Journal of Applied Microbiology 2005, **99,** 502–508 doi:10.1111/j.1365-2672.2005.02651.x

Molecular survey of aeroplane bacterial contamination

C.J. McManus and S.T. Kelley
Department of Biology, San Diego State University, San Diego, CA, USA

2005/0030: received 13 January 2005, revised 28 February 2005 and accepted 5 March 2005

ABSTRACT

C.J. MCMANUS AND S.T. KELLEY. 2005.

Aims: To examine bacterial contamination of passenger aircraft and to identify aeroplane environments posing the greatest potential health risk.

Methods and Results: DNA was extracted from ten environmental samples collected on four different flights (three domestic, one international) from a variety of surfaces frequently touched by passengers. PCR clone libraries were made from the DNA samples using bacterial-specific 16S ribosomal DNA (rDNA) primers. A total of 271 bacterial rDNA sequences were obtained. We used BLAST analysis of the rDNA clone sequences to identify sequences in Genbank with the highest sequence similarity. The majority of the rDNA clones obtained from aeroplane environments were more than 97% identical to rDNA sequences from cultured bacterial species. Samples collected from the cabin surfaces (e.g., tray tables and arm rests) had undetectable levels of DNA and produced no PCR products. Bacterial diversity was highest on lavatory surfaces, including door handles, toilet handles, and sink faucets. Sequence data from these surfaces detected species from 58 different bacterial genera, and many of the best BLAST hits matched rDNA sequences of cultured species known to be opportunistic pathogens. The most frequently observed species came from five genera commonly associated with humans: *Streptococcus, Staphylococcus, Cornybacterium, Proprionibacterium* and *Kocuria*.

Conclusions: The results show that there is a large diversity of bacterial contamination on aeroplanes, including organisms known to be opportunistic pathogens.

Significance and Impact of the Study: Our results indicate that aeroplanes have the potential to spread an enormous diversity of bacterial species among passengers and destinations. Aeroplane lavatories present an especially significant concern to public health.

Keywords: airline travel, microbial diversity, polymerase chain reaction, public health, ribosomal RNA.

INTRODUCTION

Over the past five centuries, Europeans travelling to the New World introduced measles, small pox and tuberculosis with devastating consequences (Crosby 1972). In modern times, commercial travel has spread viral diseases such as dengue fever, SARS, and HIV, as well as bacterial pathogens such as *Staphyloccus*, *Shigella* and *Salmonella* (Wilson 2003). Aeroplane travel, in particular, is a major reason these emerging and reemerging diseases have spread so widely and so rapidly (Wilson 2003). Aeroplanes pack a diversity of passengers, including some who may be symptomatic or asymptomatic carriers of disease as well as others who may be immunocompromised, into a confined space for long periods of time. The close seating arrangement combined with the low humidity in the pressurized cabin appears to aid the spread of droplet nuclei 1–5 μm in diameter, increasing the likelihood of spreading respiratory infections (Wilson 2003). In the late 1990s, a single passenger carrying antibiotic resistant tuberculosis infected 30% of passengers seated within two rows and an additional 3% of passengers on the flight (Kenyon *et al.* 1996).

Culturing studies of aeroplane environments have revealed high levels of bacterial contamination in aeroplane

Correspondence to: Scott Kelley, Department of Biology, San Diego State University, San Diego, CA 92182-4614, USA (e-mail: skelley@sciences.sdsu.edu).

water supplies (http://www.epa.gov/airlinewater/summary table.html, 9/22/04). Although culturing methods are useful for detecting microbes with known growth conditions, studies of environmental samples have shown that <1% of bacterial species are cultivatable using current methods (Pace *et al.* 1985). As a result, culturing methods routinely miss the vast majority of microbial diversity present in any given environment (Amann *et al.* 1995; Torsvik *et al.* 1998). The development of culture-free molecular methods based on PCR technology has revolutionized the field of microbiology and exposed an enormous breadth of microbial diversity (Amann *et al.* 1995; Kelley *et al.* 2001). Culture-free methods are just beginning to be used in the arena of public health and preliminary studies suggest they will prove extremely valuable in identifying microbial diversity and potential pathogenic organisms present in human environments (Kelley *et al.* 2004).

To determine the composition and location of bacterial contamination on aeroplanes, we used a PCR-based molecular approach to examine environmental DNA samples collected from aeroplane environments. Using evolutionarily conserved bacterial small subunit ribosomal DNA (rDNA) primers, we created PCR clone libraries from 10 environmental samples collected from four flights: Honolulu to San Francisco/Denver (HO–SFO/DEN), Mexico to San Diego (MEX–SAN), Oakland to San Francisco (OAK–SFO) and Chicago to Washington DC (CHI–WAS). The samples included a variety of potentially contaminated sites frequently touched by passengers, and the results of this study provide insight on the latent health risks involved in aircraft transportation.

MATERIALS AND METHODS

Sample preparation

Samples were collected from various flights in the summer of 2004. Sampled areas included armrests, tray tables, toilet seats, bathroom sinks, floors, handles on the outside of the lavatories and door knobs within the lavatories, and an unused paper towel. Collections were performed at the end of the flight using kimwipes to wipe the surfaces. Prior to collection, the person sampling the surface washed their hands thoroughly with soap and water before using the kimwipe to minimize contamination. The entire surface of the toilet handle, sink handle and door knob were wiped down. Just the top of the tray table, armrest and toilet seat were sampled; *c.* 100 cm^2 of the lavatory floor was sampled and, finally, we extracted DNA from the entire paper towel. After collections were made, kimwipes were stored in either a sterile polypropylene 15 ml tube or a fresh resealable plastic bag. After the end of the flight, samples were placed on ice and shipped to San Diego State University where they were frozen at 20°C until DNA extractions were performed.

The DNA was extracted from the kimwipes using an UltraClean Soil DNA Kit Mega Prep (MoBio Laboratories, Solana Beach, CA, USA). The manufacturer's protocol was followed except for two small adjustments: the 50-ml bead tubes were attached to a vortex for 30 min, and the filters were eluted using 2 ml as not to over-dilute the sample. The MoBio Soil DNA kit uses a bead-beating method for mechanically shearing microbial cell walls and has proven effective for isolating DNA from challenging sources such as hot spring environments and coral mucus (Casas *et al.* 2004; Spear *et al.* 2005). Bead-beating procedures have also proven effective with acid-fast bacteria, such as the *Mycobacteria* (Angenent *et al.* 2005).

For the PCR amplifications, we used two bacterial-specific 16S primers, 8F and 805R (Weisburg *et al.* 1991). All reactions contained sterile purified DNA-free water, 1x buffer (Tris, KCl and $MgCl_2$), 0·8 mg ml^{-1} BSA, 160 μmol l^{-1} dNTPs, 0·1 μmol l^{-1} of each primer, half a unit of *Taq* polymerase and varying amounts of DNA. The amount of DNA was titrated to establish the most productive reaction conditions for each sample. To avoid potential DNA contamination, PCR master mixes were exposed to UV light for 15 min to cross-link double-stranded DNA before the addition of *Taq* polymerase and template DNA. The thermal cycling parameters included a hot start at 95°C for 5 min, followed by a three step cycle (95°C 1 min, 55°C 45 s, 72°C 1 min 30 s) that was repeated between 30 and 35 times and the programme ended with a 20 min extension at 72°C. PCR amplifications producing very faint bands after 30 cycles were repeated using 35 cycles. Products were cleaned using Qiagen's QIAquick PCR Purification Kit (Qiagen Corporation, Valencia, CA, USA).

The clean PCR products were cloned using pGEM-T Easy Vector System (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. Specifically, the cleaned PCR products were placed in a thermal cycler at 72°C for 20 min with 1 μl of polymerase, and 1 μl of dNTPs to facilitate the T/A cloning procedure. Transformed XL1B competent *Escherichia coli* cells were plated on LB-agar plates containing 50 μg ml^{-1} ampicillin and top-plated with X-gal and IPTG. Using sterile toothpicks, white colonies were cultured into a 96-well plate with 150 μl of LB containing 6% glycerol and 100 μg ml^{-1} ampicillin. The inoculated media was incubated overnight at 37°C. Miniprep and sequencing reactions were completed by the San Diego State Microchemical Core Facility.

The sequence information was analysed using XplorSeq 2·0, a program written by Dan Frank at the University of Colorado. This program imports chromatograms and uses automatic base calling software to determine the quality of the sequence. Xplorseq also runs a batch BLAST through

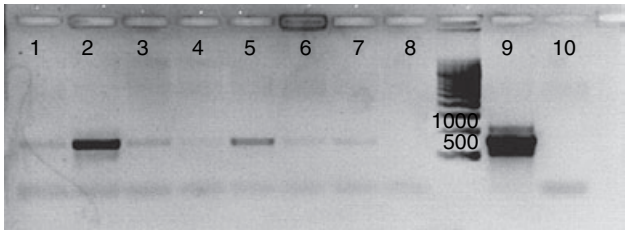

Fig. 1 The PCR products from reactions containing 5 μl of sample. Lanes 3 and 5 are samples from Mexico to San Diego (MEX–SAN) flight, and the rest are from the Honolulu to San Francisco/Denver (HO–SFO–DEN) flight (see Materials and methods). Lane 1, toilet seat; lane 2, doorknob; lane 3, paper towel; lane 4, floor; lane 5, bathroom sample; lane 6, handle; lane 7, sink; lane 8 was a PCR amplification with a negative extraction control on a clean kimwipe; *Escherichia coli* DNA was used as a positive control in lane 9 and lane 10 was the negative PCR control

NCBI and produces tab delimited output files that can be imported into Excel.

RESULTS

Figure 1 shows the results of a typical series of PCR amplifications using DNA isolated from aeroplane samples. The DNA extraction and PCR negative controls had no visible products under any reaction conditions (Fig. 1). With the exception of the tray table and armrest, all the sampled aeroplane environments produced PCR product after adjusting the DNA concentration and the PCR cycling parameters. Tray table and armrest samples from two different flights (four DNAs total) did not produce any PCR amplification under any conditions. In fact, spectrophotometer analysis was not able to detect any DNA in these samples, suggesting an absence of bacteria. Figure 2 shows the results of clone insert screening for one of the environmental samples positive for bacterial DNA. The cloning efficiency ranged from 85 to 95%.

Single pass sequencing of the clone inserts using the 8F primer provided *c.* 600 bp of high quality sequence for BLAST analysis. Most of the BLAST 'hits' were >97% identical to the 16S sequences in Genbank and gave us a high level of confidence concerning the phylogenetic relationships of the determined sequences. Collectively, the diversity proved considerable; based on rDNA identifications, we found 58 genera of bacteria from numerous divisions (Table 1). Calculations of the Chao1 estimation based on dereplicated sequence information at the 97% level estimated that the samples contained anywhere from 50 to 200 bacterial species. As a comparison, we note that this level of diversity is similar to that detected in the human mouth and gut (Hughes *et al.* 2001). The dereplicated rDNA sequences have been deposited in Genbank under accession numbers AY957663–AY957771.

DISCUSSION

The organismal diversity present in the various aeroplane samples was surprisingly complex. However, we found some consistent patterns in terms of the type and abundance of bacteria. In each area, specific-bacterial genera represented a significant proportion of the sampled diversity (Fig. 3). The most frequent types of organisms present in the samples are commonly associated with humans: *Streptococcus*, *Staphylococcus*, *Corynebacterium*, *Propionibacterium* and *Kocuria*. In some instances, these bacteria represented >30% of the sample (Fig. 3).

The organisms that we detected with the greatest known potential for harm were the ones related to *Staphylococcus* spp. *Staphylococcus epidermis* and *Staphylococcus hominis* were both detected in several samples (Table 1) and these species have been associated with serious infections (Pfaller and Herwaldt 1988). *Staphylococcus epidermis* is of particular concern. An organism that was once dismissed as a laboratory contaminant is now considered an opportunistic

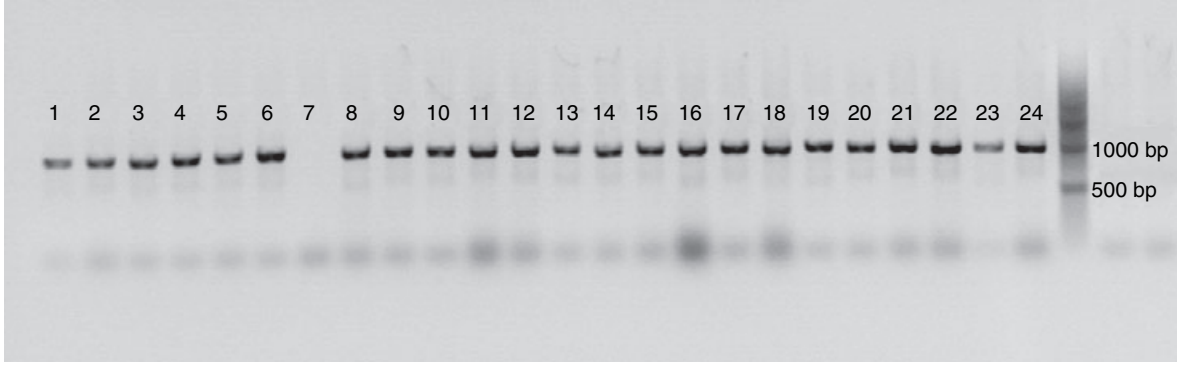

Fig. 2 Results of PCR screening using M13 primers for 24 clones inserts from a doorknob sample from the Honolulu to San Francisco/Denver (HO–SFO–DEN) flight

Table 1 The genus and species of the bacteria in which the cloning sequences matched 97% and above, and the amount of times that sequence was discovered

Genus and species	Door knob*	Floor*	Handle*	Paper towel†	Sink*	Toilet seat*
Abiotrophia sp.	1					
Achromobacter xylosoxidans				1		
Acidithiobacillus thiooxidans				1		
Aerococcus viridans					1	
Agrobacterium tumefaciens	1					
Anaerococcus octavius		1				1
Anaerococcus vaginalis			1			1
Bacillus psychrosaccharolyticus						1
Bacteroides ureolyticus			1			
Brachybacterium paraconglomeratum	1			1		
Brachybacterium sp.	1				1	
Bradyrhizobium sp.					1	
Brevibacterium aurantiacum					1	
Comamonas aquatica						1
Comamonas terrigena			3	1		
Comamonas testosteroni		3	1			
Corynebacterium imitans						2
Corynebacterium sp. 61720		10	8		3	2
Corynebacterium sp. 96447		4	1		2	
Corynebacterium sp. CIP107067		1				
Delftia tsuruhatensis strain ARI_7		3	1			
Dermabacter hominis			1			
Dietzia psychralcaliphila	2					
Dietzia sp.						
Finegoldia magna			1		1	1
Flavobacterium sp.					1	
Fusobacterium periodonticum				1		
Gemella haemolysans				1		
Granulicatella elegans				1		
Haemophilus parainfluenzae				2		
Kocuria carniphila	11					
Kocuria palustris	3			1	1	
Lactobacillus iners		2	5			
Leptotrichia genomosp. C1						1
Leuconostoc mesenteroides						1
Micrococcus sp.	5					
Moraxella osloensis	3				1	1
Mycobacterium sp.	1					
Neisseria elongata			1			
Neisseria flava			2			
Neisseria subflava				1		
Paracoccus sp.					1	
Peptoniphilus sp.	1					
Prevotella genomosp.						1
Prevotella sp.	1			1		
Propionibacterium acnes		1	2	3		14
Pseudomonas sp.	1					
Psychrobacter ikaite	2					
Sphingobacterium antarcticum			1			
Sphingomonas sp.						1
Staphylococcus epidermidis			2			
Staphylococcus equorum					3	
Staphylococcus hominis		3			1	1
Staphylococcus sp.					1	

Table 1 (*Continued*)

Genus and species	Door knob*	Floor*	Handle*	Paper towel†	Sink*	Toilet seat*
Stenotrophomonas maltophilia		2	1	1	1	
Stenotrophomonas sp.		1				
Streptococcus gordonii			1			
Streptococcus mitis	1	1		7		
Streptococcus oralis						1
Streptococcus sanguinis	1			1		1
Streptococcus thermophilus						1
Sulfobacillus disulfidooxidans					2	
Weissella minor					1	
Uncultured bacteria		2	3		7	2

*Honolulu to San Francisco/Denver (HO–SFO–DEN).
†Mexico to San Diego (MEX–SAN).

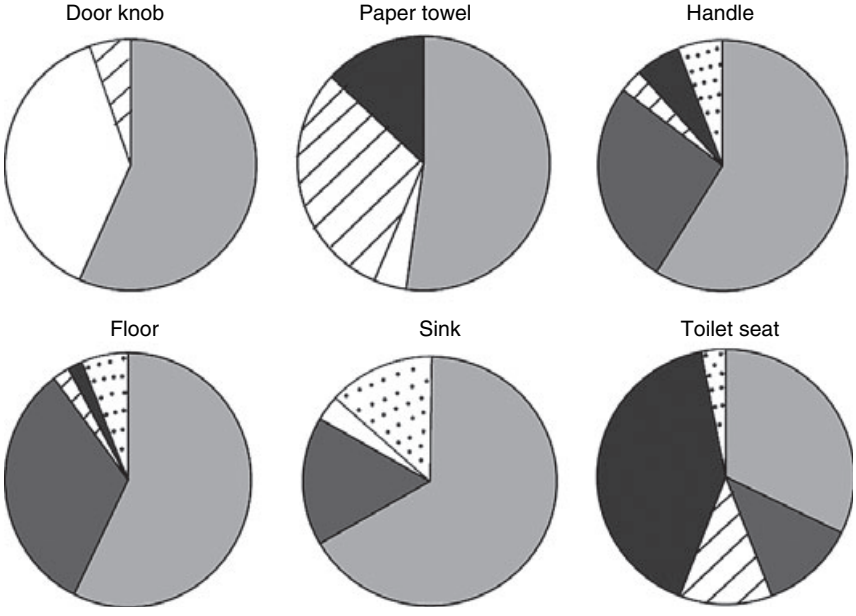

Fig. 3 Graphical representation of common bacterial genera found in aeroplane environments. Bacterial genus names: ■, other bacteria; ■, *Corynebacterium*; □, *Kocuria*; ▨, *Streptococcus*; ■, *Propionibacterium*; ▩, *Staphylococcus*

pathogen that can cause bacteraemia, mediastinitis, meningitis and endocarditis in patients with prosthetic devices, and general infections in immunocompromised patients (Pfaller and Herwaldt 1988; Tanner *et al.* 1999). Our results suggest that the potential for the spread of these organisms via aeroplanes is extremely high. Although we did not detect the medically challenging antibiotic resistant *Staphylococcus aureus* in our samples, we see no reason why this and other disease threats could not spread in aeroplanes in the same manner as these other *Staphylococcus*.

The next most abundant organisms of concern were the streptococci. Many species of *Streptococcus* are known pathogens, especially *Streptococcus pneumoniae*. Although we did not find sequences from this particular organism in our samples, we did find a number of other *Streptococcus* spp. known to be opportunistic pathogens, such as *Streptococcus mitis* and *Streptococcus oralis* (Table 1). *Streptococcus mitis* is frequently associated with respiratory disease, and both *S. mitis* and *S. oralis* cause infections in immunocompromised patients (Whatmore *et al.* 2000).

Other common genera found in this study included *Corynebacterium*, *Kocuria* and *Propionibacterium* (Table 1; Fig. 3). *Corynebacterium* is very difficult to culture and, as a result are not well understood (Tanner *et al.* 1999). However, *Corynebacterium* has been identified in patients with blood infections, urethritis, renal failure and prostatitis (Funke *et al.* 1997). *Kocuria* is in the family Micrococcaceae and in humans they can be found living on the skin, mucosae and oropharynx. These bacteria are thought to be associated with prosthetic valve endocarditis and

catheter-related bacteremias (Basaglia *et al.* 2002). *Propionibacterium* is most popularly known as the bacteria that cause acne and are part of the normal skin flora. *Propionibacterium* sp. is rarely life-threatening but can affect the psychological well-being of highly infected individuals (Kim *et al.* 2002).

In addition to exposing the diversity of bacterial species that may be found in aeroplane environments, our results also shed light on the most troublesome aeroplane environments. The substantial diversity of human-associated bacterial species found on numerous lavatory surfaces on every flight tested, including a paper towel, suggests that lavatories represent the biggest potential hazard for passengers and crew (Table 1; Fig. 3). This makes sense given the purpose of the lavatory and the fact that it is the most commonly visited area of the aeroplane. Interestingly, a significant number of the species that we identified appeared to typically inhabit human oral cavities. The passengers possibly spread these organisms around the confined space of the lavatory via aerosols by activities such as coughing or blowing their noses.

Although culture-free molecular techniques are highly useful for determining microbial diversity, they cannot by themselves assess whether the organisms detected are still viable. However, other research studies indicate that bacteria can stay viable for some time on similar artificial surfaces. Scott and Bloomfield (1990) found that coliform bacteria can survive for up to 24 h on a laminate surface. In another study, Rusin *et al.* (1998) were able to culture a variety of different bacterial species by swabbing household bathroom and kitchen surfaces (Scott and Bloomfield 1990; Rusin *et al.* 1998). Thus, it appears that many, if not most, of the human-associated bacteria species we discovered in aeroplane environments could potentially be viable for the duration of the flight and certainly long enough to transfer among passengers.

With the exception of the rising numbers of immunocompromised patients, the organisms we detected are not especially threatening to the general public. However, we see serious potential for ill persons with more threatening conditions to spread disease to other passengers on planes. Based on the findings of our study and the conclusions of other aeroplane environment research, we suggest the aeroplane lavatories pose a particular threat to public health. We recommend that the airlines take this matter very seriously and institute periodic in-flight cleanings of lavatory door, toilet and sink handles using a 10% bleach solution to reduce contamination.

Future studies in this area would also be enhanced by using more rigorous sampling methods. For instance, the use of sterile latex gloves during our sampling procedure would have been preferable, but we avoided using gloves so as not to alarm fellow passengers unnecessarily. We decided to risk the potential for cross-contamination by avoiding the use of laboratory gloves and relied on thorough hand-washing instead. The fact that our negative kimwipe extraction controls, which had been handled by samplers, and armrest and tray table samples were negative for DNA and PCR products indicates that we successfully minimized cross-contamination. Also, as our principle aim in this study was to prove the utility of culture-free methods and make an initial survey of diversity, we sampled as much of each surface as possible. Future studies should also make greater attempts to quantify the amount of bacteria on surface areas and standardize the amount of surface area sampled. Finally, the sampling method could be improved by standardizing the time from sampling to storing the DNA on ice.

In addition to improvements in the sampling methods, future research in this area should also pursue more thorough investigation of the bacterial and viral diversity in aeroplane environments. As a preliminary survey of just four flights, this study exposed a remarkable diversity of human-associated bacteria (even discovering a few species without cultured relatives; Table 1) and suggested that many more species may be found commonly on aeroplanes. Future studies should investigate the types and frequency of cleaning regiments effective for minimizing exposure and transmission of microbes. Other questions to address include: what is the importance of biofilms in maintaining bacterial viability on aeroplanes? How many bacterial cells are aerosolized at any given time? How do the number of passengers, the destination and duration of flights impact bacterial contamination? Is the risk greater on international flights compared with domestic flights? What physical factors (e.g. toilet flushing) are the most important for spreading microbes? There is also a need to quantify the viability of many of the microbial species we discovered. The answers to these questions should prove useful for minimizing the spread of bacteria and potential diseases.

ACKNOWLEDGEMENTS

We would like to thank the anonymous airline travelers who helped us obtain samples. We also thank F. Rohwer for the use of his lab facilities, and V. Thackray, B. Lovitt and two anonymous reviewers for comments on the manuscript.

REFERENCES

Amann, R.I., Ludwig, W. and Schleifer, K.H. (1995) Phylogenetic identification and *in situ* detection of individual microbial cells without cultivation. *Microbiol Rev* **59**, 143–169.

Angenent, L., Kelley, S.T., St Amand, A., Pace, N.R. and Hernandez, M. (2005) Molecular identification of potential pathogens in water and air of a hospital therapy pool. *Proc Nat Acad Sci USA* **102**, 4860–4865.

Basaglia, G., Carretto, E., Barbarini, D., Moras, L., Scalone, S., Marone, P. and De Paoli, P. (2002) Catheter-related bacteremia due to *Kocuria kristinae* in a patient with ovarian cancer. *J Clin Microbiol* **40**, 311–313.

Casas, V., Kline, D.I., Wegley, L., Yu, Y., Breitbart, M. and Rohwer, F. (2004) Widespread association of a Rickettsiales-like bacterium with reef-building corals. *Environ Microbiol* **6**, 1137–1148.

Crosby, A.W.J. (1972) *The Columbian Exchange*. Westport, CT: Greenwood Press.

Funke, G., von Graevenitz, A., Clarridge, J.E. III and Bernard, K.A. (1997) Clinical microbiology of coryneform bacteria. *Clin Microbiol Rev* **10**, 125–159.

Hughes, J.B., Hellmann, J.J., Ricketts, T.H. and Bohannan, B.J. (2001) Counting the uncountable: statistical approaches to estimating microbial diversity. *Appl Environ Microbiol* **67**, 4399–4406.

Kelley, S.T., Harris, J.K. and Pace, N.R. (2001) Evaluation and refinement of tmRNA structure using gene sequences from natural microbial communities. *RNA* **7**, 1310–1316.

Kelley, S.T., Theisen, U., Angenent, L.T., St Amand, A. and Pace, N.R. (2004) Molecular analysis of shower curtain biofilm microbes. *Appl Environ Microbiol* **70**, 4187–4192.

Kenyon, T.A., Valway, S.E., Ihle, W.W., Onorato, I.M. and Castro, K.G. (1996) Transmission of multidrug-resistant *Mycobacterium tuberculosis* during a long airplane flight. *N Engl J Med* **334**, 933–938.

Kim, J., Ochoa, M.T., Krutzik, S.R., Takeuchi, O., Uematsu, S., Legaspi, A.J., Brightbill, H.D., Holland, D. *et al.* (2002) Activation of toll-like receptor 2 in acne triggers inflammatory cytokine responses. *J Immunol* **169**, 1535–1541.

Pace, N.R., Stahl, D.A., Lane, D.J. and Olsen, G.J. (1985) Analyzing natural microbial populations by rRNA sequences. *ASM News* **51**, 4–12.

Pfaller, M.A. and Herwaldt, L.A. (1988) Laboratory, clinical, and epidemiological aspects of coagulase-negative staphylococci. *Clin Microbiol Rev* **1**, 281–299.

Rusin, P., Orosz-Coughlin, P. and Gerba, C. (1998) Reduction of faecal coliform, coliform and heterotrophic plate count bacteria in the household kitchen and bathroom by disinfection with hypochlorite cleaners. *J Appl Microbiol* **85**, 819–828.

Scott, E. and Bloomfield, S.F. (1990) The survival and transfer of microbial contamination via cloths, hands and utensils. *J Appl Bacteriol* **68**, 271–278.

Spear, J.R., Walker, J.J., McCollom, T.M. and Pace, N.R. (2005) Hydrogen and bioenergetics in the Yellowstone geothermal ecosystem. *Proc Natl Acad Sci USA* **102**, 2555–2560.

Tanner, M.A., Shoskes, D., Shahed, A. and Pace, N.R. (1999) Prevalence of corynebacterial 16S rRNA sequences in patients with bacterial and 'nonbacterial' prostatitis. *J Clin Microbiol* **37**, 1863–1870.

Torsvik, V., Daae, F.L., Sandaa, R.A. and Ovreas, L. (1998) Novel techniques for analysing microbial diversity in natural and perturbed environments. *J Biotechnol* **64**, 53–62.

Weisburg, W.G., Barns, S.M., Pelletier, D.A. and Lane, D.J. (1991) 16S ribosomal DNA amplification for phylogenetic study. *J Bacteriol* **173**, 697–703.

Whatmore, A.M., Efstratiou, A., Pickerill, A.P., Broughton, K., Woodard, G., Sturgeon, D., George, R. and Dowson, C.G. (2000) Genetic relationships between clinical isolates of *Streptococcus pneumoniae*, *Streptococcus oralis*, and *Streptococcus mitis*: characterization of 'atypical' pneumococci and organisms allied to *S. mitis* harboring *S. pneumoniae* virulence factor-encoding genes. *Infect Immun* **68**, 1374–1382.

Wilson, M.E. (2003) The traveller and emerging infections: sentinel, courier, transmitter. *J Appl Microbiol* **94**(Suppl.), 1S–11S.