times were extremely reproducible. There was no statistical difference between the mean gastric emptying times, measured by t_{50} (time taken for 50% of the Clinutren ISO to leave the stomach and t_{90} (time taken for 90% of the Clinutren ISO to leave the stomach) in the fed and fasted state (Table 1). A robust and reproducible model of the gastric emptying of a new nutrient liquid meal is described, which shows similar emptying kinetics in the fasted and the post-prandial conditions.

This study was completed as part of a MRC CASE studentship funded by GSK.

Table 1 Mean gastric emptying times

Fasted t_{50} (min)	Fed t_{50} (min)	Fasted t_{90} (min)	Fed t_{90} (min)
60 ± 6.6	63 ± 7.8	120.6 ± 13.2	135 ± 19.2

Data are means $\pm$ s.d, n = 8.

May, H. A. et al (1984) *Int. J. Pharm.* **19**: 169–176

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The Gram-negative bacterial flora of the cotton production environment and relevance to occupational lung disease

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To grow cotton commercially, a temperate climate is required with ample water supply. Similar conditions favour the growth of the majority of bacteria; couple this with the nutrition provided by the large quantities of plant and insect sugars present on the fibres and the ideal environment for the proliferation of microbial populations is created. Many of these microbes are Gram-negative bacteria, which release endotoxin (known as lipopolysaccharide or LPS in the purified form) from their outer membrane. Inhalation of endotoxin primarily targets alveolar macrophages causing a decrease in lung function (Rylander 1992). The contaminated cotton fibres enter the cotton mill, where associated bacterial cells and fragments thereof are released with dust created during cotton processing. Prolonged exposure to this environment can result in the textile worker's lung disease byssinosis, a chronic and irreversible condition. This study involved the quantification and identification of Gram-negative bacterial cells in numerous cotton samples from diverse countries. The aim was to investigate the diversity of cotton bacteria, as well as to identify differences in viable counts and hence assess risk to respiratory health and how this varies across the world. Ten cotton lint samples were provided by the Liverpool Cotton Research Corporation from countries within Africa, the Middle East and North and South America. Bacteria were extracted from the fibres by vigorous shaking in phosphate-buffered saline, followed by spread plating onto over-dried tryptic soy agar plates seeded with cycloheximide ($50\,\mu g\,mL^{-1}$) and vancomycin ($15\,\mu g\,mL^{-1}$) to prevent the growth of fungi and Gram-positive bacteria, respectively. Plates were incubated for 18–24 h at 37°C (±2°C) and colony-forming units then counted manually. Colonies appearing morphologically different were subcultured for a further 18–24 h. Isolates were then identified using microscopy and Analytical Profile Index (API) strips. Viable cell counts on the samples varied significantly from a high of $2.2 \pm 0.3 \times 10^5\,CFU\,g^{-1}$ on cotton from Zimbabwe, to $7.1 \pm 2.1 \times 10^2\,CFU\,g^{-1}$ on the cotton sample from Tajikistan. The mean viable count was significantly higher on cotton samples from countries within Africa compared with those within Asia ($1.4 \pm 0.3 \times 10^5\,CFU\,g^{-1}$ compared with $2.4 \pm 0.6 \times 10^3\,CFU\,g^{-1}$) ($P < 0.05$). This is of importance as African countries exhibit high levels of occupational lung disease (Abebe & Seboxa 1995) and these regions lack the means to effectively monitor and protect their workforce. Five genera of bacteria were identified on the samples: *Aeromonas, Enterobacter*, *Pantoea*, *Psuedomonas*, and *Serratia*, all common bacteria found in soil, water or on plants. The most widely distributed genus was *Enterobacter*, identified in cotton from seven countries (Table 1). This is also significant, as endotoxin from different bacterial genera varies in its ability to induce an inflammatory response upon inhalation, and that from *Enterobacter* has been shown to be one of the most potent (Helander et al 1980). Hence, a significant health risk is posed to cotton workers across the world.

We gratefully acknowledge the support of the Liverpool Cotton Research Corporation and the British Cotton Growing Association.

Table 1 Geographic distribution of cotton bacteria

Bacterial genera	Number of countries
Enterobacter	7
Pseudomonas	4
Serratia	3
Aeromonas	2
Pantoea	2

Abebe, Y., Seboxa, T. (1995) *Ethiopian Med. J.* **33**: 37–49
Helander, I. et al (1980) *Infect. Immun.* **29**: 859–862
Rylander, R. (1992) *Tuber. Lung Dis.* **73**: 21–26

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The fungal profile of cotton fibres, associated toxins and wider implications for indoor health

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Cotton plant material is an ideal substrate for the proliferation of fungal colonies. Many of these are soil-borne saprophytes, which survive on plant debris. Therefore, these organisms are available to contaminate cotton fibres while they are exposed to field weathering before harvest. Further to this, several fungi can also develop while organic material is stored. This results in the unavoidable introduction of these organisms to the industrial environment. Many common filamentous fungi are capable of producing mycotoxins, which are secondary metabolites of low molecular weight, able to cause a number of effects including kidney failure, damage to the central nervous system and some are known carcinogens (Fischer & Dott 2003). This study involved the quantification and identification of fungal cells on numerous cotton samples from diverse countries. The aim was to enumerate the cotton fungal cells, as well as to identify the genera present, to assess potential health risks. Ten cotton lint samples were provided by the Liverpool Cotton Research Corporation from countries within Africa, the Middle East and North and South America. Fungal cells were extracted from the fibres by vigorous shaking in phosphate-buffered saline, followed by spread plating onto over-dried malt extract agar plates seeded with chloramphenicol ($30\,\mu g\,mL^{-1}$) to prevent the growth of bacteria. Plates were incubated for four days at 25°C (±2°C), then colony forming units were counted manually. Colonies appearing morphologically different were subcultured as before for 4 days. Isolates were then mounted using tape-lift procedures and stained with lactophenol blue solution; identification was carried out using macroscopic and microscopic morphology with comparison to taxonomic reference material. Viable cell counts on the samples varied significantly, from a high of $9250 \pm 820\,CFU\,g^{-1}$ on cotton from Benin to $281 \pm 29\,CFU\,g^{-1}$ on the cotton sample from Uzbekistan. The mean viable count was significantly higher on cotton samples from countries within Africa, compared with those within Asia ($4233 \pm 668\,CFU\,g^{-1}$ compared with $1033 \pm 67\,CFU\,g^{-1}$) ($P < 0.05$). Five fungal genera were identified in the samples; these were *Aspergillus, Cladosporium*, *Fusarium*, *Penicillium* and *Rhizopus*, all common soil and plant fungi. The most widely distributed genus was *Aspergillus*, identified in every cotton sample involved in the study, with *Aspergillus niger* the most common species, followed by *A. flavus*. These *Aspergillus* species are able to produce several mycotoxins, the most potent of which is aflatoxin, a carcinogen that has been shown to produce lung neoplasms upon inhalation (Bennett & Klich 2003). *Fusarium* and *Penicillium* are also potential toxin producers (Table 1). The presence of such fungi in organic material has significant implications for the health of workers in a number of occupational environments, and further research into these organisms and the toxins they produce is required.

We gratefully acknowledge the support of the Liverpool Cotton Research Corporation and the British Cotton Growing Association.

Table 1 Toxins produced by cotton fungi

Fungal taxa	Toxin
Aspergillus flavus	Aflatoxin B1 and B2
Aspergillus niger	Malformin C, ochratoxin A
Fusarium	Fumonisin, trichothecenes
Penicillium	Citrinin

Bennett, J. W., Klich, M. (2003) *Clin. Microbiol. Rev.* **16**: 497–516
Fischer, G., Dott, W. (2003) *Arch. Microbiol.* **179**: 75–82

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Disinfection of biofilms using Toluidine Blue O and Red Light

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As current antimicrobial agents are gradually being rendered ineffective by resistance developing in target organisms, there is an urgent need for alternative antimicrobial approaches. Toluidine Blue O (TBO) is a light-activated antimicrobial agent that has been shown to be effective against a wide range of bacteria. The aim of this investigation was to determine the efficacy of TBO against biofilms grown on silicone surfaces when impregnated into the substrate and when applied externally. Silicone discs were impregnated by swelling the discs with chloroform for 2 h and then applying 1 mg mL^{-1} TBO solution (or water for controls) for 16 h. Discs were then rinsed of excess TBO. Biofilms of *Proteus mirabilis* and *Staphylococcus epidermidis* were grown by seeding for 4 h with the appropriate culture in TSA at 37°C (1×10^5 CFU mL^{-1}). The culture was then removed from each disc and replaced with fresh medium. Discs were incubated for 48 h. Photo-activated disinfection (PAD) was initiated by activating the biofilms for 15 min with red light from a diode laser (wavelength: 633 ± 2 nm) to apply a total energy dose of 59 Joules. In an alternative treatment protocol, discs were washed with a TBO solution (25 μg mL^{-1}) followed by light activation as before. Following treatment, biofilm viability was assessed by removal of adherent bacteria and enumeration by viable counts. *S. epidermidis*: washing with TBO led to a 3.2 log reduction in cell numbers. Impregnated discs not exposed to red light resulted in a 1.1 log reduction and when exposed to red light a 1.2 log reduction was observed. Washing of biofilms on impregnated discs gave 1 log reduction in the non-light-activated control and 2.4 log reduction when exposed to red light. *P. mirabilis*: washing led to a 1.1 log reduction in viability. Impregnated discs showed no significant reduction. Washing *P. mirabilis* biofilms on impregnated discs gave no reduction in the control and a 1 log reduction when exposed to red light. Successful disinfection according to the British Standard (BS EN 1276, 1997) occurs when there is a five log reduction in cell number. This did not occur in any of these data. When you compare the two organisms, it can be clearly demonstrated that *S. epidermidis* is more susceptible to disinfection using PAD than *P. mirabilis*. Presently, washing the biofilms with TBO is the most successful method of applying the photoactivated dye to use PAD technology for the disinfection of silicone biofilms.

British Standard (1997) *Chemical disinfectants and antiseptics – quantitative suspension test for the evaluation of bactericidal activity of chemical disinfectants and antiseptics used in food, industrial, domestic and institutional areas – Test method and requirements (phase 2/step 1)*. BS EN 1276

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Conformable semisolids for photodynamic inactivation of MRSA

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Methicillin-resistant strains of *Staphylococcus aureus* (MRSA) are a primary aetiological factor in nosocomial infections, being difficult to treat and leading to prolonged hospital stays. Widespread resistance has been demonstrated, leaving some forms of MRSA susceptible only to glycopeptide antibiotics. Increasing resistance to vancomycin and teicoplanin has now been reported, with resistance to all antistaphyloccal agents a distinct possibility. MRSA may be present in nares and on skin of healthy people, as well as in wounds, burns and venous ulcers, and may be transmitted from patient-to-patient, doctor-to-patient or surface-to-patient. Clearly, there is an urgent need to develop alternative treatments for MRSA infections that are selective for the organism over human cells and have a low propensity for resistance development. Photodynamic antimicrobial chemotherapy (PACT) is a potentially novel approach for the selective killing of MRSA. In PACT, a combination of a sensitizing drug and visible light causes the selective destruction of microbial cells via singlet oxygen production. Importantly, as singlet oxygen is a non-specific oxidizing agent and is only present during illumination, development of resistance to this treatment is unlikely. As a result of increasing antibiotic resistance, PACT has recently come to the fore as a potential alternative antimicrobial therapy. However, due to the rapid development of the field, drug delivery research in this area is virtually non-existent (Donnelly et al 2005a). The primary objective of this study was to determine the susceptibility of a clinical MRSA isolate growing planktonically to photodynamic inactivation using a combination of methylene blue (MB) and visible light (635 nm, 100 J cm^{-2}). The secondary objective was to determine the susceptibility of biofilm cultures of the clinical isolates to MB-mediated lethal photosensitization. As the intended lesions for this photodynamic eradication are topical and usually venous in origin, exudation is a problem for effective drug delivery. To overcome this problem, a shear-sensitive PVA-borax gel was evaluated as a potential drug delivery system for MB. Suspension cultures of MRSA 180 were grown overnight in nutrient broth and centrifuged to pellets before resuspension (1×10^7 cfu mL^{-1}) in photosensitizer solutions of various concentrations. Various incubation times (37 °C, in the dark) were investigated and irradiation was performed in 96-well plates with back well walls and clear bottoms, as described previously for *Candida albicans* (Donnelly et al 2005b). Biofilms were grown over 24 h on PVC discs in nutrient broth, which was then removed and replaced with photosensitizer solutions of various concentrations for defined time periods before irradiation. Incubation of planktonic MRSA cells with MB concentrations of 0.25 mg mL^{-1} and 0.005 mg mL^{-1}, respectively, for 30 min before irradiation achieved greater than 6 $\log_{10}$ reductions in numbers of viable organisms in both cases. Incubation of MRSA biofilms with MB concentrations of 0.25 mg mL^{-1} and 0.005 mg mL^{-1}, respectively, for 30 min before irradiation again achieved greater than 6 $\log_{10}$ reductions in numbers of viable organisms in both cases. Drug release studies demonstrated that PVA-borax gels could accommodate MB and release almost 60% within 15 min. In addition, they possessed tensile properties that would make them ideal for both application to, and removal from, exudative lesions.

Donnelly, R. F. et al (2005a) *Photochem. Photobiol.* In press
Donnelly, R. F. et al (2005b) *J. Control. Release* **103**: 381–392

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Photodynamic inactivation of planktonic and biofilm-grown *Pseudomonas aeruginosa* isolated from cystic fibrosis patients

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Cystic fibrosis patients accumulate thick mucus in their lungs, providing an ideal environment for development of antibiotic-resistant *Pseudomonas aeruginosa* biofilms. Persistent infection leads to pulmonary inflammation and eventually death due to respiratory failure. Recent evidence indicates that photodynamic antimicrobial chemotherapy (PACT) may be useful for infections caused by antibiotic-resistant bacteria and those in multi-species biofilms (O'Neill et al 2002). In PACT, a combination of a sensitizing drug and visible light cause the selective destruction of microbial cells via singlet oxygen production. Importantly, as singlet oxygen is a non-specific oxidizing agent and is only present during illumination, development of resistance to this treatment is unlikely (Wainwright 1998). The primary objective of this study was to determine the susceptibility of clinical *P. aeruginosa* isolates growing planktonically to photodynamic inactivation using a combination of either toluidine blue O (TBO) or meso-tetra (N-methyl-4-pyridyl) porphine tetra tosylate (TMP) and visible light (635 nm, 100 J cm^{-2}). The secondary objective was to determine the susceptibility of biofilm cultures of the clinical isolates to TBO-mediated lethal photosensitization. Suspension cultures of six clinical *P. aeruginosa* isolates were grown overnight in nutrient broth and centrifuged to pellets before resuspension in photosensitizer solutions of various concentrations. Various incubation times were investigated and irradiation was performed in 96-well plates with back well walls and clear bottoms, as described previously for *Candida albicans* (Donnelly et al 2005). Using a TBO concentration of 0.05 mg mL^{-1} and a 30-s incubation time, the highest percentage kill achieved was 99.9% for isolate 12A and the lowest was 89.96% for isolate 6A. With TMP, the highest percentage kill achieved was 99.77% for isolate 7–6087B, and the lowest was 86.34% for isolate 6A. However, a TMP concentration of 5 mg mL^{-1} was required to achieve these percentage kills, due to the poor absorptivity of TMP at 635 nm and possibly also its non-ionic nature. Biofilms were grown over 24 h on PVC discs in nutrient broth, which was