight and age were treated as fixed effects and a minimum prevalence of 0.4 was applied. Multiple comparisons were corrected using FDR, and all other settings were used as default. Where the difference between more than four taxa was identified by MaAsLin2, the coefficient of change was plotted as horizontal column graph. For interpretation, the absolute value of the coefficient was plotted with the signed direction of change illustrated by column colour. We chose to set both $\alpha$ and the adjusted-$\alpha$ at a rate of 1 in 20.
To compare the top 20 taxa between groups at each timepoint, we calculated the difference for each taxa using a Kruskal-Wallis test, as the KW test is a generalised Mann-Whitney U test. The p-value for each test was adjusted after calculation by applying the FDR correction, with q-values reported.

**Analysis of resistance genes:**

To focus the analysis of AMR, genes involved in resistance to non-clinically relevant compounds such as heavy metals, detergents, cleaning compounds and tetracyclines were excluded. Statistical analysis and visualization of AMR genes followed the methods outlined previously.

Genes detected with ARIBA were compiled for each patient and measured in Reads per Kilobase gene per Megabase of sequencing (RPKM).

Sequencing and analysis statistics of PICNIC patients is available in Supplementary Table 1

Sequencing and analysis statistics from samples obtained from described by Clark et al [12] are available in Supplementary Table 2.


