

Face mask sampling reveals antimicrobial resistance genes in exhaled aerosols from patients with chronic obstructive pulmonary disease and healthy volunteers

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ABSTRACT

Introduction The degree to which bacteria in the human respiratory tract are aerosolised by individuals is not established. Building on our experience sampling bacteria exhaled by individuals with pulmonary tuberculosis using face masks, we hypothesised that patients with conditions frequently treated with antimicrobials, such as chronic obstructive pulmonary disease (COPD), might exhale significant numbers of bacteria carrying antimicrobial resistance (AMR) genes and that this may constitute a previously undefined risk for the transmission of AMR.

Methods Fifteen-minute mask samples were taken from 13 patients with COPD (five paired with contemporaneous sputum samples) and 10 healthy controls. DNA was extracted from cell pellets derived from gelatine filters mounted within the mask. Quantitative PCR analyses directed to the AMR encoding genes: *blaTEM* (β -lactamase), *ErmB* (target methylation), *mefA* (macrolide efflux pump) and *tetM* (tetracycline ribosomal protection protein) and six additional targets were investigated. Positive signals above control samples were obtained for all the listed genes; however, background signals from the gelatine precluded analysis of the additional targets.

Results 9 patients with COPD (69%), aerosolised cells containing, in order of prevalence, *mefA*, *tetM*, *ErmB* and *blaTEM*, while three healthy controls (30%) gave weak positive signals including all targets except *blaTEM*. Maximum estimated copy numbers of AMR genes aerosolised per minute were *mefA*: 3010, *tetM*: 486, *ErmB*: 92 and *blaTEM*: 24. The profile of positive signals found in sputum was not concordant with that in aerosol in multiple instances.

Discussion We identified aerosolised AMR genes in patients repeatedly exposed to antimicrobials and in healthy volunteers at lower frequencies and levels. The discrepancies between paired samples add weight to the view that sputum content does not define aerosol content. Mask sampling is a simple approach yielding samples from all subjects and information distinct from sputum analysis. Our results raise the possibility that patient-generated aerosols may be a significant means of AMR dissemination that should be assessed further and that consideration be given to related control measures.

Key messages

- Aerosols generated from the human respiratory tract are a potential source for the dissemination of antimicrobial resistance (AMR) genes.
- Patients with and to a lesser extent, healthy individuals, aerosolise bacteria carrying high numbers of AMR genes (up to thousands/per minute) without exerting any special respiratory effort.
- We describe use of a simple face mask-based aerosol sampling system compatible with routine clinical practice with 23 individuals and demonstrate its utility to detect AMR genes by quantitative PCR.

BACKGROUND

The threat of antimicrobial resistance (AMR) in infectious diseases has been extensively documented.^{1–4} Available antimicrobial therapy for commonplace infections such as those affecting the lower respiratory tract may become greatly restricted, compromising clinical management. Chronic obstructive pulmonary disease (COPD) is a ‘common, treatable respiratory disease characterized by persistent respiratory symptoms and airflow limitation that is due to airway and/or alveolar abnormalities.’⁵ Patients frequently experience exacerbations in symptoms associated with the isolation of bacterial pathogens from their sputum for which they are given antibiotics.

Mortality associated with bacteria exhibiting AMR is increasingly observed in intensive care patients treated for exacerbations of COPD (EOCOPD)⁶; risk factors for EOCOPD with AMR include previous antimicrobial therapy, prolonged or systemic corticosteroid therapy, previous intubation and advanced COPD with severe lung function impairment.⁷

We have been developing the use of face masks to sample exhaled patient aerosols. The approach has been used to detect *Mycobacterium tuberculosis*⁸ and has potential applications in diagnosis and in assessing patient infectiousness. During our studies on the microbiome associated with COPD, we have detected numerous AMR encoding sequences (manuscript in preparation). Based on these experiences, we speculated that patients with COPD might exhale bacteria carrying AMR genes. Exhaled bacteria, therefore, may represent a previously unassessed means of AMR dissemination for which control measures should be considered. Our principal aim here was to explore the feasibility of this sampling technique to detect the presence of AMR genes in expired aerosols. We report results of sampling from patients with stable COPD and from healthy volunteers.

METHODS

Samples

Between October and December 2016, samples were collected from 13 patients with COPD attending an advanced COPD outpatient clinic (OPC) at Glenfield Hospital, University Hospitals of Leicester.

Sample collection

Synthetic fibre single-use face mask (FFP1) (Moldex, Culver City, USA), were fitted with gelatine filters (Sartorius, Goettingen, Germany) mounted on locally manufactured metal holders (figure 1). Direct aerosol sampling took place in consultation rooms in the OPC. Patients were fitted with the mask, covering both nose and mouth; no contact was made between the face and the filter prior to sampling. Masks were worn for 15 min with no restrictions on talking or coughing. Details of recent exacerbation of symptoms and antimicrobial treatment were recorded. Those expectorating sputum provided samples (stored at 4°C for up to 72 hours prior to DNA extraction). After sampling, face masks were immediately replaced in double grip seal bags and stored at 4°C. In healthy volunteers, face mask sampling was performed as mentioned above with volunteers asked to read a set text aloud for 15 min.

Face mask processing

Face masks were processed within 24 hours of sampling. In a class 2 safety cabinet, gelatine filters were removed with forceps and placed into a 40 mL crystallising glass dish. Up to 2 mL of sterilised collagenase buffer (50 µg/mL collagenase A (COLLA-RO, Roche Sigma, UK), 50 mM N-Tris-methyl-2-aminoethanesulfonic acid, 0.36 mM calcium chloride, pH=7.4) was added. Covered dishes were placed on a heated block at 37°C for 15 min until the gelatine filters were digested. The lysates were pipetted into 2 mL screw cap microfuge tubes and centrifuged for 10 min at 15 000×g. The DNA extract was obtained by



Figure 1 FFP1 face mask with gelatine filter.

centrifugation of the 2 mL lysate. The supernatants were discarded and the pellet stored at -80°C.

DNA extraction

The stored pellets were defrosted at room temperature and DNA was extracted using the QIAcube system and the Gram-positive protocol (QIAamp DNA mini kit, Qiagen, Valencia, USA).⁹ Sputum samples were weighed and homogenised with 1 mL of 0.1% dithiothreitol per gram prior to extraction. DNA extracted samples (100 µL) were stored at -20°C until quantitative PCR (qPCR) analysis.

Quantitative PCR

EOCOPD are routinely treated with β-lactams, macrolides or tetracyclines. On this basis and based on their detected prevalence in sputum studies (manuscript in preparation), 10 AMR encoding genes were selected for analysis: *AmpC*, *blaTEM*, *CfxA*, *FOX-5*, *PBP2X*, *ErmB*, *mefA*, *tetM*, *tetA* and *AcrA-05*. Up to 25 µL PCR mixture of each reaction tube was prepared in 0.1 mL Rotor-Gene PCR tubes (QIAGEN, UK) containing 12.5 µL of 2X SensiFAST SYBR No-ROX (BIOLINE, UK), 1 µL of each 10 µM forward and reverse primers (table 1; Integrated DNA Technologies), 1 µL of DNA template and 9.5 µL of molecular grade water; and run in technical triplicates on Rotor-Gene 6000 real-time DNA analysis system using

Table 1 AMR targeted qPCR primers

AMR gene	Oligonucleotide sequence	
	Forward	Reverse
<i>blaTEM</i>	AGCATCTTACGGATGGCATGA	TCCTCCGATCGTTGTCAGAAGT
<i>ErmB</i>	TAAAGGGCATTTAACGACGAAACT	TTTATACCTCTGTTTGTAGGGAATTGAA
<i>mefA</i>	CCGTAGCATTGGAACAGCTTTT	AAACGGAGTATAAGAGTGCTGCAA
<i>tetM</i>	TAATATTGGAGTTTTAGCTCATGTTGATG	CCTCTCTGACGTTCTAAAAGCGTATTAT
<i>AcrA-05</i>	CGTGCGCGAACGAACA	ACTTTGCGCGCCATCTTC
<i>AMPc-04</i>	TCCGGTGACCGCACAGA	CAGCACGCCGGTGAAAAGT
<i>cfxA</i>	TCATTCCTCGTTCAAGTTTTTCAGA	TGCAGCACCAAGAGGAGATGT
<i>FOX-5</i>	GGTTTGCCGCTGCAGTTC	GCGGCCAGGTGACCAA
<i>PBP2X</i>	TTTCATAAGTATCTGGACATGGAAGAA	CCAAAGGAACTTGCTTGAGATTAG
<i>TetA-01</i>	GCTGTTTGTCTGCCGAAA	GGTTAAGTTCCTTGAACGCAAAC

AMR, antimicrobial resistance; qPCR, quantitative PCR.

Corbett PCR machine (Corbett Life Science, QIAGEN). The cycling conditions were as described by Xu *et al.*¹⁰ Pathogen-directed qPCR for *Haemophilus influenzae* and *Staphylococcus aureus* (SYBR green) and *Moraxella catarrhalis*, *Pseudomonas aeruginosa* and *Streptococcus pneumoniae* (Taqman) were performed in technical triplicates as previously described.¹¹

Analyses for *blaTEM*, *ErmB*, *mefA* and *tetM* were repeated using IDT gBlock standards (Integrated DNA Technologies) to enable quantification. Calibration curves converting cycling threshold (C_t) values to gene copy numbers were generated as described by Gunawardana *et al.*¹² A custom DNA oligomer-gBlock (Integrated DNA Technologies) was synthesised to span the region of the AMR genes covered by the forward and reverse primers for each of the four AMR genes assayed. Tenfold serial dilutions of these oligomers were used as qPCR standards. qPCR cycling conditions were modified for assay optimisation by increasing the annealing temperature to 63°C for 30 s. qPCR readings with an $R_s > 0.985$ and efficiency $\geq 85\%$ were accepted. Blank gelatine filters due to their organic composition contained some intrinsic AMR genes (predominantly β -lactamase) which produced C_t values ranging from 29 to 33. However, these signals were at least three C_t values above those obtained for the 10^2 standard. The C_t value of the 10^2 standard was considered as the lower limit for detection. All technical replicates ($n=3$) showed coefficients of variation below 10%. Mask and sputum analyses are quoted as copies per mask and as copies per gram, respectively. The calculated total number of copies per face mask sample was derived from the calculated number of copies per qPCR reading (copy/ μ L) multiplied by the total volume of DNA extract (200 μ L).

RESULTS

Ten AMR genes were initially selected for analysis (*AmpC*, *blaTEM*, *CfxA*, *FOX-5*, *PBP2X*, *ErmB*, *mefA*, *tetM*, *tetA* and *AcrA-05*). Six of these (*AcrA-05*, *AmpC*, *CfxA*, *FOX-5*,

PBP2X, *tetA*) generated multiple amplicons in blank filter control samples. While melt curves consistent with the specific targets were observed within the mixtures, additional work to confirm their identities has not been undertaken. In contrast, blank filters produced no amplicons for the remaining four targets (*ErmB*, *mefA*, *tetM* and *tetA*) and single bands were obtained from positive samples on gel electrophoresis.

Thirteen 15 min mask aerosol and sputum samples collected contemporaneously from five patients were obtained and these were compared with mask samples from 10 healthy volunteers. Demographics of the subjects are shown in table 2.

Detection of AMR genes

Positive detections in the mask and sputum samples are shown in figure 2. Nine patients with COPD (69%) were found to have aerosolised cells containing AMR genes while only three (30%) of the healthy volunteers did so. Of the patients with positive aerosols, one, three, two and one respectively aerosolised all four, three, two and one of the four target genes. Although samples from the four remaining patients showed some amplification, the C_t values were below the established limit of detection. The frequency of positive mask samples was *mefA* > *tetM* > *ErmB* > *blaTEM* and this followed the relative frequency of positives observed in sputum. Although positives for three targets were found in the healthy volunteers, the frequencies were consistently lower than in the patient samples.

The quantitative results for COPD (aerosol and sputum for five subjects) and healthy volunteers (aerosol only) are shown in table 3. *MefA* was detected in all samples. In the paired sputum and aerosol analyses, four target positive results gave numerically higher copy numbers per gram of sputum than those per mask, while in five pairs the aerosol counts were higher. In two cases, the aerosol was positive and the sputum negative. We note that in patient 9 the *mefA* mask signal was higher while

Table 2 COPD cohort demographic data

Sample size	13	10
Age (years) (SD)	63.5 (10.07)	Between 18 and 48*
Male (%)	5 (38)	2 (20)
Current smoker (%)	3 (23)	NA
FEV ₁ % predicted	30.6 (12.6)	NA
Mean FEV ₁ /FVC ratio (SD)	31.4 (7.7)	NA
Exacerbations per year (SD)	5.2 (4.5)	NA
Antimicrobials		None within 6 months
Prophylactic macrolides	2	NA
Self-management	10	NA
Amoxicillin†	5	NA
Doxycycline	5 (1 in combination)	NA
Levofloxacin	1	NA

*Individual ages of healthy volunteers not collected.

†Includes one on co-amoxiclav.

COPD, chronic obstructive pulmonary disease; FEV₁, forced Expiratory Vvolume in 1 s; FVC, forced vital capacity; NA, not applicable.

the *tetM* signal showed the converse pattern. If the expelled aerosol reflects sputum content then we would expect the ratio between signals for these two targets to be the similar in both samples. The discrepant *mefA/tetM* ratios observed between aerosol and sputum in patients 2 (1.1 vs 0.15) and 9 (6.2 vs 0.02) and the aerosol positive, sputum negative analyses argue against the view that aerosol is a direct sample of sputum (table 3).

DISCUSSION

We have detected four bacterial AMR encoding genes in aerosols generated by patients with COPD and, to a lesser extent, by healthy volunteers. To our knowledge, this is the first direct recognition that expired air is a potential means by which AMR genes may be disseminated. Our approach was applied in an outpatient setting and required only 15 min sampling without detailed instruction to the sampler or the subject.

While we initially selected 10 targets for analysis, we were only able to validate the assays for four of these in the mask sample system. This was due to intrinsic contamination of the gelatine filters we used to collect the samples. It is disconcerting that we obtained such high signals for multiple targets that we could not analyse our aerosol samples for six of our selected targets (*AcrA-05*, *AmpC*, *CfxA*, *FOX-5*, *PBP2X*, *tetA*).

Presence of AMR genes

We hypothesised that patients with COPD might aerosolise significant quantities of AMR genes as both frequent and prophylactic antimicrobial therapy in this group provides strong selection pressure for colonisation with resistant microorganisms.¹³ One or more AMR genes were identified in the aerosol samples from 69% of patients with COPD. Non-detection of AMR genes in the remaining four patient samples may reflect a lesser tendency to aerosolise bacteria from the lower airways in these individuals or absence of resistance. In the former regard, large differences in aerosolisation rates have been reported between individuals with smear positive tuberculosis,¹⁴ while in the latter, multiple complex factors beyond antimicrobial exposure contribute towards the evolution and persistence of AMR genes within the resistome.³

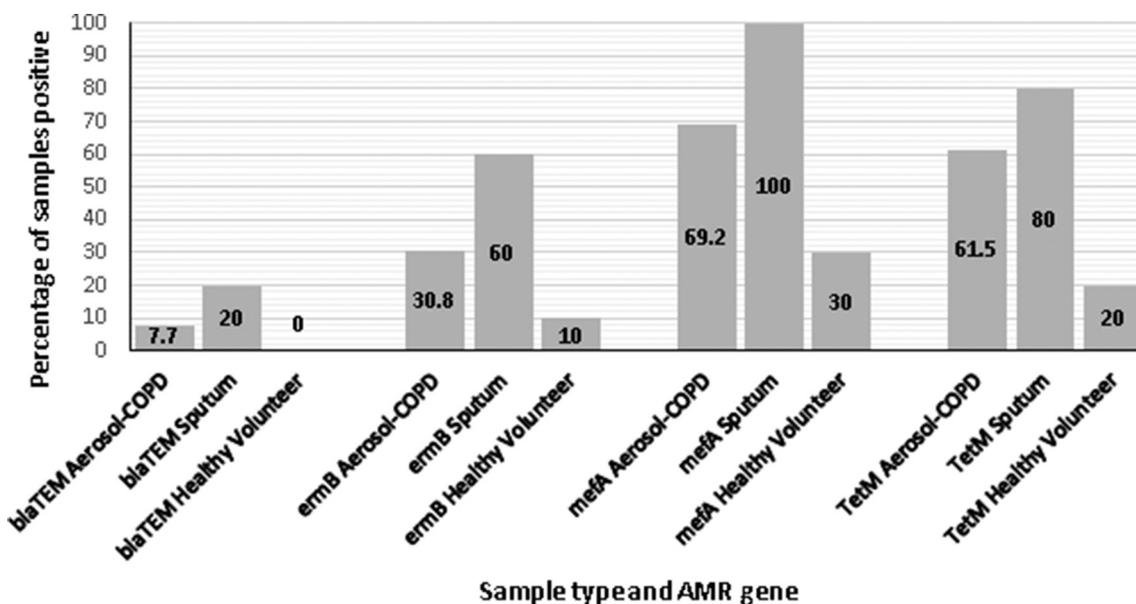


Figure 2 Prevalence of resistance genes in COPD aerosol, COPD sputum and healthy volunteer aerosol samples. AMR, antimicrobial resistance; COPD, chronic obstructive pulmonary disease.

Table 3 Samples with positive qPCR signals obtained from mask and sputum samples

Target/sample	Patients with COPD (9/13)*									HV (3/10)*		
	Paired mask-ae (copies/mask) and sputum (sp) samples									Mask only		
	2	6	9	12	13	3	4	7	10	4	8	10
<i>mefA</i> ae	114	1514	45 159	8724	3574	254	120	478	5923	163	181	829
<i>sp</i>	6727	162	728	6253	74 722	–	–	–	–	–	–	–
<i>tetM</i> ae	101	334	7296	365	1362	258	<	308	247	134	<	290
<i>sp</i>	45 295	<	37 828	48†	40 590	–	–	–	–	–	–	–
<i>ErmB</i> ae	<	<	<	<	1375	239	<	1379	273	<	<	1470
<i>sp</i>	<	<	<	<	17 352	–	–	–	–	<	<	<
<i>blaTEM</i> ae	<	<	<	<	<	<	<	<	365	<	<	<
<i>sp</i>	<	<	<	<	<	–	–	–	–	–	–	–
Ratio <i>mefA</i> / ae	1.1	4.5	6.2	23.9	2.62	–	–	–	–	–	–	–
<i>tetM</i> <i>sp</i>	0.15	–	0.02	56.7	1.84	–	–	–	–	–	–	–
Proph/SM	Tet	Amox	Nil	Nil	Amox	Tet	Nil	Amox	Co-Amox	–	–	–
Pathogen qPCR	Nil	Mc+Pa	Nil	Hi	Nil	Nil	Nil	Nil	Nil	–	–	–

–, not done; <, below limit of detection (100 copies). Note: pt 11-prophylactic amoxicillin-antimicrobial resistance (AMR) genes below limit of detection.

*Figures in parentheses show number of subjects positive/total number of subjects sampled.

†>100 copies detected.

ae, mask aerosol sample (copies per mask); Amox, amoxicillin; COPD, chronic obstructive pulmonary disease; Co-Amox, co-amoxiclav; HV, healthy volunteers; Hi, *Haemophilus influenzae*; Mc, *Moraxella catarrhalis*; Pa, *Pseudomonas aeruginosa*; Proph/SM, prophylaxis/self-management; sp, sputum (copies per gram). Tet, tetracycline; qPCR, quantitative PCR;

MefA was identified in all of the positive COPD aerosol samples. This AMR gene is prevalent in streptococci¹⁵ and recognised as the most frequent macrolide resistance in *S. pneumoniae*, the PROTEKT study demonstrated 70.8% macrolide resistance in *S. pneumoniae*.¹⁶ Streptococci make a major contribution to the Firmicute signals obtained from lower respiratory tract samples but only *S. pneumoniae* is recognised as a commonly pathogenic in COPD.^{17 18} Interestingly, the pneumococcus was not detected in any of our aerosol samples here, indicating that the detected *mefA* signals presumably derived from other streptococci. It seems likely that the high prevalence of *mefA* in aerosols detected here, the natural transformability of *S. pneumoniae*¹⁹ and the high frequency of macrolide resistance in this pathogen are connected.

Among the three remaining targets *tetM* was the most frequently detected; this has been reported as the most widely phylogenetically distributed of the AMR genes analysed here (table 4). In this pilot study we have not attempted to determine the bacterial hosts of the genes we detected. We note in table 4 that all four of our targets have been detected in *Pseudomonas* spp, all except *mefA* in *Haemophilus* spp and all except *blaTEM* in *Streptococcus* spp. While resistance in known pathogens clearly represents the greatest threat, our purpose here has been to add knowledge to understanding of AMR dissemination. The presence of these genes in aerosols clearly presents a previously undefined means of their dissemination whichever host the signals are derived from. Moreover,

we have yet to determine the degree to which resistance determinants, particularly degradative enzymes, expressed in the lower respiratory microbiota other than recognised pathogens, may affect the outcome of therapy directed to apparently sensitive pathogens.

Utility of mask sampling

The present study builds on our experience in respiratory microbiology which has predominantly derived from sputum analyses. We have been surprised by the abundance of bacterial signals collected on face masks, notably the possibility that such collections may be of value in diagnosing and assessing infectivity in tuberculosis.⁸ Indeed, reviewing the data for patient 9 in table 3, it appears that this patient with COPD exhaled over 3000 copies of *mefA* and 480 of *tetM* per minute. Both these AMR determinants have been reported to be encoded on Tn916-related transposons,^{20 21} further emphasising the potential for their dissemination across strain and species barriers. It should also be noted that in contrast to the Cough Aerosol Sampling System (CASS) described by Fennelly and colleagues,²² subjects were not asked to perform specific respiratory efforts to obtain mask samples. Thus, our results represent AMR genes exhaled while subjects were breathing normally and may therefore represent natural dissemination rates.

Results from CASS studies have provided initial evidence that patient-produced aerosols are not a simple sample of

Table 4 Reported family and genus level distribution of AMR genes detected in this study

Gene	Reported distribution
<i>mefA</i>	<i>Acinetobacter</i> , <i>Bacteroides</i> , <i>Citrobacter</i> , <i>Clostridium</i> , <i>Corynebacterium</i> , <i>Enterococcus</i> , <i>Enterobacter</i> , <i>Escherichia</i> , <i>Fusobacterium</i> , <i>Gemella</i> , <i>Klebsiella</i> , <i>Lactobacillus</i> , <i>Micrococcus</i> , <i>Morganella</i> , <i>Neisseria</i> , <i>Pantoea</i> , <i>Providencia</i> , <i>Proteus</i> , <i>Ralstonia</i> , <i>Pseudomonas</i> , <i>Salmonella</i> , <i>Serratia</i> , <i>Staphylococcus</i> , <i>Streptococcus</i> , <i>Stenotrophomonas</i> ^{15 31}
<i>TetM</i>	<i>Abiotrophia</i> , <i>Acinetobacter</i> , <i>Actinomyces</i> , <i>Aerococcus</i> , <i>Aeromonas</i> , <i>Afipia</i> , <i>Arthrobacter</i> , <i>Bacillus</i> , <i>Bacterionema</i> , <i>Bacteroides</i> , <i>Bifidobacterium</i> , <i>Brachybacterium</i> , <i>Catenibacterium</i> , <i>Clostridium</i> , <i>Corynebacterium</i> , <i>Edwardsiella</i> , <i>Eikenella</i> , <i>Enterobacter</i> , <i>Enterococcus</i> , <i>Erysipelothrix</i> , <i>Escherichia</i> , <i>Eubacterium</i> , <i>Flavobacterium</i> , <i>Fusobacterium</i> , <i>Gardnerella</i> , <i>Gemella</i> , <i>Granulicatella</i> , <i>Haemophilus</i> , <i>Kingella</i> , <i>Klebsiella</i> , <i>Kurthia</i> , <i>Lactobacillus</i> , <i>Lactococcus</i> , <i>Listeria</i> , <i>Microbacterium</i> , <i>Mycoplasma</i> , <i>Neisseria</i> , <i>Paenibacillus</i> , <i>Pantoea</i> , <i>Pasteurella</i> , <i>Peptostreptococcus</i> , <i>Photobacterium</i> , <i>Prevotella</i> , <i>Pseudoalteromonas</i> , <i>Pseudomonas</i> , <i>Ralstonia</i> , <i>Selenomonas</i> , <i>Serratia</i> , <i>Shewanella</i> , <i>Staphylococcus</i> , <i>Streptococcus</i> , <i>Streptomyces</i> , <i>Ureaplasma</i> , <i>Veillonella</i> , <i>Vibrio</i> ^{15 32}
<i>ermB</i>	<i>Aggregatibacter</i> , <i>Acinetobacter</i> , <i>Aerococcus</i> , <i>Arcanobacterium</i> , <i>Bacillus</i> , <i>Bacteroides</i> , <i>Citrobacter</i> , <i>Corynebacterium</i> , <i>Clostridium</i> , <i>Enterobacter</i> , <i>Escherichia</i> , <i>Eubacterium</i> , <i>Enterococcus</i> , <i>Fusobacterium</i> , <i>Gemella</i> , <i>Haemophilus</i> , <i>Klebsiella</i> , <i>Lactobacillus</i> , <i>Micrococcus</i> , <i>Neisseria</i> , <i>Pantoea</i> , <i>Pediococcus</i> , <i>Peptostreptococcus</i> , <i>Porphyromonas</i> , <i>Proteus</i> , <i>Pseudomonas</i> , <i>Ruminococcus</i> , <i>Rothia</i> , <i>Serratia</i> , <i>Staphylococcus</i> , <i>Streptococcus</i> , <i>Treponema</i> , <i>Wolinella</i> ^{15 31}
<i>blaTEM</i>	Enterobacteriaceae, Neisseriaceae, <i>Pseudomonas</i> , <i>Haemophilus</i> ³³

AMR, antimicrobial resistance.

sputum¹⁴ and there is further evidence for this here. As noted above, if aerosol is a direct sample of sputum, then the ratio between signals for different AMR genes should be the same in both. This was clearly not the case for two of the five paired samples shown in table 3. While it is possible that the sampling procedure and processing of sputum and mask might bias the results, this effect would be expected to be consistent across all the samples and again this was not the case. We therefore suggest that the differing *mefA/tetM* ratios observed provide further

support to the view that aerosol is not a simple sample of sputum and that bacteria collected in masks have been selected by a process distinct from those present in sputum. One property potentially underpinning this is cell surface hydrophobicity, a property known to affect the potential of mycobacteria to enter into aerosols.²³

Limitations

While this initial study on 23 individuals has illustrated the feasibility of the mask sampling approach, several aspects of our results should be interpreted with caution. In particular, our sample size was too small and demographic matching insufficient to allow formal comparisons of the AMR gene positive frequencies between our healthy and COPD subjects. A further important limitation was the presence of contaminating DNA in our gelatine filters which prevented analysis of 6 out of 10 of our initial targets. Gelatine is derived from animal collagen and is a by-product of the meat and leather industries. The tissues involved are certain to be contaminated with microbes, both during husbandry and in abattoirs. While our filters were guaranteed sterile by the manufacturers we know from this and other analyses that abundant bacterial DNA is present. Although further work will be needed to confirm the identity of the contaminating DNA the background signals we have detected raise further concern over the contribution of animal husbandry to the AMR problem. We have now developed a different sampling matrix that has no significant background and preliminary studies indicate that several of the targets excluded here can be detected in aerosols. A further shortcoming is that we have not optimised the efficiency of mask sampling by asking the patients to perform respiratory manoeuvres that maximise the collection yield of exhaled bacteria. Studies to identify the capacity of such manoeuvres to yield samples from different parts of the respiratory tract are in progress.

We have not identified the host organisms for the AMR genes studied here. This could be achieved with a metagenomic approach or by more limited sequencing studies but these were beyond the scope of this exploratory study.

Caution should be exercised in interpreting the low signals that were detected in many mask samples. However, confidence that these were true positives in the COPD subjects is reinforced by the detection of the same target in sputum and correspondence between high frequency of positives in the latter sample and detection in aerosols. We re-emphasise that we found no background positive signals for our target genes on blank filters. We note that free bacterial DNA may be present in aerosolised samples²⁴ and that this would not have been detected by our cell pellet directed procedure.

Infection prevention and control implications

Although viral spread is generally regarded as the principal airborne infection hazard, many bacterial respiratory pathogens are transmitted by this route.²⁵ Nosocomial

outbreaks of resistant bacterial respiratory infections²⁶ and influenza²⁷ are well documented. Approaches to reduce both pathogen and AMR transmission via aerosol in healthcare and other setting are clearly desirable. The contribution of human-generated aerosols containing bacterial cells or free bacterial DNA towards the growing AMR crisis is currently undefined.

Gilbert *et al.*²⁸ isolated and amplified *ermF*, *ermX* and *tetG* genes from airborne samples within hospital rooms. The originating organism(s) were unidentified, but hypothesised to arise from the genome of unculturable bacteria. Air sampling of four Iranian hospitals revealed airborne β -lactamase-resistant bacteria harbouring *OXA-23* and *OXA-51* genes associated with *Acinetobacter* spp. The authors suggested the potential role for of airborne bacteria in the transmission of drug-resistant nosocomial infections, with identification of dissemination sources important in reducing transmission within the healthcare setting.²⁹ Aerosolisation of AMR genes from patients may contribute to the burden of AMR circulating in the environment, and the airborne route has been identified as a potential reservoir of AMR elements necessitating further surveillance studies.³⁰

Our study has demonstrated that patients with COPD and healthy volunteers can be a source of aerosolised AMR genes; our limited study indicates that the former groups are a more abundant source. The AMR genes detected here are already widely disseminated and the clinical impact of their continued aerosol spread is probably small. However, the potential to spread AMR genes with a greater clinical and public health impact is clearly established. There is growing recognition of the likely healthcare-associated airborne spread of highly resistant opportunistic pathogens such as *Burkholderia cepacia* and *Mycobacterium abscessus* in vulnerable patient groups including those with cystic fibrosis. We suggest that the mask aerosol sampling system (MASS) used here provides an amenable approach to assessing the scale and importance of human-exhaled microbial hazards at both individual and population levels. Such information will inform appropriate control measures.

CONCLUSION

We have demonstrated that patients with COPD aerosolise bacteria carrying AMR genes at rates up to thousands of copies per minute without exerting special respiratory effort. The simple MASS we describe is readily applicable in clinical settings and could be used extensively to measure respiratory output of microbes. This approach has potential to enable better control of agents spread by the respiratory route and could contribute to limiting the spread of AMR.

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Contributors MK: study conceptualisation, data collection, laboratory processing of COPD and volunteer samples, data interpretation, statistical analysis and drafting of manuscript. MYR, CMLW and JA: assistance with laboratory processing of samples. MA: collection of volunteer samples and extraction of DNA. KH: assistance with laboratory processing of samples and manuscript review. CEB: project supervision. MRB: study conceptualisation and manuscript drafting and review.

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Patient consent Obtained.

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Data sharing statement All data collected as part of this study and used to reach the above conclusions are included within this article and in the online supplementary material. Data displayed within this article are available upon request from MK.

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REFERENCES

- Davies J, Davies D. Origins and evolution of antibiotic resistance. *Microbiol Mol Biol Rev* 2010;74:417–33.
- Dodds DR. Antibiotic resistance: a current epilogue. *Biochem Pharmacol* 2017;134:139–46.
- Holmes AH, Moore LS, Sundsfjord A, *et al.* Understanding the mechanisms and drivers of antimicrobial resistance. *Lancet* 2016;387:176–87.
- Theuretzbacher U. Global antibacterial resistance: the never-ending story. *J Glob Antimicrob Resist* 2013;1:63–9.
- Global Initiative for Chronic Obstructive Lung Disease. Pocket guide to copd diagnosis, management, and prevention: a Guide for Health Care Professionals. 2017.
- Nseir S, Di Pompeo C, Cavestri B, *et al.* Multiple-drug-resistant bacteria in patients with severe acute exacerbation of chronic obstructive pulmonary disease: prevalence, risk factors, and outcome. *Crit Care Med* 2006;34:2959–66.
- Nseir S, Ader F. Prevalence and outcome of severe chronic obstructive pulmonary disease exacerbations caused by multidrug-resistant bacteria. *Curr Opin Pulm Med* 2008;14:95–100.
- Williams CM, Cheah ES, Maikin J, *et al.* Face mask sampling for the detection of Mycobacterium tuberculosis in expelled aerosols. *PLoS One* 2014;9:1–7.
- Qiagen. *QIAamp® DNA Mini and Blood Mini Handbook*. 2010.
- Xu L, Ouyang W, Qian Y, *et al.* High-throughput profiling of antibiotic resistance genes in drinking water treatment plants and distribution systems. *Environ Pollut* 2016;213:119–26.
- Bafadhel M, McKenna S, Terry S, *et al.* Acute exacerbations of chronic obstructive pulmonary disease: identification of biologic clusters and their biomarkers. *Am J Respir Crit Care Med* 2011;184:662–71.
- Gunawardana M, Chang S, Jimenez A, *et al.* Isolation of PCR quality microbial community DNA from heavily contaminated environments. *J Microbiol Methods* 2014;102:1–7.
- Brill SE, Law M, El-Emir E, *et al.* Effects of different antibiotic classes on airway bacteria in stable COPD using culture and molecular techniques: a randomised controlled trial. *Thorax* 2015;70:930–8.
- Jones-López EC, Namugga O, Mumbowa F, *et al.* Cough aerosols of Mycobacterium tuberculosis predict new infection: a household contact study. *Am J Respir Crit Care Med* 2013;187:1007–15.
- van Hoek AHAM, Mevius D, Guerra B, *et al.* Acquired antibiotic resistance genes: an overview. *Front Microbiol* 2011;2:1–27.
- Farrell DJ, Couturier C, Hryniewicz W. Distribution and antibacterial susceptibility of macrolide resistance genotypes in Streptococcus pneumoniae: PROTEKT Year 5 (2003–2004). *Int J Antimicrob Agents* 2008;31:245–9.

17. Cameron SJ, Lewis KE, Huws SA, *et al.* Metagenomic sequencing of the chronic obstructive pulmonary disease upper bronchial tract microbiome reveals functional changes associated with disease severity. *PLoS One* 2016;11:1–16.
18. Cabrera-Rubio R, Garcia-Núñez M, Setó L, *et al.* Microbiome diversity in the bronchial tracts of patients with chronic obstructive pulmonary disease. *J Clin Microbiol* 2012;50:3562–8.
19. Santoro F, Vianna ME, Roberts AP. Variation on a theme; an overview of the Tn916/Tn1545 family of mobile genetic elements in the oral and nasopharyngeal streptococci. *Front Microbiol* 2014;5:1–10.
20. Domenech A, Ardanuy C, Balsalobre L, *et al.* Pneumococci can persistently colonize adult patients with chronic respiratory disease. *J Clin Microbiol* 2012;50:4047–53.
21. Calatayud L, Ardanuy C, Cercenado E, *et al.* Serotypes, clones, and mechanisms of resistance of Erythromycin-resistant streptococcus pneumoniae isolates collected in Spain. *Antimicrob Agents Chemother* 2007;51:3240–6.
22. Wainwright CE, France MW, O'Rourke P, *et al.* Cough-generated aerosols of *Pseudomonas aeruginosa* and other Gram-negative bacteria from patients with cystic fibrosis. *Thorax* 2009;64:926–31.
23. Jankute M, Nataraj V, Lee OY-C, *et al.* The role of hydrophobicity in tuberculosis evolution and pathogenicity. *Sci Rep* 2017;7:1–10.
24. Zhen H, Han T, Fennell DE, *et al.* Release of free DNA by membrane-impaired bacterial aerosols due to aerosolization and air sampling. *Appl Environ Microbiol* 2013;79:7780–9.
25. Tang JW, Li Y, Eames I, *et al.* Factors involved in the aerosol transmission of infection and control of ventilation in healthcare premises. *J Hosp Infect* 2006;64:100–14.
26. Weiss K, Restieri C, Gauthier R, *et al.* A nosocomial outbreak of fluoroquinolone-resistant *Streptococcus pneumoniae*. *Clin Infect Dis* 2001;33:517–22.
27. Sartor C, Zandotti C, Romain F, *et al.* Disruption of services in an internal medicine unit due to a nosocomial influenza outbreak. *Infect Control Hosp Epidemiol* 2002;23:615–9.
28. Gilbert Y, Veillette M, Duchaine C. Airborne bacteria and antibiotic resistance genes in hospital rooms. *Aerobiologia* 2010;26:185–94.
29. Mirhoseini SH, Nikaeen M, Shamsizadeh Z, *et al.* Hospital air: a potential route for transmission of infections caused by β -lactam-resistant bacteria. *Am J Infect Control* 2016;44:898–904.
30. Messi P, Sabia C, Anacarso I, *et al.* Prevalence of multi-drug-resistant (MDR) bacteria in air samples from indoor and outdoor environments. *Aerobiologia* 2015;31:381–7.
31. Roberts M. 2016. Location of the various genes 14/2/2017 [cited 2017 19/09/2017]; Macrolide resistance gene database]. <http://faculty.washington.edu/marilynr/ermweb4.pdf>
32. Roberts M. Mechanism of resistance for characterized tet and otr genes. Tetracycline resistance genes 2016 [cited 2017 19/09/2017]; 13/7/2016:[Tetracycline resistance gene database]. <http://faculty.washington.edu/marilynr/tetweb1.pdf>
33. Salverda ML, De Visser JA, Barlow M. Natural evolution of TEM-1 β -lactamase: experimental reconstruction and clinical relevance. *FEMS Microbiol Rev* 2010;34:1015–36.
34. Schroeder MR, Stephens DS. Macrolide resistance in *Streptococcus pneumoniae*. *Front Cell Infect Microbiol* 2016;6:1–9.