SUPPLEMENT MATERIAL

Materials & methods

DGGE

For denaturing gradient gel electrophoresis (DGGE) analyses, an approximately 200 bp fragment of the 16S rRNA gene was amplified using universal bacterial primers, one of which contained a GC clamp (1). The PCR products were separated with 8 % (v/v) polyacrylamide gel (acrylamide:bisacrylamide 37.5:1, Sigma-Aldrich, Germany). The DGGE band patterns were visualised by SYBR Gold staining and Dark Reader transilluminator (Claire chemical research, USA). Digital images were captured with Canon PowerShot G9 and the images were analysed using the Bionumerics 4.7 software (Applied Maths, Belgium).

Cloning and Sequencing

Selected DGGE bands were excised from the polyacrylamide gel and amplified using PCR. PCR product (0.8 ul) was ligated directly to 10 ng of blunt-ended pJET plasmid vector using CloneJET^{TM} PCR Cloning Kit (Thermo Fischer Scientific, USA) according to manufacturer's instructions. Bacteria from 24 colonies were re-inoculated into fresh agar plates. Cloned fragments were reamplified using following PCR conditions: 1 x DreamTaq Mastermix (Thermo Fischer Scientific, USA), 200 µM dNTP, 1 µM each flanking vector primer (pJET-F: 5'-CGA CTC ACT ATA GGG AGA GCG GC-3', pJET-R: 5'-AAG AAC ATC GAT TTT CCA TGG CAG-3'), 5% DMSO, 0.5 mM betaine, 0.5 U Phusion DNA Polymerase (Thermo Fischer Scientific, USA), and 5 µl DNA template in total volume of 25 ul. Sequencing was committed in one direction using pJET-F primer and BigDye Terminator cycle sequencing kit. Sequencing reactions were run on ABI3700 automated DNA sequencer (Applied Biosystems, Foster City, CA, USA). Sequence preprocessing including quality check and vector and primer sequence exclusion was done using Pregap and Gap4 programs of the Staden Package (2). The Pyrosequencing pipeline Classifier and Seqmatch tools of Ribosomal Database Project (RDP) package (3) were used to assign the sequences to bacterial taxa above species level and select reference sequences for phylogenetic analysis. Sequenced products were rerun to DGGE gels to check the purity and correct placing in the gel.

qPCR assays

QPCR assays were developed or optimized from existing assays based on the results retrieved from sequencing of the DGGE bands of interest (Table 2). Technical details of the qPCR assays are described in the Table S1. *In silico* testing was done using BLAST database and Ribosomal Database Project.
Clostridium cluster XI (Clost XI) assay has been developed and tested in an earlier publication (4) and is specific for species allocated in cluster XI. In our current Clost XI assay one base in forward primer was changed from the original publication (see Table S1). This assay detects (zero mismatches in database comparison) following cluster species, but no other bacteria: *Clostridium sordellii, C. bifermentans, C. difficile, C. glycolicum, C. irregulare, C. ghonii, C. bartlettii, C. lituseburense* and *Eubacterium tenue*.
The *Staphylococcus* (Staph) assay has been developed and published by Matsuda et al. (5) and detailed tests of the assay are presented in the original paper. In *in silico* testing primers were matching (no mismatch) to *Staphylococcus aureus* and *Staphylococcus argentus* only. Based on the original publication and our in vitro tests this assay can detect a few other *Staphylococcus* species, including *Staphylococcus aureus*, *S. epidmermidis and S. schleiferi* subsp. *coagulans*. and *S. simulans.*
Corynebacterineae/Pseudonocardianeae group specific assay (Cory/Pseu) was developed to detect widely bacteria of the family *Corynebacteriacea*. Based on the *in silico* tests primers amplify (without mismatch) in addition to the target species of *Corynebacteriaceae* also species of closely related families, such as *Dietziaceae*, *Mycobacteriaceae*, *Tsukamurellaceae*, *Nocardicaeae* and *Pseudonocardiaceae*.

The herein developed *Corynebacteria amycolatum* cluster assay (Camy), based on *insilico* testing is specific – allowing no mismatch – for *Corynebacterium amycolatum*. With one mismatch allowed, the assay will also detect *Corynebacterium hansenii,* which we were able to confirm in *in vitro* tests.

In our laboratory all these assays were tested with several bacterial and fungal pure strains as presented in Table S2. Assays were optimized and tested with several annealing temperature and primer concentrations, and final conditions of the qPCR reactions are presented in Table S1.

Geotrichum candidum (EPA 400) was used as an internal control and added to the samples before DNA extraction. The total volume of the qPCR reactions in all assays were 20 µl. TaqMan based assays (Clostridia cluster XI "Clost XI" and *Corynebacterium amycolatum* cluster "Camy") consisted of 10 µl of Environmental Master Mix kit (Applied Biosystems, USA) and HyClone® HyPure™ nuclease free water (HyClone Laboratories Inc., USA). Clost XI assay contained 250 nM forward and reverse primers and 125 nM probe. Camy assay contained 250 nM each primer and probe. Both SYBR based assays consisted 10 µl master mix volyme; Cory/Pseu assay contained SYBR Select Master Mix (Thermo Fisher Scientific, USA) and Staph assay DyNAmo Flash SYBR green qPCR Master Mix kit (Thermo Fisher Scientific, USA). In Cory/Pseu assay final concentration for each primer was 250nM and in Staph assay 500 nM. Finally, 2 µl of template DNA was added in each well. All qPCR assays were performed using the Agilent Stratagene Mx3005P QPCR System (Agilent Technologies Inc, USA).

Numbers of detected cell equivalents in the samples were calculated using relative quantification as described earlier (6). To calculate the microbial cell equivalents in the dust sample, target Ct values were normalised using the internal reference (*Geotrichum candidum*). Ct value and the average of the standard curve were used as the control sample. Standard curves were produced using from one to three different pure strains of the detected microbes per assay (Table S1). For the standard curves DNA was isolated from 100 µl of the bacterial suspensions with a known concentration, and tenfold serial dilutions of the isolated DNA were analysed in triplicate. The amplification efficiency (Ae) of the primer/probe set was calculated using the formula: $Ae=10(-1/slope)$, in which the slope was calculated by regression analysis of the obtained Ct values versus calculated log number of cells in the serial dilutions.

Results

Table S1. Technical details of qPCR assays

Assay name (target taxa)	Abbreviation	Primers and probe sequences	qPCR cycles	Reference strains for standard curves	Reference for assay	NB!
Clostridium cluster XI	Clost XI	F: ACGGTACTTGAGGAGGA R: GAGCCGTAGCCTTTCACT P: FAM-GTGCCAGCAGCCGCGGTAATACG-BHQ1	50 °C 2min, 95 °C 10min, *95 °C 15 sec +* *58 °C 60 sec (40 x)*	*C.glycolicum* (DSM1288), *C. bifermentans* (NC00506), *C. sordellii* (ATCC9714)	Song et al. 2004 (4)	F-primer modified from the original publication
Corynebacterineae/ Pseudonocardineae	Cory/ Pseu	F: CAAGCGGCGGAGCATGTGGA R: TTGCGCTCGTTGCGGGACTT	50 °C 2min, 95 °C 2min, *95 °C 5 sec +* *60 °C 30 sec (40 x),* *95* °C 60 sec, ramp 55-95	*Corynebacterium amycolatum (*DSM 6922), *Corynebacterium hansenii* (DSM 45109)	Novel	
Corynebacterium amycolatum cluster	Camy	F: TTCGGGTTGTAAACTCCTTTCAC R. CGTAGTTAGCCGGTGCTTCTTCT P: FAM-ATCGACGAAGGGTTTCTGACGGTAGATG-TAMRA	95 °C 10min, *95 °C 15 sec +* *60 °C 60 sec (40 x)*	*Corynebacterium amycolatum (*DSM 6922), *Corynebacterium hansenii* (DSM 45109	Novel	
Staphylococcus	Staph	F: ACGGTCTTGCTGTCACTTATA R:TACACATATGTTCTTCCCTAATAA	50 °C 2min, 95 °C 2min, *95 °C 5 sec +* *60 °C 30 sec (40 x),* *95* °C 60 sec, ramp 55-95	*Staphylococcus aureus* (ATCC 25923)	Matsuda et al. 2007 (5)	

F= Forward primer, R= reverse primer, P= probe

Table S2. Bacterial strains tested in laboratory with qPCR assays used in this study

Microbial strain	Camy	Cory/Pseu	Staph	Clost XI
Corynebacterium amycolatum (DSM 6922)	+	+	-	
Corynebacterium hansenii (DSM 45109)	+	+		
Corynebacterium afermentas subsp. afermentas (DSM 44280)	-	+		
Corynebacterium jeikeium (DSM 7171)	-	+		
*Corynebacterium glutamicum (*DSM 20300)	-	+		
*Staphylococcus aureus (*ATCC 25923)	-	-	+	-
*Staphylococcus pasteuri (*ATCC 51129)			-	
*Staphylococcus hominis subs. hominis (*ATCC 27844)			-	
*Staphylococcus simulans (*ATCC 27848)			+	
Staphylococcus sciuri subs. sciuri (ATCC 29062)			-	
*Staphyloccus epidermidis (*ATCC 14990)			+	
*Bacillus cereus (*ATCC 11778)	-	-		-
*Clostridium sordellii (*ATCC 9714)				+
*Clostridium bifermentas (*NC 00506)				+
Clostridium glycolicum (DSM 1288)				+
*Clostridium perfringens (*ATCC 13124)				-
*Clostridium beijerinckii (*DSM 791)				-
Clostridium subterminale (DSM 6970)				-

Table S3. Association of the four microbial groups measured using qPCR with BHR, wheezing, and asthma score

	BHRslope			Wheezing or whistling		Asthma score	
Marker	N	**estimate**	**(95% CI)**	**N**	**OR (95% CI)**	**N**	**OR (95% CI)**
Cory/Pseu (cells/mg)							
I ≤ 19340	68	0		98	1	98	1
II 19341-49099	70	0.298	(-0.521-1.118)	97	0.83 (0.40-1.75)	96	0.86 (0.65-1.14)
III 49100-124999	66	0.613	(-0.197-1.424)	97	0.59 (0.28-1.23)	97	0.97 (0.73-1.30)
IV ≥ 125000	67	0.365	(-0.520-1.249)	96	0.44 (0.20-0.98)**	96	0.79 (0.58-1.07)
Camy (cells/mg)							
I 0	105	0		146	1	146	1
II 1-1319	82	-0.604	(-1.261-0.053)*	121	1.30 (0.70-2.44)	120	1.11 (0.87-1.41)
III ≥ 1320	84	-0.196	(-0.880-0.487)	121	0.78 (0.42-1.46)	121	0.91 (0.70-1.18)
Clost XI (cells/mg)							
I ≤ 5.50	66	0		97	1	96	1
II 5.51-18.35	68	-0.032	(-0.732-0.668)	96	0.89 (0.41-1.94)	96	1.12 (0.86-1.45)
III 18.36-71.46	69	-0.202	(-1.097-0.693)	96	0.60 (0.27-1.32)	96	0.80 (0.59-1.08)
IV ≥ 71.47	68	0.202	(-0.566-0.969)	99	0.49 (0.22-1.08)*	99	1.10 (0.81-1.49)
Staph (cells/mg)							
I ≤ 52000	65	0		97	1	97	1
II 52001-124000	67	-0.065	(-0.823-0.694)	96	0.64 (0.31-1.34)	95	0.92 (0.68-1.26)
III 124001-318450	66	0.495	(-0.314-1.305)	97	0.72 (0.32-1.59)	97	1.08 (0.81-1.43)
IV ≥ 318451	73	0.348	(-0.485-1.181)	98	0.66 (0.31-1.45)	98	0.87 (0.64-1.19)

*p<0.1, **p<0.05
Adjusted for parental allergy, smoking status, household density, gender, age and centre. Asthmascore analyses adjusted also for case status.

Cory/Pseu = *Corynebacterineae/Pseudonocardianeae* group specific assay

Camy = *Coreynebacterium amycolatum* cluster spesific assay

Clost XI = *Clostridium* cluster XI specific assay

Staph= *Staphylococcaceae* group specific assay

Table S4. Levels of *Clostridium* cluster XI (Clost XI) and *Corynebacteriales/Pseudonocardiales* group (Cory/Pseu) in four repeated samples from 5 urban and 4 rural homes

	Clost XI Mattress dust concentrations (CE/mg)				
	N	Mean	25th	50th	75th
all	45	72	2	9	64
urban	25	13	0.4	3	17
rural	20	147	7	64	228

	Cory/Pseu Mattress dust concentrations (CE/mg)				
	N	Mean	25th	50th	75th
all	45	629 544	210 791	472 501	806 400
urban	25	762 142	150 385	423 186	1 057 495
rural	20	463 780	240 718	482 507	699 798

	Clost XI Floor dust concentrations (CE/mg)				
	N	Mean	25th	50th	75th
all	45	168	5	21	197
urban	25	29	2	7	19
rural	20	340	42	165	326

	Cory/Pseu Floor dust concentration (CE/mg)				
	N	Mean	25th	50th	75th
all	44	331 781	86 002	215 959	487 977
urban	25	367 860	71 508	280 660	606 218
rural	19	284 309	138 050	213 798	395 454

	Clost XI Personal air samples (CE/m3)				
	N	Mean	25th	50th	75th
all	42	99	0	0	37
urban	24	0.3	0	0	0
rural	18	231	14	53	375

	Cory/Pseu Personal air samples (CE/m3)				
	N	Mean	25th	50th	75th
all	42	312 409	4 713	11 711	63 473
urban	24	7 854	3 697	5 000	9 352
rural	18	718 483	20 466	120 040	230 299

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