related cancer pathways including chromosome instability, p53 signalling and Wnt/β-catenin signalling. MMP12 and LMO7 were found within the highest significantly differently expressed genes and were therefore chosen to pursue studies focused on understanding the potential mechanisms leading to the development of lung SCC. In agreement with the gene expression data the expression of MMP12 and LMO7 proteins were up-regulated and down-regulated, respectively, in progressive when compared with regressive lesions. Inhibiting MMP12 by MMP12 knockdown significantly reduced the migration and invasion of different squamous cell carcinoma cell lines (A431, H357 and H376). We also established HBECs knockdown targeting LMO7. We observed a significant increase in the migration and invasion of HBECs cells in the LMO7 shRNA knockdown compared to control.

Our results suggest that MMP12 and LMO7 may be potential therapeutic markers for lung cancer at early stage.

Infection of the pleural space in disease and on purpose

S113 PREDICTORS OF BACTERIAL 'LOAD' IN PLEURAL INFECTION

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Pleural infection is usually defined using pleural fluid biochemical characteristics, given that only ~30% of cases are culture positive, but the relationship between these characteristics and pleural space bacterial concentration is unclear.

We developed an assay to estimate bacterial 'load' using quantitative polymerase chain reaction (PCR) to determine 16S rRNA gene copy number in pleural fluid samples (this gene is present in all bacteria). This enabled us to explore the relationship between patient characteristics and pleural fluid bacterial 'load'.

Methods Pleural infection samples were obtained from the Second Multicentre Intrapleural Sepsis randomised controlled Trial (MIST2), REC no. 04/MRE5/53. DNA was extracted using the FastDNA SPIN Kit. Quantitative PCR (qPCR) of the 16S rRNA gene was undertaken using the ultra-pure Power SYBR Green PCR reagent and primers that amplified the 467 nt V3–4 region of the 16S rRNA gene. A 3-step thermal cycling profile was empirically determined to give optimal results. Ten-fold dilutions of *Acidothermus cellulolyticus* DNA were used to estimate sample 16S rRNA gene concentration. All PCRs were performed in duplicate. Melt-curve analyses and agarose gel electrophoresis of qPCR amplicons were used to ensure absence of non-specific PCR products.

Results 172 pleural fluid samples were analysed. Pleural fluid pH, culture status, appearance, LDH and glucose were all predictive of bacterial load (see Table). Patient C-reactive protein (CRP) and white cell count (WCC) were not significantly associated with bacterial load.

Conclusions Bacterial 'load' was associated with acknowledged predictors for pleural infection. Such findings add further support to the utility of pH, glucose and LDH values as proxies for pleural infection, in the correct clinical context. Patient WCC and CRP were not significantly associated with bacterial 'load'.

This assay is limited in that it assesses total bacterial DNA (from viable and dead bacteria), rather than quantifying viable

Abstract S113 Table 1 Relationships between copies of 16S rRNA gene (base 10 logarithmic values) and characteristics of patients and pleural fluid (PF) samples

		Copies of 16S rRNA gene, measured by qPCR				
	Number	% change per unit[1]	95% CI	Geometric mean	95% CI	*p* value
PF pH	97	-89.6	-97.6, -55.5			0.003
PF culture status						
Negative	110			2.8×10^5	1.9×10^5, 4.0×10^5	
Positive	44			2.2×10^6	1.2×10^6, 4.0×10^6	<0.0001
PF appearance						
Non-purulent	74			1.9×10^5	1.2×10^5, 2.9×10^5	
Purulent	76			1.4×10^6	8.8×10^5, 2.2×10^6	<0.0001
PF LDH						
≤1000	26			1.4×10^5	6.9×10^4, 2.8×10^5	
1000–5000	41			4.1×10^5	2.3×10^5, 7.1×10^5	
>5000	36			6.2×10^5	3.4×10^5, 1.1×10^6	0.007
PF glucose						
≤1.0	43			5.9×10^5	3.4×10^5, 1.0×10^6	
1.0–2.2	14			2.9×10^5	1.1×10^5, 7.3×10^5	
>2.2	35			1.5×10^5	8.5×10^4, 2.8×10^5	0.007
Patient CRP						
<100	28			3.5×10^5	1.6×10^5, 7.5×10^5	
100–160	27			3.5×10^5	1.6×10^5, 7.7×10^5	
≥160	87			5.8×10^5	3.7×10^5, 8.9×10^5	0.372
Patient WCC						
≤11.0	41			3.7×10^5	1.9×10^5, 7.0×10^5	
11.0–16.5	58			4.6×10^5	2.7×10^5, 7.9×10^5	
>16.5	53			7.7×10^5	4.4×10^5, 1.4×10^6	0.215

[1]% change in 16S rRNA gene copies number per unit increase in the specified variable. CI = confidence interval. *p* values for tests of linear trend (continuous variables) and for tests of heterogeneity (categorical variables). LDH units – IU/L; glucose units – mmol/L; CRP units – mg/L; WCC units – x 10^9/L

bacteria. Further, bacteria vary in their copy number of the 16S rRNA gene, dependent on species. Despite these limitations, our associations have reached a strong level of significance.

S114 PREVIOUSLY UNRECOGNISED ORAL ANAEROBES IN PLEURAL INFECTION

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Laboratory culture of pleural infection samples is positive in only 30% of cases, probably related to antibiotic usage and fastidious or unculturable organisms such as some anaerobes. Previous studies using capillary sequencing of the 16S rRNA gene improves rates of organism identification, but is unable to resolve the polymicrobiality thought to be present in anaerobic infection.

We used ultra-deep pyrosequencing to definitively characterise anaerobic pleural infection.

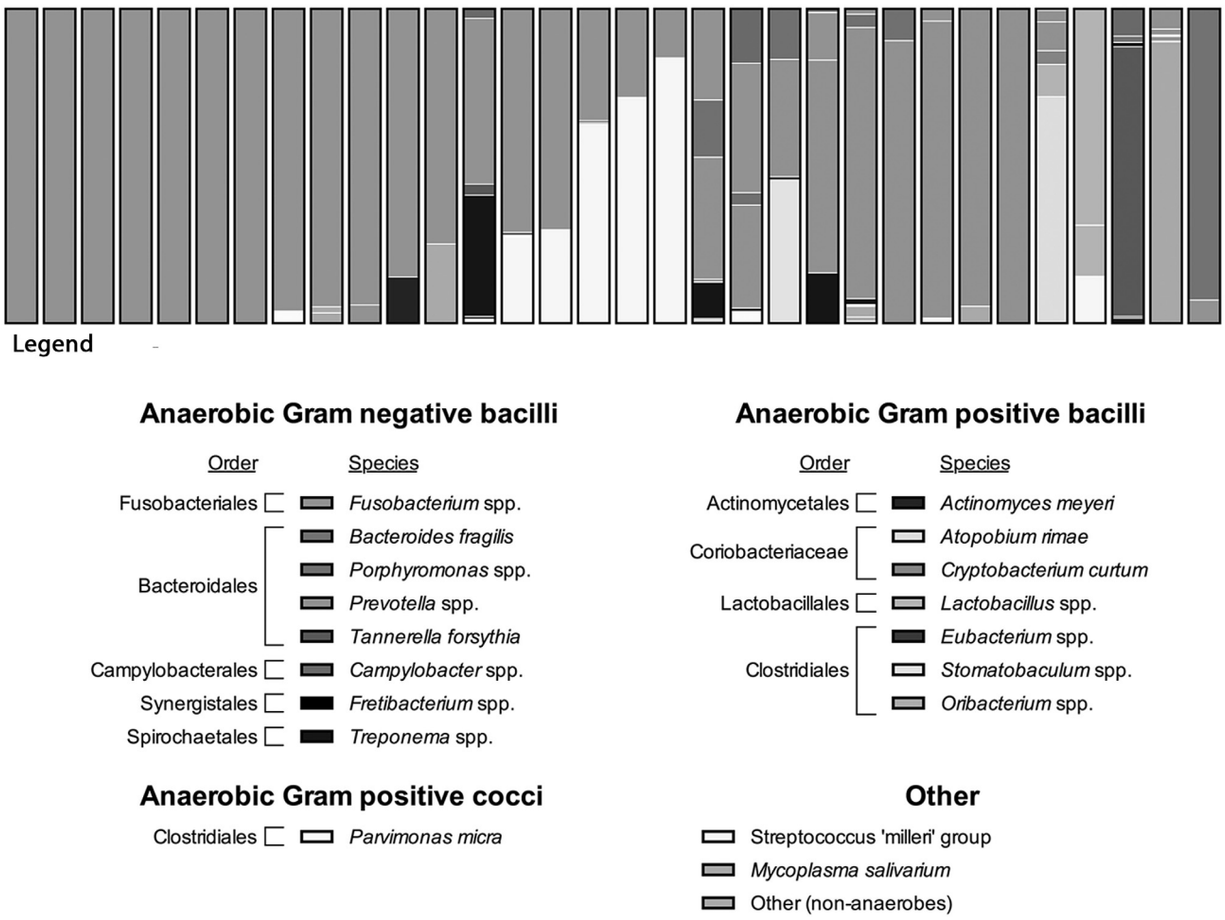
Methods Pleural infection samples were obtained from the Second Multicentre Intrapleural Sepsis randomised controlled Trial (MIST2), REC no. 04/MRE5/53. DNA was extracted using the FastDNA SPIN Kit. Modified 'fusion' primers amplified the V4–6 regions of the 16S rRNA gene. Subsequent pyrosequencing was performed on the Roche 454 GS FLX instrument. Data analyses were performed using the open souce 'Quantitative Insights Into Microbial Ecology' platform. Strategies were used to control for contamination.

Results 172 pleural fluid samples were available, 98 of which were successfully sequenced. 32/98 samples contained anaerobes (defined when ≥10% of sequences in a sample represented anaerobes).

Fusobacteriales, particularly *Fusobacterium nucleatum*, and Bacteroidales, particularly *Prevotella* spp. were commonly found although other anaerobes were seen (see Figure).

Anaerobic pleural infection was usually polymicrobial, with an estimated 4–5 operational taxonomic units ("species") per sample. Particular patterns of co-infection were *Fusobacterium nucelatum* and Streptococcus 'milleri' group although *Prevotella* spp. ± *Fusobacterium* spp. ± *Porphyromonas* spp. ± *Treponema* spp. also co-infected several samples.

Many species were found that have not been previously documented, including *Atopobium rimae, Cryptobacterium curtum, Lactobacillus spp., Stomatobaculum* spp., *Oribacterium* spp., *Prevotella baroniae, Prevotella dentalis/Hallella seregens, Prevotella scopos, Fretibacterium* spp., *Tanerella forsythia, Treponema denticola, lecithinolyticum, maltophilum, medium and socranskii*. Intriguingly, the original isolation and description of almost all these anaerobes were from the oropharynx and some have never been detected at other body sites.



Abstract S114 Figure 1 Individual bacteriology of all samples that had anaerobes account for at least 10% of sequence reads