The Bacteriology of Pleural Infection by Genetic and Standard Methods and Its Mortality Significance

Nick A. Maskell, Sarah Batt, Emma L. Hedley, Christopher W. H. Davies, Stephen H. Gillespie, and Robert J. O. Davies

Department of Respiratory Medicine, Southmead Hospital, North Bristol NHS Trust, Bristol; Department of Microbiology, Royal Free Hospital, London; Oxford Pleural Unit, Oxford Centre for Respiratory Medicine, Oxford Radcliffe Hospital, Headington, Oxford; and Department of Respiratory Medicine, Royal Berkshire Hospital, Reading, United Kingdom

Background: **Antibiotic choices for pleural infection are uncertain as its bacteriology is poorly described.**
Methods: **Pleural fluid from 434 pleural infections underwent standard culture and a screen for bacteria by amplification and sequencing of bacterial 16S ribosomal RNA gene.**
Results: **Approximately 50% of community-acquired infections were streptococcal, and 20% included anaerobic bacteria. Approximately 60% of hospital-acquired infections included bacteria frequently resistant to antibiotics (methicillin-resistant *Staphylococcus aureus*, 25%; Enterobacteriaceae, 18%; *Pseudomonas* spp., 5%, enterococci, 12%). Mortality was increased in hospital-acquired infection (hospital, 17/36 [47%]; community, 53/304 [17%]; relative risk, 4.24; 95% confidence interval, 2.07–8.69; $p < 0.00001$; χ^2, 1 df = 17.47) and in gram-negative (10/22 [45%]), *S. aureus* (15/34 [44%]), or mixed aerobic infections (13/28 [46%]), compared with streptococcal infection (23/137 [17%]) and infection including anaerobic bacteria (10/49 [20%]; $p < 0.00001$, χ^2, 4 df = 23.35).**
Conclusion: **Pleural infection differs bacteriologically from pneumonia and requires different treatment. Antibiotics for community-acquired infection should treat aerobic and anaerobic bacteria. Hospital-acquired, gram-negative *S. aureus* and mixed aerobic infections have a high mortality rate.**

Keywords: empyema; ISRCTN 39138989; MIST1 trial; parapneumonic effusion; pleural infection

Bacterial pleural infection has been a substantial clinical challenge since ancient times. Descriptions of its treatment date from Hippocrates and perhaps ancient Egypt (1, 2). Today, it affects up to 65,000 patients each year in the United Kingdom and United States (3) and has a 12-mo mortality of 22%, with another 15% of patients requiring surgical abscess drainage (4). Appropriate antibiotic choices are important to minimize this morbidity, and these choices are likely to be different from those of pneumonia because small series suggest these syndromes differ bacteriologically (5–15). However, this difference in antibiotic choice is unclear because these studies were small, biased by case selection, or retrospective (5–15), and do not take into account the increasing prevalence of antibiotic-resistant pathogens. Antibiotic selection is also problematic because about 40% of patients with convincing pleural infection have no pathogens identified on standard laboratory culture (4).

The First Multicenter Intrapleural Sepsis Trial (MIST1) assembled a large, well-characterized patient cohort (4), and this article presents the bacterial microbiology of this cohort, including prognostic significance of different bacterial isolates. The results include a screen for bacterial pathogens from pleural fluid samples by the amplification and sequencing of the bacterial 16S ribosomal RNA gene, which has been shown to improve the identification of pathogens from cases of pleural infection (15).

This analysis provides a detailed description of the microbiology of pleural infection using current techniques, describes how this differs from pneumonia, and determines the influence of different bacterial species on prognosis, allowing clinicians to make logical therapeutic choices.

METHODS

Medical Research Council/BTS MIST1 Trial

This article reports the bacteriology from the Medical Research Council/British Thoracic Society (BTS) MIST1 trial of streptokinase in pleural infection (4). This trial showed no treatment effect from streptokinase and so patient outcome is not confounded by this factor. Briefly, the MIST1 trial recruited 454 patients from 52 centers in the United Kingdom. Entry criteria were macroscopically purulent, or bacterial culture, or Gram stain–positive pleural fluid, or a pleural fluid of pH < 7.2, in the presence of clinical evidence of infection. Apart from trial intrapleural streptokinase, patients received standard clinical care. All patients received antibiotics at the discretion of their local managing physician, but antibiotic guidelines were provided in the trial protocol (available in the online supplement).

Baseline microbiological information included the results of pleural fluid Gram stain, and aerobic and anaerobic pleural fluid culture, performed in the recruiting center. The trial outcomes included mortality, which is used in the survival analyses presented here. At randomization, pleural fluid was collected and transferred to the coordinating center and frozen at −70°C. Samples were frozen within 48 h of being taken. Culture-negative pleural fluid samples were also gathered from 20 patients with pleural effusions due to noninfectious causes to act as control samples. The trial was approved by the Anglia and Oxford Multicentre Research Ethics Committee (MREC) (ref: 98/5/61), the Oxford Local Research Ethics Committee, and the local research ethics committees for each center. All participants gave informed, written consent.

Nucleic Acid Amplification and Identification

Bacterial DNA was extracted, amplified, cleaned, and sequenced using standard techniques (15, 16).

Pleural fluid DNA extraction. Pleural fluids were thawed from –70°C storage and equilibrated to ambient temperature. Bulk nucleic acid was promptly extracted using the Qiagen QIAmp Mini Kit following the manufacturer's guidelines (Qiagen, Hilden, Germany). The nucleic acid was eluted in 100 μl of buffer.

Polymerase chain reaction. The polymerase chain reaction (PCR) was modified from Woo and colleagues (17). The PCR reaction mixture consisted of 5 μl Bioline KCl buffer (Bioline, London, UK), 27.3 μl PCR-grade water, and final concentrations of 0.5mM $MgCl_2$, 0.2mM

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Correspondence and requests for reprints should be addressed to R.J.O. Davies, D.M., F.R.C.P., Reader and Consultant in Respiratory Medicine, Oxford Pleural Unit, Oxford Centre for Respiratory Medicine, Churchill Hospital Site, Oxford Radcliffe Hospital, Headington, Oxford, OX3 7LJ UK. E-mail: robert.davies@ndm.ox.ac.uk

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dNTPS (Promega), and 0.5 μM each primer (Sigma Genosys, Poole, UK) and 1 unit per reaction Taq polymerise (Bioline). The PCR reaction mixture was aliquoted and exposed to ultraviolet light for 2 min. Ten microliters of nucleic acid extract were added to give a final volume of 50 μl. The cycling conditions were as follows: 94°C for 5 min, followed by 30 cycles of 94°C for 1 min, 60°C for 1 min, and 72°C for 2 min, with a final elongation at 72°C for 5 min (Progene Thermal Cycler; Techne, Cambridge, UK).

Sequencing. PCR amplimers were cleaned using QIAquick PCR clean-up (QIAquick PCR purification kit; Qiagen), and the size of the amplion was verified using Hyperladder I (Bioline). Sequencing reactions were performed using the BigDye Terminator Cycle Sequencing Ready Reaction DNA sequencing kit (Applied Biosystems, Inc., Foster City, CA) and analyzed on the ABI 377 Genetic Analyser (Applied Biosystems, Warrington, UK). Sequences obtained were in the region of 400–700 base pairs. Sequencing was performed twice in the forward direction only, and the sequences aligned to ensure base pair accuracy using the CLUSTALW algorithm.

Cloning. Twelve samples that had mixed sequences underwent cloning using the TOPO TA cloning kit (Invitrogen, Paisley, UK). This cloning strategy was limited to 12 samples for reasons of cost. Cloning reactions contained the following: 1 μl fresh PCR product, 1 μl salt solution, 2 μl sterile water, and 1 μl TOPO vector. The mixtures was incubated at room temperature for approximately 20 min and then placed on ice. The 2 μl of the cloning mixture were added to the competent cells (that had been thawed on ice) and heat-shocked at 42°C for 30 s and then incubated with 250 μl media at 37°C on an orbital shaker. The 10- and 50-μl volumes were then spread on Luria-Bertani (LB) agar containing 50 μg/ml ampicillin. Plates were incubated overnight at 37°C.

M13 PCR to analyze transformants. Twelve colony picks of transformants per sample were transferred directly in M13 PCR mixtures. The 50-μl M13 PCR reaction mixtures contained the following: 0.2 μM each primer, 0.2 mM deoxynucleotide triphosphates (dNTPs), 2 mM $MgCl_2$ (Bioline), 5 μl buffer, and 1 unit Taq polymerase (Bioline). Primer sequences were as follows: M13(f) 5′CAGGAAACAGC TATGA and M13(r) GTAAAACGACGGCCAG. The cycling conditions were as follows: initial denaturation, 94°C for 5 min; then 30 cycles of 94°C for 40 s; 55°C for 40 s; and 72°C for 1 min followed by a final elongation at 72°C for 7 min. Amplimers were resolved through 1% agarose, and successful transformants were assumed to be those with bands at 1,300 bp. Amplimers were then cleaned and sequenced using the methods described above (using 16S primers).

Bacterial identification. Identification of bacteria from pleural fluids was performed by the comparison of bacterial DNA sequences coding for the 16S ribosomal RNA subunit with the GenBank database available at the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov). A positive identification was recorded when the sequence completely matched a database entry and other matches showed significantly less homology. Sequences that showed clear amplification and strong signal but were unreadable (i.e., where base pairs could not be assigned due to two peaks in the same position) were recorded as "mixed." Twelve of these samples were then cloned and resequenced and the identities of the bacteria present clarified. Details of the identity percentage data for the genetic bacterial identification are available from the corresponding author, if required.

Data Analysis

Combination of the results of standard culture and nucleic acid amplification. To compare the results of the standard pleural fluid bacterial culture and the nucleic acid amplification, *pre hoc* rules defining these comparisons were established. These rules were as follows:

1. Where the bacteria identified by culture and nucleic acid amplification were identical (or both were negative), this was defined as "agreement."
2. Where the identified bacterium was consistent, but one technique provided better information, this was defined as "agreement but superior" (e.g., methicillin-resistant *Staphylococcus aureus* by culture/*S. aureus* by nucleic acid amplification = "agreement, culture superior", or *S. milleri* by culture/*S. intermedius* by nucleic acid amplification = "agreement, nucleic acid amplification superior").
3. Where one technique identified a bacterium that was not detected by the other, this was defined as "superior" (e.g., nucleic acid amplification negative/*S. aureus* by culture = "culture superior").
4. Where the two techniques identified different bacteria, it was considered impossible to assess which test was more informative (e.g., *Streptococcus pneumoniae* by culture/*Haemophilus influenzae* by nucleic acid amplification = "indeterminable").

Survival analyses in bacterial subgroups. The survival of patients in the different bacterial pathogen groups was defined by selecting groups of subjects with bacteria of a particular class blind to these subjects' clinical outcome. Mortality was then studied in these groups. The following groups were identified to this analysis:

No pathogen identified (n = 71)

S. pneumoniae (n = 59)

S. intermedius group (n = 55)

Other streptococci (n = 23)

Anaerobic or mixed aerobic/anaerobic infection (n = 49)

S. aureus (n = 34)

Mixed aerobic bacteria (n = 28)

Gram-negative bacteria (n = 22)

Survival was also compared between cases acquired in the community and in hospital.

Statistical analysis. The χ^2 analysis and Fisher exact test were used when comparing proportions and overall mortalities. Cohort survivals were described using Kaplan-Meier survival curves. Statistical analysis was performed with SPSS, version 10 (SPSS, Inc., Chicago, IL).

RESULTS

Subjects

The clinical and blood culture characteristics of the subjects with pleural infection are shown in Table 1, and elsewhere (4). Of the patients, 82 of 454 (18%) had chronic lung disease. The bacteriology was similar in this group to the whole sample.

Negative control subjects for the molecular microbiology. Twenty age-matched control patients (11 male; mean age, 69 yr [SD, 20 yr]), with pleural effusion believed clinically to be of a noninfectious cause, gave samples to act as negative controls. Seven were transudative effusions (by "Light's criteria" [18]) and 13 had histocytologically proven malignant pleural effusion. These controls were selected on the basis of the security of their diagnosis and negative standard cultures for aerobic and anaerobic bacteria, mycobacteria, and fungi.

TABLE 1. CHARACTERISTICS OF THE SUBJECTS

Age, yr, mean (SD)	60.5 (18)
Male, n (%)	302 (67)
Duration of symptoms prior to presentation, median (IQR)	14 (8–28)
Comorbidity, n (%)	
Diabetes mellitus	48/454 (11)
Excessive alcohol consumption	43/454 (9)
Etiology of pleural infection, n (%)	
Community-acquired infection	394 (87)
Hospital-acquired pneumonia	29 (6)
Post–thoracic surgery	11 (2)
Post–abdominal surgery	5 (1)
Iatrogenic	4 (1)
Unclassified	11 (2)

Definition of abbreviation: IQR = interquartile range.
Total: n = 454.

Overall Bacteriology

Of cases, 434 of 454 (95%) had standard microbiological pleural cultures and Gram's stain performed, of which 250 of 434 (58%) were positive, as follows: 151 (35%) produced a single aerobic growth, 29 (9%) produced a single anaerobic growth, 52 (12%) produced polymicrobial cultures, and 18 (2%) were Gram-stain positive only. In 20 subjects, pleural fluid was not available for culture. Seventy-seven subjects received antibiotics before pleural fluid sampling, and culture was negative in 47 of (61%) of these patients. Pleural fluid was available for molecular microbiological analysis in 404 of 434 (93%) subjects. Seventy (16% of total sample) of culture-negative cases had bacteria identified by subsequent nucleic acid amplification, leaving 114 of 434 (26%) cases still bacteriologically obscure.

Among the 12 of 404 (3%) cases where nucleic acid amplification showed more than one bacterial DNA sequence, cloning suggested the following bacteria to be present. Six samples revealed only one bacterium: *Proechimys oris* in two cases; and one case each of *Staphylococcus intermedius, Streptococcus pneumoniae, Streptococcus pyrogenes*, and *Filifactor micros*. Six samples revealed more than one bacterium: *Fusobacterium nucleatum* and *Peptostreptococcus micros* in three cases; and one case each of *Bacteriodes fragilis* + *P. oris*; *F. nucleatum* subsp. *vincentii* + *P.* oral clone; *Peptostreptococcus micros* + *Peptostreptococcus* oral clone + *Fusobacterium* oral clone + *Bacteroides* oral clone. These results were combined with those from nucleic acid amplification.

The summary bacteriology derived from both standard culture and nucleic amplification is presented in Table 2. *S. intermedius*, *S. anginosis*, and *S. constellatus* were grouped together as the "*S. intermedius* group."

TABLE 2. DESCRIPTION OF THE BACTERIOLOGY OF COMMUNITY- AND HOSPITAL-ACQUIRED PLEURAL INFECTION

Organism	Community Acquired (*no. isolates*)	Hospital Acquired (*no. isolates*)
Aerobes		
Streptococcus	176	11
Streptococcus intermedius–anginosus–constellatus ("milleri") group	80	4
Streptococcus pneumoniae	71	3
Streptococcus pyogens	9	0
Other *Streptococcus* species	16	4
Staphylococcus	35	21
S. aureus	27	6
Methicillin-resistant *S. aureus*	7	15
S. epidermidis	1	
Enterococcus spp.	4	7
Gram negatives	29	14
Escherichia coli	11	2
Other coliforms	4	6
Proteus	6	2
Enterobacter spp.	5	1
Pseudomonas aeringosa	3	3
Anaerobes	67	5
Fusobacterium	19	1
Bacteroides	16	1
Peptostreptococcus	9	
Mixed anaerobes, unclassified	8	2
Prevotella spp.	13	1
Clostridium spp.	2	
Mycobacterium tuberculosis	2	
Actinomyces spp.	4	
Other*	17	2
Total	336	60

Both infections differ bacteriologically from pneumonia.

* Includes *Burkholderia anthina*, *Eikenella*, *Haemophilus influenzae*, oral bacterium, *Pasterella multocida*, *Klebsiella* spp.

Comparison of Community- and Hospital-acquired Infection

The bacteriologies of hospital- and community-acquired infection differed substantially (Table 2), and both differ from pneumonia, which is consistent with previous small reports (5–15). Pleural infections acquired in the community were most frequently due to streptococcal infection (*S. pneumoniae*, 71/336 [21%]; *S. intermedius* group, 80/336 [24%]; other streptococcal species, 25/336 [7%]), and infections due to *S. aureus* and enterococci were more prevalent in the infections acquired in hospital. Fifteen of the 60 (25%) isolates in hospital-acquired infection were due to methicillin-resistant *S. aureus*.

Overall mortality was substantially increased in subjects who acquired their infection in hospital compared with those who acquired their infection in the community (hospital mortality, 17/36 [47%]; community, 53/304 [17%]; relative risk, 4.24; 95% confidence interval [CI], 2.07–8.69; $p < 0.00001$; χ^2, 1 df = 17.47). Survival curves are shown in Figure 1.

Survival in Different Bacterial Subsets

One-year mortality outcome data were available in 438 of 440 (99.5%) subjects in the survival analyses. All the streptococcal subsets showed similar mortalities (*S. pneumoniae*, 10/59 [17%]; *S. intermedius* group, 9/55 [16%]; other streptococci, 4/23 [17%]; $p = 0.92$; χ^2, 2 df = 0.17; *see* survival curve in Figure E1 of the online supplement). These groups were combined in later comparisons.

The mortality in the group in whom no pathogen was identified was 9 of 71 (13%), similar to that in streptococcal infection, which was 23 of 137 (17%; relative risk, 1.27; 95% CI, 0.53–3.06; $p = 0.60$; χ^2, 1 df = 0.28; survival curve in Figure E2).

Mortality at 1 yr varied substantially between the culture-positive bacterial groups ($p < 0.00001$; χ^2, 4 df = 23.35; survival curve in Figure 1). This was due to a statistically significant increase in mortality in three groups: those with gram-negative bacteria (10/22 [45%]), *S. aureus* (15/34 [44%]), or mixed aerobic bacteria (13/28 [46%]), compared with the others; those with streptococcal infection (23/137 [17%]); and those with infection including anaerobic bacteria (10/49 [20%]).

Hospital-acquired Infection, Bacterial Class, and Mortality

The pathogens causing hospital-acquired infection differ from those causing community-acquired infection and are generally from bacterial classes with a worse outcome. To clarify whether the bacterial mortality effect and the hospital-acquired mortality effect were independent, we have explored this interaction in Table 3. This shows that the place of infection and the type of bacterial infection are independently associated with variations in mortality.

Comparison of Conventional Culture and Nucleic Acid Amplification

In 140 of 404 (35%) cases, the same organism was found by both nucleic acid amplification (or cloning) and standard culture. Of these, using the rules previously described, in 120 of 140 cases, the standard culture and nucleic acid amplification were in agreement; in 9 of these 140, the standard culture was superior; and in 11 of 140 cases, the nucleic acid amplification was superior.

In 107 cases, the standard culture was superior to the nucleic acid amplification because it identified a bacterium not identified from the DNA studies. In 54 cases, nucleic acid amplification was superior to standard culture because it identified a bacterium not identified by culture. In 50 cases, the standard culture and

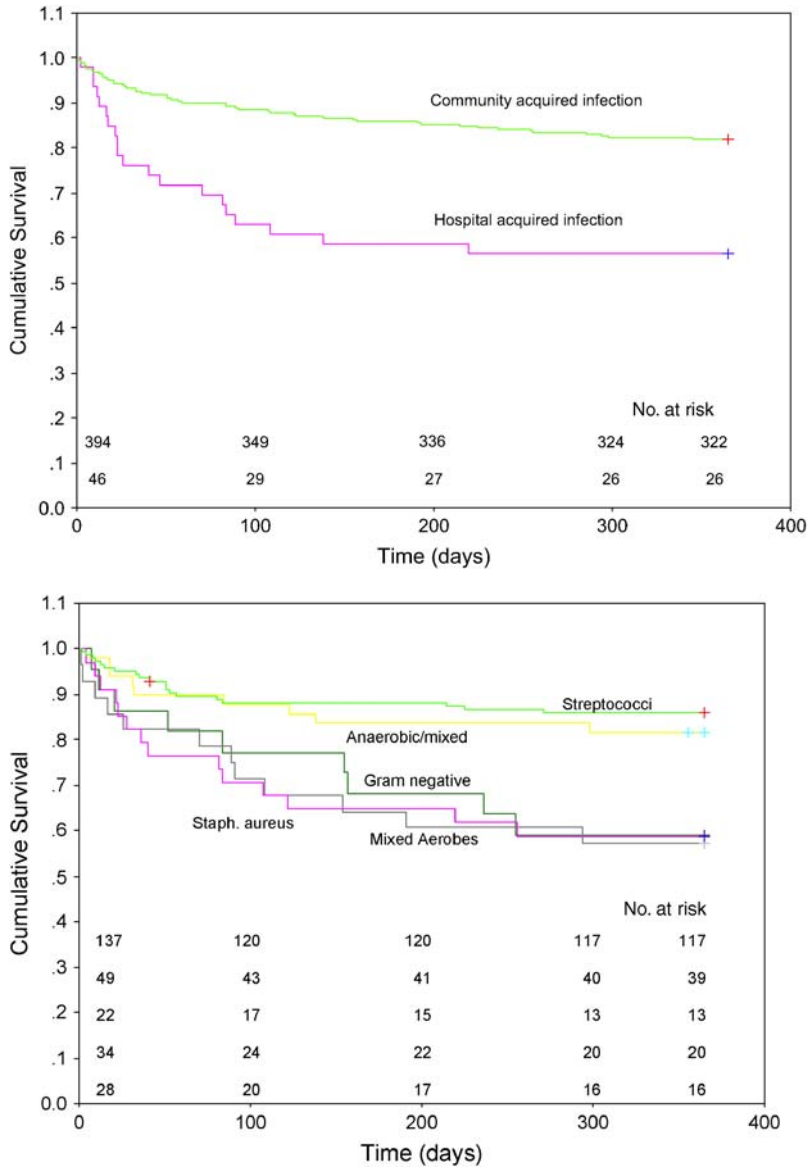

Figure 1. Survival curves in patients with community- and hospital-acquired pleural infection (*upper panel*) and in patients with infections with differing bacterial etiology (*lower panel*).

the nucleic acid amplification identified different bacteria, and it could not be assessed which method was more informative. The results of conventional culture and nucleic acid amplification are compared in Table 4.

Nucleic Acid Amplification of Culture-negative Pleural Fluid Control Cases

In 19 of the 20 culture-negative and presumed uninfected control subjects, no bacterial DNA was identified. In one, a *Prevotella* spp. was identified (from a patient with malignant pleural mesothelioma). *Post hoc* review of the case notes showed this patient was febrile at the time of pleural fluid sampling, although this fever had been attributed to the tumor.

DISCUSSION

This report has clarified the bacteriology of pleural infection and may improve antibiotic choices for the 65,000 people who develop this infection each year in the United Kingdom and the United States, helping minimize the 22% mortality associated with pleural infection (4). It is the first study to examine the standard culture and genetic bacteriology of pleural infection in a large generalizable cohort, and to relate this to patient mortality. These data provide a foundation for clinical trials to define whether better bacterial diagnosis and antibiotic choices improve outcome in pleural infection.

The cases studied here were accumulated from 52 centers in the United Kingdom, including both teaching and district hospitals, and so are likely to be representative. This study's results include DNA sequencing and cloning to clarify the bacteriology in detail, a technique that has proved effective in other smaller samples (15). This has reduced the number of cases that are bacteriologically undiagnosed from 42 to 26%, a diagnostic improvement that is likely to be clinically valuable.

This analysis confirms smaller studies (5–15), which suggest the bacteriology of pleural infection differs from that of pneumonia (reviewed in Reference 21), and that these syndromes should be considered separate. Because pleural infection follows bacterial migration from beneath the visceral pleura, it is often termed "complicated parapneumonic effusion," which could be taken to imply a bacteriologic etiology similar to that of pneumonia. This study shows that this is simplistic and antibiotic choices

TABLE 3. MORTALITY AT 12 MONTHS IN DIFFERENT CULTURE-POSITIVE BACTERIAL SUBGROUPS IN SUBJECTS WHO ACQUIRED THEIR INFECTION IN THE COMMUNITY OR IN HOSPITAL

	Community-acquired Infection	Hospital-acquired Infection	Statistical Significance in Hospital- and Community-acquired Infection (χ^2)
Streptococcal infection	19/131	0/6	p = 0.60 (χ^2, 1 df = 1.01)
Staphylococcal infection	6/22	8/12	p = 0.04 (χ^2, 1 df = 4.98)
Infections including anaerobic bacteria	5/44	2/4	p = 0.13 (χ^2, 1 df = 3.37)
Infections with mixed aerobic bacteria	5/18	7/10	p = 0.05 (χ^2, 1 df = 4.68)
Infections with gram-negative bacteria	9/20	0/2	p = 0.49 (χ^2, 1 df = 1.52)
Statistical significance across bacterial groups (χ^2)	p = 0.01 (χ^2, 4 df = 13.13)	p = 0.03 (χ^2, 4 df = 10.93)	

targeted at the typical range of pneumonic pathogens are not ideal for pyogenic pleural infection. The differences in the bacteriology are probably due to the acidic and hypoxic environment of the infected pleural space favoring selected pathogens. Many of the anaerobic bacteria of pleural infection are strictly anaerobic and cannot tolerate the Po_2 of lung parenchyma, whereas streptococci of the "*intermedius–anginosus–constellatus*" group characteristically flourish in low pH and Po_2 tissue environments and favor these conditions in artificial culture (19). The microbiological differences from pneumonia suggest that future studies should try to define which clinical phenotypes are associated with which pattern of pathogens. For example, preliminary data are beginning to suggest that different radiographic patterns of lung disease are associated with different bacterial pathogens (20).

Interestingly, even the genetic bacterial analysis presented here may underestimate the variety of bacteria present in pleural infection. Our limited cloning strategy suggests even greater bacterial diversity than the 16S amplification, and may provide more clinical information in the future.

Antibiotic Choices in Pleural Infection

Of the cases with a confirmed bacteriology, community-acquired pleural infection is caused by penicillin-sensitive streptococci in about 50% of cases, with the other 50% being due to organisms that are usually penicillin resistant, including staphylococci and Enterobacteriaceae. About 25% of community-acquired pleural infections include anaerobic bacteria. Appropriate empiric antibiotic choices for these patients should therefore cover streptococci, penicillin-resistant staphylococci, and Enterobacteriaceae and should usually also include anaerobic bacterial therapy.

By contrast, hospital-acquired infection includes more staphylococcal infection (more than 70% of which is due to methicillin-resistant *S. aureus*) and Enterobacteriaceae, organisms that

TABLE 4. RESULTS OF 316 OF 404 CASES WHERE BOTH STANDARD CULTURE AND NUCLEIC ACID AMPLIFICATION WERE PERFORMED AND A SINGLE ORGANISM WAS IDENTIFIED

	Number of Positive Isolates		
Organism	Bacteria Isolated by Conventional Culture and Nucleic Acid Amplification	Bacteria Isolated by Conventional Culture But Not by Nucleic Acid Amplification	Bacteria Isolated by Nucleic Acid Amplification But Not by Culture
Aerobes			
Streptococcal species			
S. pneumoniae	27	4	28
S. intermedius–anginosus–constellatus group	23	26	10
S. pyogenes	3		3
Other/unidentified *Streptococcus*	8	2	
Staphylococcus			
S. aureus	11	6	4
Methicillin-resistant *S. aureus*	3	5	
Enterococcus spp.	1		1
Anaerobes			
Mixed (anaerobes only)	4	4	1
Bacteroides spp.	1	3	
Fusobacterium	2		6
Peptostreptococcus		1	1
Prevotella spp.	2	1	3
Clostridium spp.		1	
Gram negatives			
E. coli	2	1	
Proteus spp.		3	
Pseudomonas		3	
Enterobacter spp.		5	
All other	2	5	4
No organism identified (negative)	89		

To allow direct comparison of the two techniques, the 88 polymicrobial cases have been omitted. References defining the use of this methodology for anaerobe detection include References 2 and 3.

are often multiple-drug resistant. This may contribute to the poor prognosis in these patients. Here, empiric antibiotic therapy should be effective against these multidrug-resistant organisms. The differences in the bacteriology and the prognosis between hospital- and community-acquired empyema are sufficiently marked that these syndromes should probably be considered separate clinical entities.

Mycoplasma spp., and *Legionella* spp. are respiratory pathogens that are difficult to isolate using routine culture methods. The molecular bacterial techniques found no evidence of either of these species, which suggests that these pathogens are not likely causes of pleural infection and that empiric antibiotic regimes targeted at them are probably not required. By contrast, molecular diagnostics increased the yield of anaerobic bacteria in comparison to routine culture methods, which is expected given the difficulties of isolating these fastidious organisms in a clinical microbiological laboratory. Therefore, empiric antibiotics targeted at anaerobic infection should be usual. An exception to this is where routine culture has identified *S. pneumoniae* (because we found no evidence of any coincidental anaerobic infection in these cases, either by standard culture or nucleic acid amplification). Here, specific pneumococcal therapy would be appropriate provided the prevalence of pneumococcal penicillin resistance is low.

Survival in Different Bacterial Subgroups

There are substantial differences in mortality in different bacterial subgroups. Patients with streptococcal infection generally have the best prognosis, with 83% of these patients alive at 12 mo (Figure E1). A similar 87% survival is seen in subjects in whom no pathogen can be identified by either culture or nucleic acid amplification (Figure E2). This suggests that the majority of culture- and nucleic acid amplification–negative cases may be of streptococcal origin, with antibiotic therapy having suppressed bacterial numbers to undetectable concentrations.

Most previous reports of the bacteriology of pleural infection have suggested that infections including anaerobic bacteria have a particularly high mortality (7, 8, 11). The data reported here contradict this view, showing that infections including anaerobic bacteria have an 80% survival, which is similar to streptococcal infections (Figure 1). The groups with the highest mortality were those patients with staphylococcal, enterobacterial, and mixed aerobic infections: about 45% at 1 yr (Figure 1). Because standard clinical care (with chest tube drainage and antibiotics) is associated with such a high mortality in this group, rapid, aggressive empyema drainage (e.g., with early surgical intervention) should be considered for these patients.

Standard Culture and Nucleic Acid Amplification/Cloning

Our study demonstrates that bacterial nucleic acid amplification allows the identification of the pathogen in up to 75% of cases. This rate could have been improved further if the cloning strategy had been applied to all the cases where mixed sequences were detected. False-positive rates with nucleic acid amplification have been low. Sterile laboratory saline controls have been reliably negative, and only one of the fluids from patients not believed to have pleural infection identified any bacterial DNA. Interestingly, with hindsight, this subject was febrile when the pleural fluid sample was collected, with a fever attributed to his tumor. This result suggests it could have been infectious in origin. Larger studies relating clinical phenotypes with genetic bacteriology in patients with sterile pleural fluid on standard culture are needed to resolve whether such isolates are true or false positives.

The substantial increase in the number of bacteria identified by nucleic acid amplification when compared with normal culture in this study is similar to the advantage seen when this strategy was applied to a small sample of pediatric empyema fluids (15), strengthening the case for its use.

CONCLUSIONS

This study presents the largest comprehensive description of the bacteriology of pleural infection using conventional and molecular methods. The results confirm pleural infection is bacteriologically significantly different from pneumonia, and that hospital-acquired pleural infection is a subentity with a high mortality. Over 40% of hospital-acquired infections were due to multiantibiotic-resistant pathogens. Three bacterial subgroups were associated with a substantially increased mortality and should probably be targeted for early surgical abscess drainage. In contrast to previous reports, we found that infections including anaerobic bacteria do not have a poor outcome. This improved description of the bacteriology of this disease allows better antibiotic and therapeutic strategies to reduce the high morbidity and mortality associated with this disease.

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O.A. Afolabi (North Tyneside General Hospital); N. Ali (Kingsmill Hospital, Mansfield); M. Allen (North Staffordshire Hospital); A. G. Arnold (Castle Hill Hospital, Cottingham); D. Baldwin (City Hospital, Nottingham); J. R. Bateman (Derby City General Hospital); J. Bennett (Derby Royal Hospital); A. Bentley (North Manchester Hospital); D. Boldy (Pilgrim Hospital, Boston); M. Bone (South Tyneside District Hospital); M. Britton (St. Peters Hospital, Surrey); S. Burge (Birmingham Heartlands Hospital); R. Butland (Gloucester Royal Hospital); I. Campbell (Llandough Hospital, Cardiff); J. Congleton (Worthing Hospital); D. C. Currie (Dewsbury & District Hospital); R. J. O. Davies (Oxford Radcliffe Hospital); C. W. H. Davies (The Royal Berkshire Hospital, Reading); A. G. Davison (Southend Hospital); O. Dempsey (Aberdeen Royal Infirmary); R. G. Dent (QEII, Welwyn Garden City); A. P. Greening (Western General Hospital, Edinburgh); J. Hadfield (Chesterfield & Derbyshire Royal Hospital); B. D. W. Harrison (Norfolk and Norwich Hospital); J. Harvey (Southmead Hospital, Bristol); M. Hetzel (Bristol Royal Infirmary); H. Hosker (Airedale General Hospital, Keighley); P. Hughes (Derriford Hospital, Plymouth); A. M. Hunter (York District Hospital); M. Jackson (Brighton General Hospital); W. J. M. Kinnear (Queens Medical Centre, Nottingham); C. Laroche (West Suffolk Hospital, Bury St. Edmonds); C. Laroche (Papworth Hospital, Cambridge); A. Leonard (Great Western Hospital, Swindon); M. C. I. Lipman (Royal Free Hospital, London); D. Lomas (Addenbrookes Hospital, Cambridge); A. Malin (Royal United Hospital, Bath); D. McLeod (Sandwell General Hospital, West Bromwich); M. L. D. Morgan (Glenfield Hospital, Leicester); M. F. Muers (The Leeds General Infirmary); N. C. Munro (University Hospital of North Durham); E. Neville (St. Mary's Hospital, Portsmouth); D. J. Newbury (Ashford Hospital); D. Peckham (St. James University Hospital, Leeds); M. Pond (Bridlington & District Hospital); S. Saboor (Chase Farm Hospital, Enfield); D. Seaton (Ipswich Hospital); R. Smith (Horton General Hospital, Banbury); S. Spiro (The Middlesex Hospital, London); B. Stack (West Glasgow Hospital); D. Thickett (Queen Elizabeth Hospital, Birmingham); C. Wathen (Wycombe General Hospital); J. H. Winter (Nine Wells Hospital, Dundee); N. Withers (Royal Devon & Exeter Hospital); M. Woodhead (Manchester Royal Infirmary); F. Zaman (Pendle Community Hospital, Burnley).

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