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# Periodic screening of donor faeces with a quarantine period to prevent transmission of multidrug-resistant organisms during faecal microbiota transplantation: a retrospective cohort study



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## Summary

**Background** On June 13, 2019, the US Food and Drug Administration issued a warning after transfer of faeces containing an extended-spectrum  $\beta$ -lactamase (ESBL)-producing *Escherichia coli* by faecal microbiota transplantation led to bacteraemia in two immunocompromised patients. Consequently, we evaluated the effectiveness of the faeces donor-screening protocol of the Netherlands Donor Faeces Bank, which consists of screening of donors for multidrug-resistant organisms every 3 months, combined with additional screening on indication (eg, after travelling abroad) and application of a quarantine period for all faecal suspensions delivered within those 3 months.

**Methods** We did a retrospective cohort study of data collected between Jan 1, 2015, and Oct 14, 2019, on the multidrug-resistant organism testing results of donor faeces. Additionally, we tested previously quarantined faecal suspensions approved for faecal microbiota transplantation between Dec 12, 2016, and May 1, 2019, for the presence of multidrug-resistant organisms using both aselective and selective broth enrichment media. Whole-genome sequencing with core-genome multilocus sequence typing (cgMLST) was done on all multidrug-resistant isolates.

**Findings** Among initial screenings, six (9%) of 66 tested individuals were positive for multidrug-resistant organisms and 11 (17%) of 66 tested individuals were positive for multidrug-resistant organisms at any timepoint. Multidrug-resistant organisms were detected in four (25%) of 16 active donors, who had a median donation duration of 268 days (IQR 92 to 366). Among all screening results, 14 (74%) of 19 detected multidrug-resistant organisms were ESBL-producing *E coli*. 170 (49%) of 344 approved faecal suspensions had corresponding research faeces aliquots available and were tested (from 11 active donors with a median of eight [IQR five to 26] suspensions per donor). No multidrug-resistant organisms were detected in the 170 approved faecal suspensions (one-sided 95% CI 0 to 1.7). cgMLST revealed that all multidrug-resistant organisms were genetically different.

**Interpretation** Healthy faeces donors can become colonised with multidrug-resistant organisms during donation activities. Our screening protocol did not result in approval of multidrug-resistant organism-positive faecal suspensions for microbiota transplantation.

**Funding** None.

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## Introduction

Faecal microbiota transplantation is an effective treatment for multiple recurrent and therapy-refractory *Clostridioides difficile* infections<sup>1,2</sup> and is currently under investigation for several other diseases, such as hepatic encephalopathy and ulcerative colitis.<sup>3</sup> Recurrence of *C difficile* infections is facilitated by a disturbed microbiota, which can be restored by administration of healthy donor faeces via faecal microbiota transplantation. Numerous stool banks have been founded worldwide to provide ready-to-use donor faecal suspensions, which are produced in a standardised manner.

On June 13, 2019, the US Food and Drug Administration (FDA) issued a safety communication regarding faecal microbiota transplantation.<sup>4</sup> Two immunocompromised

patients who received donor faeces developed bacteraemia after transfer of faeces containing extended-spectrum  $\beta$ -lactamase (ESBL)-producing *Escherichia coli* (sequence type 131) by faecal microbiota transplantation. Five (42%) of 12 other tested patients who received faecal microbiota transplantation with faeces from the same donor also tested positive for this ESBL-producing *E coli*. Importantly, the donor faeces was not tested for ESBL-producing organisms before use.<sup>4,5</sup> According to the FDA, donors need anamnestic screening to identify risk factors for multidrug-resistant organism carriage, and donor faeces should be tested for multidrug-resistant organisms.<sup>4</sup>

The Netherlands Donor Faeces Bank was founded in 2015 and has adhered to strict donor screening protocols from initiation (appendix p 2).<sup>6</sup> Stool banking practices and

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See [Online](#) for appendix

### Research in context

#### Evidence before this study

Donor faeces banks and expert centres undertake donor screening to prevent the presence of transmissible pathogens or multidrug-resistant organisms in faeces used for faecal microbiota transplantation. These screening protocols are mainly based on expert opinion and are not standardised, with questions around how often rescreening should be done and which infectious agents should be screened for. Testing for multidrug-resistant organisms is not included in all donor screening protocols. In 2019, two severely immunocompromised patients in the USA died due to transfer of extended-spectrum  $\beta$ -lactamase (ESBL)-producing organisms by faecal microbiota transplantation, which led to bacteraemia. These donors were not screened for ESBL-producing organisms.

We searched PubMed from inception to April 16, 2020, for studies reporting on the presence of multidrug-resistant organisms in faeces used for faecal microbiota transplantation from regularly screened donors. The search strategy is described in full in the appendix (p 1). The search included terms related to multidrug-resistant organisms, antimicrobial resistance, ESBLs, vancomycin resistance, carbapenemase, carbapenem resistance, meticillin resistance, donors, stool banks, faeces, and the rectum. Our PubMed search yielded only one relevant study. The non-profit donor faeces bank OpenBiome published results of their initial screening in donors of faeces. Among 435 donors tested, three carried vancomycin-resistant enterococci (<1%) and none carried ESBL-producing Enterobacterales or other multidrug-resistant organisms. We are aware of another study with longitudinal data on the presence of multidrug-resistant organisms in donor faeces, which was presented as a poster at the American Society for Microbiology Microbe 2019 meeting in San Francisco, CA, USA. The Microbiota Therapeutics Outcomes Program group in Toronto, ON, Canada, reported that four periodically screened active donors delivered 80 approved donations for faecal microbiota transplantation

and that, in retrospect, two donors were found to have faeces that contained ESBL-producing *Klebsiella pneumoniae* in three samples, representing 3.8% of all approved faecal suspensions.

#### Added value of this study

The national donor faeces bank of the Netherlands periodically screens faeces of donors for multidrug-resistant organisms, with additional screening done on indication and quarantine storage of faecal suspensions until a negative test result is reported at rescreening. Most other donor faeces banks also apply periodic donor screening protocols. This study provides an answer to the question of whether this testing protocol might result in approved faecal suspensions containing multidrug-resistant organisms. To our knowledge, our study is the first to report longitudinal data on multidrug-resistant colonisation in healthy donor faeces and the effectiveness of an extensive multidrug-resistant periodic screening protocol at a national donor faeces bank.

#### Implications of all the available evidence

This study is a step towards more evidence-based screening protocols for donor faeces. International guidelines on donor screening are being developed by experts from donor faeces banks. The data indicate that colonisation rates of faeces donors might be higher than expected based on the prevalence of multidrug-resistant organism colonisation in healthy people. This observation underlines the importance of sensitive microbiological methods and appropriate protocols when screening donor faeces. Using our screening protocol, none of the tested faecal suspensions that were approved for patient use contained multidrug-resistant organisms. We propose that periodic screening, combined with additional screening on indication and application of a quarantine period, is an appropriate method to prevent transmission of multidrug-resistant organisms from donors to patients.

protocols vary considerably between stool banks all over the world.<sup>7,8</sup> The Netherlands Donor Faeces Bank largely follows the EU consensus guidelines on stool banking.<sup>7</sup> To prevent transmission of infectious agents through faecal microbiota transplantation, donor faeces and serum are screened by the Netherlands Donor Faeces Bank at 3-month intervals for bacterial (including multidrug-resistant organisms), viral, and parasitic pathogens. Additional screening is done for specific indications (eg, travelling abroad or use of antibiotics). The multidrug-resistant organism carriage rate in faeces of healthy people in the Netherlands is relatively low (4.5–8.6% for ESBL-producing bacteria).<sup>9–14</sup>

The FDA warning prompted us to evaluate our screening protocol. The aim of this study was to investigate whether periodic screening of donor faeces,

with a quarantine period of 3 months before release for use, is an effective method to prevent the presence of multidrug-resistant organisms in donor faecal suspensions approved for use in patients. The screening protocol was considered an effective method when less than 1% of all approved faecal suspensions contained a multidrug-resistant organism.

## Methods

### Study design and participants

This retrospective cohort study was done using data of, and samples from, faeces donors registered with the Netherlands Donor Faeces Bank (Leiden University Medical Center, Leiden, Netherlands). We included all donors who were tested for multidrug-resistant organisms between Jan 1, 2015 (foundation of the

Netherlands Donor Faeces Bank), and Oct 14, 2019. Tested individuals were divided into active and non-active donors. Active donors were defined as donors who delivered faecal suspensions for the treatment of patients that were approved after a quarantine period. Non-active donors were the remaining donors who did not pass the initial screening test, became positive for a pathogen at rescreening, or refrained from donorship for other reasons. Characteristics of donors, reasons for multidrug-resistant organism testing, and information on travel abroad were retrieved from the Netherlands Donor Faeces Bank donor database, including the short questionnaires before each donation and the logfiles in which communication with the donors is recorded. KEWV extracted the data.

Written informed consent was obtained from all donors for use of their faecal samples for safety assessments and scientific research. Consent was obtained after the donor had passed the eligibility interview and questionnaire. Ethical approval was granted for the protocols and practice of the Netherlands Donor Faeces Bank by the local Medical Ethics Committee at Leiden University Medical Center (reference P15.145).

#### Netherlands Donor Faeces Bank screening protocol

The routine screening protocol of the Netherlands Donor Faeces Bank is described in the appendix (p 2). Briefly, eligible donors are healthy people aged 18–60 years without relevant medical history or medication use (appendix p 2).<sup>6</sup> The Netherlands Donor Faeces Bank has an upper age limit of 60 years, assuming a substantial increase in comorbidities and a less stable gut microbiota in those older than 60 years. Before entering the donor programme, potential donors are extensively screened with a questionnaire and interview, focusing on risk factors for transmissible diseases and disorders or factors associated with a disturbed microbiota. Faeces and serum are screened for infectious pathogens, including multidrug-resistant organisms, at initial screening and at the end of 3-month intervals.

To assess the risk of multidrug-resistant organism acquisition or enteropathogen carriage in the period between screenings, questionnaires on recent health status and risk factors for these microorganisms are completed by the donor at every donation. Besides the interval screenings, additional multidrug-resistant organism screening is done after donors travel to another country (any country outside the Netherlands), since multidrug-resistant organism acquisition during travel can be substantial (eg, up to 75% after a visit to south Asia).<sup>9,10</sup> Other indications for additional screening include repeated tests after a previous positive test for an enteropathogenic microorganism, a change of health, or after antibiotic treatment. The time of additional screening for multidrug-resistant or enteropathogenic microorganisms after a positive test depends on the expected time of clearance of the pathogen, as described

in the literature. In the case of a multidrug-resistant organism-positive test, donors are temporarily excluded and usually rescreened every 1–3 months until they test negative. After antibiotic treatment, donors are temporarily excluded for 6 months and then rescreened.

The donor stools that are processed into a faecal suspension are quarantined for 3 months and are approved for patient use only when no multidrug-resistant organism or enteropathogen is identified at the beginning and the end of the 3-month interval,<sup>6</sup> as described in the EU consensus guidelines.<sup>7</sup> During quarantine, the faecal suspensions remain in the quarantine freezer. They are transported to the freezer for approved faecal suspensions after the donor tests negative on rescreening. Faecal suspensions are stored with 10% glycerol for a maximum of 2 years. In cases of a positive pathogen test during rescreening, previously prepared, quarantined faecal suspensions are tested in retrospect to assess which faecal suspensions should be excluded from patient use.

#### Definition of multidrug-resistant organisms

The Netherlands Donor Faeces Bank and this study define multidrug resistance according to the definitions of the Dutch Working Group on Infection Prevention.<sup>15</sup> Briefly, multidrug-resistant organisms include ESBL-producing Enterobacterales; Enterobacterales and *Acinetobacter* spp that are resistant to both a fluoroquinolone and an aminoglycoside or produce carbapenemases; *Pseudomonas aeruginosa* that is carbapenemase-producing or resistant to at least three of the following antibiotic classes or agents: fluoroquinolones, aminoglycosides, ceftazidime or piperacillin, and carbapenems; co-trimoxazole-resistant *Stenotrophomonas maltophilia*; vancomycin-resistant *Enterococcus faecium* or *Enterococcus faecalis* (VRE); or methicillin-resistant *Staphylococcus aureus* (MRSA).<sup>15</sup> Insights into the optimal donor screening protocol have changed over the years. Although some multidrug-resistant organisms such as MRSA were previously found to be less relevant than other organisms, they are now routinely tested for by donor faeces banks, including the Netherlands Donor Faeces Bank.

#### Multidrug-resistant organism testing of approved faecal suspensions

Since Dec 12, 2016, at each donation, two aliquots containing 1 g of faeces each, with and without 10% glycerol as cryoprotectant, have been stored at –80°C for research purposes. The faeces and corresponding faeces aliquots are processed within 6 h after defecation. In this study, we tested for multidrug-resistant organisms in all available faeces aliquots with 10% glycerol that were stored for research purposes from donations processed from Dec 12, 2016, to May 1, 2019, and for which the faecal suspensions were subsequently approved for use. The faeces aliquots of four samples (from two donors) known to contain ESBL-producing *E coli* served as positive controls.

To test for the presence of multidrug-resistant organisms, faecal aliquots were grown in broth enrichment media before subculturing on selective solid media as this approach has been shown to improve the recovery

Active donors (n=16)	
Sex	
Female	10 (63%)
Male	6 (38%)
Age, years	29.8 (25.8–40.9; 24–57)
Body-mass index, kg/m <sup>2</sup>	22.5 (20.8–24.0; 19.6–24.8)
Duration of donor activity per donor, days*	267.5 (92.3–365.5; 2–1193)
Number of approved faecal suspensions per donor	7.5 (5.3–28.5; 2–113)

Data are n (%) or median (IQR; range). \*Measured as the difference between the initial donation and the last donation.

**Table 1: Characteristics of active donors**

rate of all types of resistant bacteria by 20–30% compared with use of solid media alone.<sup>16–18</sup> Using an inoculating loop, around 10 µL sample was scraped from frozen faeces aliquots stored with 10% glycerol and deposited in the enrichment media, which consisted of tryptic soy broth (for multidrug-resistant Gram-negative bacteria and VRE detection) and brain heart infusion media supplemented with 2.5% sodium chloride and 10 mg/L colistin sulphate (for MRSA detection). Five selective growth media were used (all from BioMérieux; Marcy-l'Étoile, France): ChromID VRE agar, MacConkey agar plus tobramycin 8 mg/L plus ciprofloxacin 0.5 mg/L, ChromID ESBL agar, ChromID OXA-48 agar, and a selective MRSA-ID agar plate. All suspected colonies were investigated for species identification by matrix-assisted laser desorption ionisation-time of flight mass spectrometry with the Microflex system (Bruker Daltonik; Bremen, Germany). Automated susceptibility testing was done with the VITEK2 system (BioMérieux), using

	Multidrug-resistant organism	Reason for screening at first positive test	Time between previous multidrug-resistant organism-negative test and first positive test	Time between first positive and first negative multidrug-resistant organism test	Foreign country visits in year before positive multidrug-resistant organism test*
<b>Active donors</b>					
Donor 1	ESBL-positive <i>E coli</i>	Additional screening†	1 year	No rescreening	Italy‡
Donor 2	ESBL-positive <i>E coli</i>	Additional screening§	3 months	6 months	Finland
Donor 3	ESBL-negative fluoroquinolone-resistant and aminoglycoside-resistant <i>E coli</i> (initial screening) and EBSL-positive <i>E coli</i> (rescreening)	Initial screening	Not tested before	4 months	England‡, Turkey‡
Donor 4	ESBL-positive <i>E coli</i>	Interval rescreening	1 month	Persistent carriage for 3 months with no further rescreening	None in the 3 months before a positive test
<b>Non-active donors</b>					
Donor 5	ESBL-positive <i>E coli</i>	Interval rescreening	1 month	Persistent carriage for 1 month with no further rescreening	None in the 1 month before a positive test
Donor 6	ESBL-positive <i>E coli</i>	Initial screening	Not tested before	No rescreening	Italy‡
Donor 7	ESBL-negative fluoroquinolone-resistant and aminoglycoside-resistant <i>E coli</i>	Initial screening	Not tested before	Persistent carriage for 2 months with no further rescreening	Unknown
Donor 8	ESBL-positive <i>E coli</i> and ESBL-negative fluoroquinolone-resistant and aminoglycoside-resistant <i>E coli</i>	Initial screening	Not tested before	No rescreening	Belgium‡, Spain‡
Donor 9	ESBL-positive <i>E coli</i>	Additional screening¶	4 months	No rescreening	Unknown
Donor 10	ESBL-negative fluoroquinolone-resistant and aminoglycoside-resistant <i>E coli</i>	Initial screening	Not tested before	4 months	Unknown
Donor 11	ESBL-positive <i>E coli</i>	Initial screening	Not tested before	No rescreening	Unknown

All tested individuals were divided into active and non-active donors. Active donors delivered faecal suspensions for the treatment of patients that were approved after a quarantine period. Non-active donors were the remaining donors who did not pass the initial screening test, became positive for a pathogen at rescreening, or refrained from donorship for other reasons. ESBL=extended-spectrum β-lactamase. *E coli*=*Escherichia coli*. \*Information is incomplete for all donors, except donor 1. †Repeated screening because of *Blastocystis hominis* detection in a previous faecal sample. ‡Country was visited after a previous negative multidrug-resistant organism test or testing had not been done before. §Repeated screening because of previous use of antibiotics for a urinary tract infection. ¶Repeated initial screening after temporary exclusion because of *Dientamoeba fragilis* detection in faeces.

**Table 2: Testing characteristics of donors positive for multidrug-resistant organisms**

the European Committee of Antimicrobial Susceptibility Testing breakpoints. The application of confirmation tests is described in the appendix (p 1).

### Genomic analysis

To assess sequence type, relatedness of strains, and the presence of antimicrobial resistance genes, all stored multidrug-resistant organism isolates detected by the Netherlands Donor Faeces Bank in donor faeces were subjected to whole-genome sequencing. Full details of the genomic analysis are given in the appendix (pp 1–2). Briefly, DNA was isolated using the QIAAsymphony DSP Virus/Pathogen Midi Kit (Qiagen; Hilden, Germany). Whole-genome sequencing was done on the Illumina NovaSeq6000 platform (Illumina; San Diego, CA, USA) at GenomeScan (Leiden, Netherlands). NEBNext Ultra II DNA Library Prep Kit (New England Biolabs; Ipswich, MA, USA) for 150-base pair paired-end sequencing was used for the preparation of sequence libraries. Antimicrobial resistance genes and plasmids were identified with staramr (version 0.5.1), using the ResFinder and PlasmidFinder databases. Genetic relatedness between strains was calculated using core-genome multilocus sequence typing (cgMLST) with SeqSphere+ version 6.0.2 (Ridom; Münster, Germany). The multidrug-resistant organism isolates of faeces donors were compared with multidrug-resistant organism isolates from the Dutch National Institute for Public Health and the Environment to assess relatedness to other isolates from the Netherlands. Minimum spanning trees based on cgMLST analysis were created with SeqSphere+ version 6.0.2. Raw sequence data for this study have been deposited in the European Nucleotide Archive at the European

Molecular Biology Laboratory-European Bioinformatics Institute under study accession number PRJEB37861.

### Statistical analysis

Binomial data from a random sample of approved faecal suspensions are presented as n (%) with 95% CIs calculated using the Clopper-Pearson method. To test for potential sample selection bias, we assessed the number of, and the reasons for, missing aliquots of approved faecal suspensions and compared the characteristics of donors with and without tested faecal suspensions.

We used SPSS version 25.0 and STATA SE version 15.1 for data analysis.

### Role of the funding source

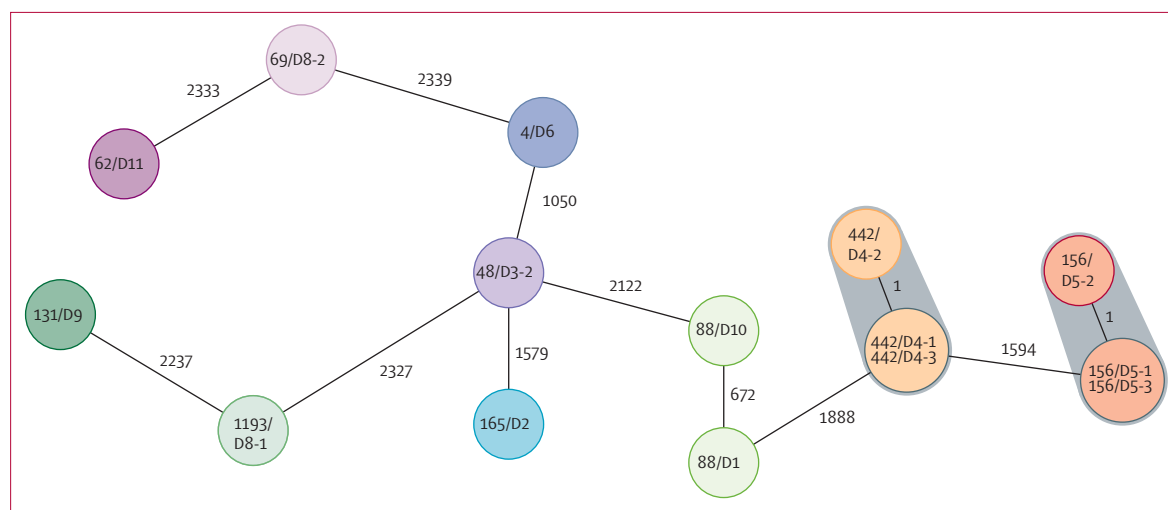
There was no funding source for this study. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

### Results

Between Jan 1, 2015, and Oct 14, 2019, 66 individuals (including 16 active donors and 50 non-active donors) were tested for colonisation with multidrug-resistant organisms. Characteristics of all active donors in the Netherlands Donor Faeces Bank are shown in table 1. A summary of the results of initial screening and (additional) rescreening is shown in the appendix (p 3).

11 (17%) of 66 tested individuals were found to be multidrug-resistant organism-positive at any timepoint. Six (55%) of these 11 individuals also had pathogens other than multidrug-resistant organisms detected in their faeces, including *Dientamoeba fragilis* (four patients), *Blastocystis*

For more on the European Committee of Antimicrobial Susceptibility Testing breakpoints see [https://www.eucast.org/clinical\\_breakpoints/](https://www.eucast.org/clinical_breakpoints/)  
For raw sequence data for this study see <https://www.ebi.ac.uk/ena/data/view/PRJEB37861>



**Figure 1: Minimum spanning tree of cgMLST results of 15 multidrug-resistant *Escherichia coli* isolates from donors**

Circles represent one isolate or identical isolates, which are connected to the closest relative. Different colours represent sequence types. The number of alleles that differ between isolates is shown on the connecting lines between circles. Numbers in circles contain the sequence type/isolate ID. Grey shaded zones around circles contain closely related isolates (ie,  $\leq 10$  allele differences). Only five isolates could be assigned a complex type by SeqSphere+, including complex type 3821 for all three multidrug-resistant organisms from donor 4, complex type 580 for the multidrug-resistant organism from donor 9, and complex type 5372 for the multidrug-resistant organism from donor 11. cgMLST=core-genome multilocus sequence typing. D=donor.



Isolate ID	Donation date	MDRO type	MLST	Aminoglycosides*												β-lactams													
				aac(3)-IId		aad A1		aad A5		ant(2'')-Ia		aph(3'')-Ib		aph(3'')-Ia		aph(6)-Id		CTX-M-1	CTX-M-15	CTX-M-65	DHA-1	OXA-1	SHV-12	TEM-1B	TEM-57				
				Gentamicin	Tobramycin	Gentamicin	Tobramycin	Gentamicin	Tobramycin	Gentamicin	Tobramycin	Gentamicin	Tobramycin	Gentamicin	Tobramycin	Gentamicin	Tobramycin												
<b>Active donors</b>																													
D1	July, 2017	ESBL-positive <i>E coli</i>	88			x	x							x	x	x	x	x	x	x	x					x			x
D2	September, 2017	ESBL-positive <i>E coli</i>	165			x	x							x	x				x	x									x
D3-1	August, 2017	ESBL-negative <i>E coli</i>	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
D3-2	August, 2017	ESBL-positive <i>E coli</i>	48																					x					
D4-1†	August, 2018	ESBL-positive <i>E coli</i>	442																	x									
D4-2†	September, 2018	ESBL-positive <i>E coli</i>	442																	x									
D4-3†	October, 2018	ESBL-positive <i>E coli</i>	442																	x									
D4-4	November, 2018	ESBL-positive <i>E coli</i>	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
<b>Non-active donors</b>																													
D5-1	March, 2016‡	ESBL-positive <i>E coli</i>	156											x	x	x	x	x	x								x	x	
D5-2	March, 2016‡	ESBL-positive <i>E coli</i>	156											x	x	x	x	x	x								x	x	
D5-3†	April, 2016	ESBL-positive <i>E coli</i>	156											x	x	x	x	x	x								x	x	
D6	October, 2017	ESBL-positive <i>E coli</i>	4																	x									
D7-1	August, 2017	ESBL-negative <i>E coli</i>	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
D7-2	November, 2017	ESBL-negative <i>E coli</i>	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
D8-1	October, 2018‡	ESBL-positive <i>E coli</i>	1193			x	x							x	x					x	x								
D8-2	October, 2018‡	ESBL-negative <i>E coli</i>	69											x	x								x				x		
D9	April, 2019	ESBL-positive <i>E coli</i>	131	x	x	x	x	x	x					x	x	x	x	x	x							x			x
D10	December, 2018	ESBL-negative <i>E coli</i>	88			x	x			x	x	x	x	x	x	x	x	x							x			x	
D11	January, 2019	ESBL-positive <i>E coli</i>	62			x	x													x							x		

(Figure 2 continues on next page)

*hominis* (one patient), rotavirus (one patient), parechovirus (one patient), and norovirus (one patient). Four (36%) of 11 individuals with positive multidrug-resistant organism tests were active donors and seven (64%) were non-active donors (appendix pp 3–4). Multidrug-resistant organisms were detected during initial screening in six (55%) donors (five non-active donors and one active donor), during rescreening after a 3-month quarantine period in two (18%) donors (one non-active donor and one active donor), and during additional screening upon indication in three (27%) donors (one non-active donor and two active donors). The 66 donors provided 155 tested faecal samples included in this study, of which 17 (11%) tested positive for multidrug-resistant organisms. Information on multidrug-resistant

organism colonisation in relation to indication for screening and risk factors is shown in table 2 and the appendix (p 4).

Overall, 14 (74%) of the 19 detected multidrug-resistant organisms were ESBL-producing *E coli* (appendix p 4). No MRSA, vancomycin-resistant enterococci, or carbapenemase-producing bacteria were detected. Three of the six individuals who tested positive for a multidrug-resistant organism at initial screening carried an ESBL-producing *E coli* (table 2; appendix p 4).

Multidrug-resistant organisms were detected in four (25%) of 16 active donors. Active donors contributed 96 tested faecal samples, of which eight (8%) tested positive for a multidrug-resistant organism. Three (75%) of four multidrug-resistant organism-positive donors had

travelled abroad in the month before the positive test, where they might have acquired the organism (table 2). The fourth donor tested negative 1 month before the positive test and had not visited any other countries in the 3 months before the positive test. In the subset of 50 non-active donors, multidrug-resistant organisms were found in seven (14%) donors, in nine (15%) of 59 samples tested. For non-active donors, previous travel was mostly unknown (table 2; appendix pp 3–4).

Between the foundation of the Netherlands Donor Faeces Bank on Jan 1, 2015, and May 1, 2019, 344 faecal suspensions were approved for patient use (15 active donors during this timeframe). 170 (49%) of 344 approved suspensions had corresponding research faeces aliquots with 10% glycerol available and were tested for multidrug-resistant organisms (from 11 active donors, with a median of 8.0 [IQR 5.0–26.0 suspensions per donor). No multidrug-resistant organisms were detected in the 170 approved faecal suspensions (one-sided 95% CI 0–1.7). Therefore, no multidrug-resistant organism-positive faecal suspensions were found in any donation period with a negative pre-test and a negative post-test. All multidrug-resistant organisms were detected at the initial screening or at the end of a quarantine period. Furthermore, all four control samples with known ESBL-producing *E coli* tested positive.

The 174 faecal suspensions that were not analysed were from four active donors (with a median of 7.0 [IQR 4.8–7.8] suspensions per donor). These suspensions were not analysed because research faeces aliquots with 10% glycerol were not available as the donor produced an insufficient amount of faeces, they were used for other research purposes, or donations were from before 2017, when samples were not yet stored for research purposes. A comparison between tested and non-tested donor groups for assessment of possible bias is supplied in the appendix (p 5). Active donors that had faeces aliquots used for other research purposes also had a faecal suspension tested in this study. The absence of faeces aliquots due to use in other studies was not associated with donor or sample characteristics. Furthermore, the tested and non-tested donor groups did not differ in the proportion of multidrug-resistant organism-positive donors. Therefore, we do not expect bias to have occurred.

10 previously detected and stored multidrug-resistant organism isolates from 11 donors were whole-genome sequenced; all were *E coli* strains. cgMLST results indicated two clusters of highly related strains from two donors, donor 4 and donor 5 (figure 1). The clusters were donor-specific but derived from separate donations from a single donor (except for strain 5-1 and strain 5-2 from donor 5, which were detected in the same donation). All other isolates were different by at least 672 alleles, suggesting no clonal relationship. One *E coli* strain, from donor 9, was identified as the multidrug resistant sequence type 131 clone producing *bla*<sub>CTX-M-15</sub> and *bla*<sub>TEM57</sub>.

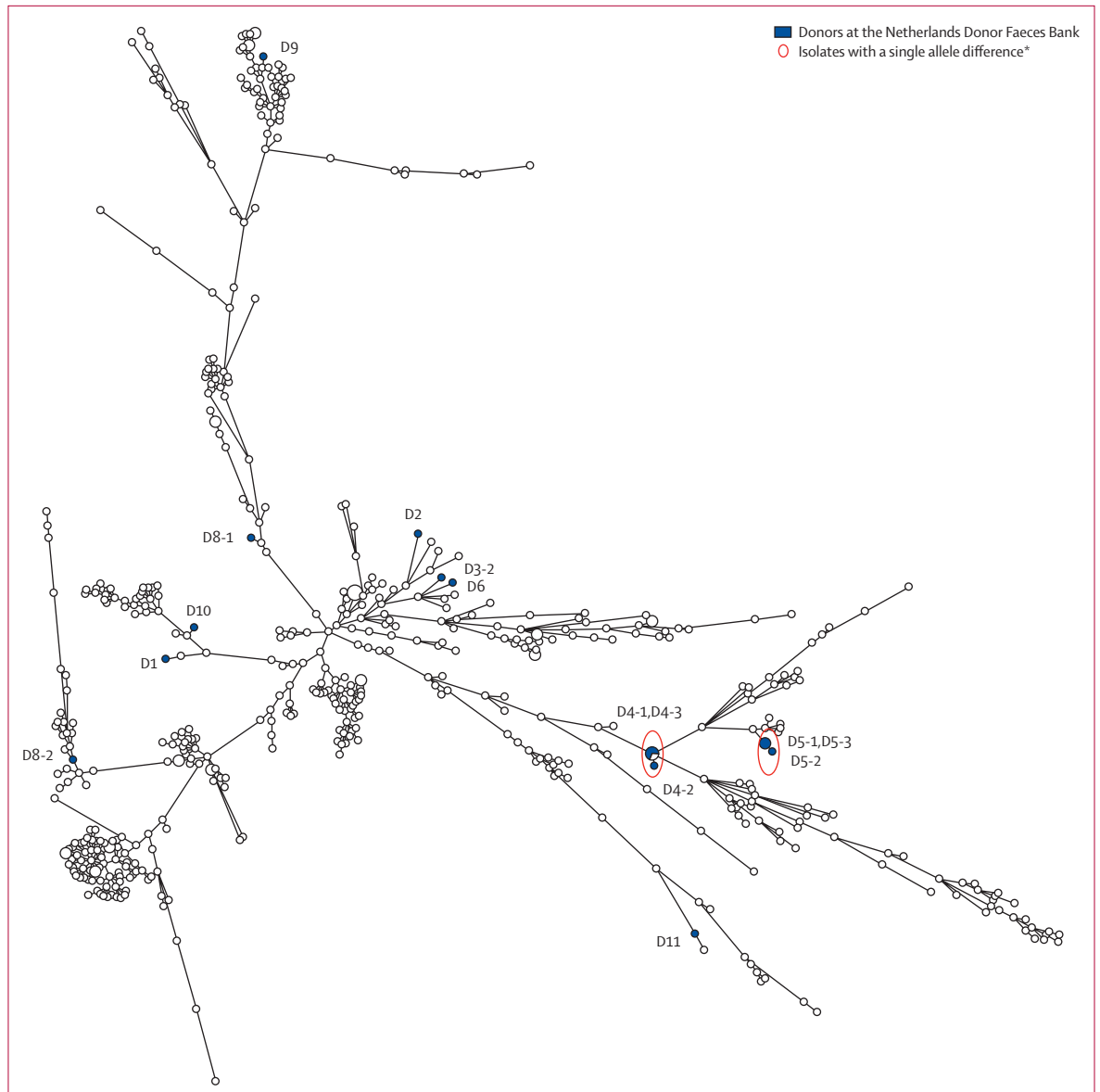
		Quinolones*						
		TEM-214	TEM-234	oqxA	oqxB	qnrB4	qnrS1	Chromosomal mutations
								parC (p.S80R), gyrA (p.S83L, p.D87N)
		NA	NA	NA	NA	NA	NA	NA
		x		x	x			parC (p.S80I), gyrA (p.S83L, p.D87N)
		x	NA	NA	NA	NA	NA	NA
							x	gyrA (p.S83L)
		NA	NA	NA	NA	NA	NA	NA
		NA	NA	NA	NA	NA	NA	NA
			x				x	parC (p.S80I), gyrA (p.S83L, p.D87N), parE (p.L416F)
						x		parC (p.S80R), gyrA (p.S83L)
								parC (p.S80I, p.E84V), gyrA (p.S83L, p.D87N), parE (p.I529L)
								parC (p.S80I), gyrA (p.S83L, p.D87N), parE (p.S458A)
								gyrA (p.S83L)

Figure 2: Relevant phenotypic and genotypic antibiotic susceptibility results of 19 MDRO isolates from active and non-active donors

Data in the far left column show donor number and, in case of several isolates per donor, a strain number. The presence of each gene is indicated by an x. Blank (white) squares indicate the genes are not present, whereas the absence of sequencing data, because the isolate was not available, is indicated by NA. To determine phenotypic resistance, results of phenotypic tests of gentamicin or tobramycin (aminoglycosides), cefotaxime or ceftazidime (β-lactam), and ciprofloxacin (quinolones) resistance are indicated by colour. Phenotypic results are indicated by green (phenotypic susceptibility), red (phenotypic resistance), or yellow (phenotypic susceptible, increased antibiotic exposure). D=donor. ESBL=extended-spectrum β-lactamase. *E coli*=*Escherichia coli*. MDRO=multidrug-resistant organism. MLST=multilocus sequence typing. NA=not available. \*A multidrug resistance translocator (mdf(A)), conferring resistance to several antibiotics, including quinolones and aminoglycosides, was found in 13 of 16 sequenced isolates. †Positive control. ‡From the same faecal sample.

For most isolates, the genotypic and phenotypic resistance matched (figure 2). Only five (31%) of 16 strains were assigned a complex type by SeqSphere+ (580 in donor 9, 5372 in donor 11, and 3821 for all three isolates from donor 4), which implies that most strains had a





**Figure 3: Minimum spanning tree of cgMLST results for 15 multidrug-resistant *Escherichia coli* isolates from donors at the Netherlands Donor Faeces Bank and 534 multidrug-resistant *E coli* isolates from the Netherlands**

For the multidrug-resistant *E coli* isolates from the Netherlands, sequence data were used from the Dutch National Institute for Public Health and the Environment. Circles represent one isolate or identical isolates, which are connected to the closest relative with a line. Larger circles represent more than one isolate. cgMLST=core-genome multilocus sequence typing. D=donor. \*Other isolates have more than 20 allele differences.

unique allele profile that had not been registered before. However, all strains were successfully typed by in-silico multilocus sequence typing. Of the two sequenced strains that were phenotypically non-ESBL-producing, strain 8-2 from donor 8 had *bla*<sub>DHA-1</sub> and *bla*<sub>TEM-1B</sub> genes and the strain from donor 10 had *bla*<sub>OXA-1</sub> and *bla*<sub>TEM-1B</sub> genes. All other sequenced strains were phenotypically ESBL-producing and had one or more  $\beta$ -lactamase genes, with most containing *bla*<sub>CTX-M-15</sub> (eight strains) or *bla*<sub>TEM-1B</sub> (six strains).

A comparison of the 15 isolates with cgMLST results from our study with 534 multidrug-resistant *E coli* isolates

from the Dutch National Institute for Public Health and the Environment showed that only three multidrug-resistant *E coli* isolates from one donor (all three isolates from donor 4) formed a cluster with another *E coli* isolate from the Netherlands (figure 3).

## Discussion

This study shows that periodic screening of donor faeces, with a quarantine period and additional screening after visiting another country or temporary exclusion for other indications, is an effective method to prevent the approval

of faecal suspensions that are positive for multidrug-resistant organisms. A quarter of active donors were colonised with multidrug-resistant organisms at some point during participation in the donor programme. cgMLST revealed that all multidrug-resistant organisms from individual donors were not clonally related to isolates from other donors.

This study evaluated the donor screening protocol of a national donor faeces bank and in doing so provides insights relevant for establishing optimal testing procedures for faeces donors. Strengths of our study are the availability of longitudinal data on the presence of multidrug-resistant organisms in healthy people and the use of a sensitive broth enrichment culture method to detect low numbers of multidrug-resistant organisms. However, there are also limitations. First, the study was done in a single centre with a small number of active donors and in a country with low endemicity for multidrug-resistant organisms.<sup>14</sup> Therefore, our observations might only be generalisable to other stool banks that use similar diagnostic procedures in countries with a similar prevalence of multidrug-resistant organisms. Second, faecal suspensions, for which no 10% glycerol research aliquot was available, were not tested. However, we expect no bias to have occurred, as a subanalysis of tested and non-tested faecal suspensions showed that both groups contained the same number of donors who had been positive for multidrug-resistant organisms at some point during participation in the donor programme. Furthermore, the only reasons for excluding a donor from the analysis because of the absence of an available aliquot were that it was released before Dec 12, 2016, or the donor produced an insufficient amount of faeces. We do not expect the use of frozen aliquots instead of fresh aliquots to have influenced the results, as glycerol was added and previous studies found little to no difference in the number of viable bacteria between frozen and fresh aliquots, as supported by our detection of multidrug-resistant organisms in positive controls.<sup>19,20</sup>

To our knowledge, only one group has reported longitudinal data on multidrug-resistant organism colonisation in donor faeces. The Microbiota Therapeutics Outcomes Program in Toronto (ON, Canada) presented the testing results of approved faecal suspensions from faeces donors who were periodically screened for multidrug-resistant organisms.<sup>21</sup> Four active donors delivered 80 donations during the study period. Two donors were found to have donated faeces that contained ESBL-producing *Klebsiella pneumoniae* in three samples (3.8% of faecal suspensions, compared to none in our study). This discrepancy between sites could be due to use of different culture methods for the detection of multidrug-resistant organisms.

Protocols used for multidrug-resistant organism testing differ between expert centres or stool banks. These differences could partly explain the low numbers of multidrug-resistant organisms reported by a US stool

bank, which found only three (<1%) of 435 people were positive for vancomycin-resistant enterococci during initial screening and no person was positive for ESBL-producing bacteria or other resistant microorganisms.<sup>22</sup> The low multidrug-resistant organism carriage rate in that study might also be due to strict primary screening of donors, including exclusion of donors who travelled abroad, before testing for multidrug-resistant organisms. However, the absence of colonisation by ESBL-producing organisms contrasts with previously reported colonisation rates in healthy individuals in the USA<sup>23</sup> and Europe.<sup>10,14</sup> In our study, ESBL-producing Gram-negative bacteria were observed in three (5%) of 66 initial screenings. The higher proportion of multidrug-resistant organisms in our study could be explained by our use of highly sensitive diagnostic methods with enrichment broth<sup>16–18</sup> and the broader definition of multidrug-resistant organisms used in the Netherlands (including Enterobacterales resistant to both fluoroquinolones and aminoglycosides). Our observed proportion of ESBL-producing Enterobacterales was similar to what was found in previous studies in healthy individuals in the Netherlands, which reported prevalence ranging from 4.5% to 8.6%.<sup>9–14</sup> In a previous study, we examined the number of antimicrobial resistance genes in donor and patient stool samples and found fewer antimicrobial resistance genes in donor stool than in patient stool, both before and after faecal microbiota transplantation.<sup>24</sup> Similar results were obtained in other studies.<sup>25,26</sup> Millan and colleagues<sup>25</sup> observed a mean of 3.4 antimicrobial resistance genes (SD 0.4) in the faeces of three stool donors compared with 6.0 (0.9) in healthy participants of the Human Microbiome Project.

As a result of our screening protocol, no faecal suspension containing a multidrug-resistant organism was approved for patient use during our study period. However, during analysis of the data in this study, we detected an administrative error that resulted in approval and administration of one faecal suspension from a multidrug-resistant organism-positive donor to a patient. We did not find multidrug-resistant organisms in the quality control sample of this faecal suspension, suggesting a low bacterial load. However, we detected the same multidrug-resistant organism strain in a faecal sample taken after faecal microbiota transplantation from the receiving patient. During follow-up (up to 3.5 years from faecal microbiota transplantation), no (infectious) adverse events were reported by the patient or treating physician. Our administrative procedure was changed shortly after this event and no similar events have occurred. This event underlines that stool banks should implement both microbiological and procedural quality control measures. Moreover, even if all faecal suspensions are screened, multidrug-resistant organisms can still be missed because of the limit of detection. Studies in people who have travelled abroad reported spontaneous decolonisation

of multidrug-resistant organisms within 3 months.<sup>10,27</sup> Multidrug-resistant organism colonisation during travel might generally be of short duration because donors who travel are typically healthy. Three of four active donors in our study had visited another country before their positive test. Studies of people who have travelled abroad showed that 25–100% of people acquired a multidrug-resistant organism during traveling.<sup>9,10,23,27</sup> Although our current screening protocol did not result in approval of multidrug-resistant organism-positive faecal suspensions, the Netherlands Donor Faeces Bank only provides completely screened faecal suspensions for patients who are severely immunocompromised, by contrast with most faecal suspensions in the study, for which the donors were only screened at 3-month intervals or on indication.

The FDA report mentioned development of a systemic infection after transfer of multidrug-resistant organisms to immunocompromised patients by faecal microbiota transplantation, but the incidence of this complication after faecal microbiota transplantation in immunocompetent patients is unknown.<sup>5</sup> Paradoxically, several studies have shown a decrease in multidrug-resistant organism colonisation or antimicrobial resistance genes after faecal microbiota transplantation.<sup>24–26,28,29</sup> These studies suggest that faecal microbiota transplantation might be a treatment option for eradication of multidrug-resistant organisms. Furthermore, the risk of developing an infection from endogenous flora after faecal microbiota transplantation is unclear. Nevertheless, transferring a multidrug-resistant organism to a patient through faecal microbiota transplantation is undesirable, and donor faeces banks should optimise donor screening protocols within the best of their abilities to minimise the risk of transmission.

In conclusion, regular screening of faeces donors for multidrug-resistant organisms is required. Retesting donors every 3 months (with a quarantine period) combined with additional screening on indication and the use of sensitive assays is an appropriate method to prevent the presence of multidrug-resistant organisms in faecal suspensions. In the future, further standardised production of faecal formulations should be implemented.

#### Contributors

The study was conceived by JvP, JJK, HWV, and EJK. KEWV helped to collect the data and samples, analysed the data, and drafted the manuscript. JvP wrote the study protocol and devised and supervised the experiments. EMT supervised the experiments and critically reviewed the study protocol. EJK also critically reviewed the study protocol. SN analysed the whole-genome sequencing data. EB-K analysed the donor data and helped to collect the data and samples. IMJGS devised and performed the experiments. EKLB provided technical support for the practices of the Netherlands Donor Faeces Bank. EvL helped to collect the data and samples. All authors critically reviewed the manuscript.

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#### Declaration of interests

We declare no competing interests.

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