Table I Univariate and multivariate analyses of risk factors for multidrug-resistant *Pseudomonas aeruginosa* acquisition

Antibiotics	Univariate analysis			Multivariate analysis[a]		
	P-value	OR	95% CI	*P*-value	OR	95% CI
Ciprofloxacin	0.027	1.8	1.06–3.04	–	NS	–
Clindamycin	0.135	1.55	0.87–2.75	0.03	2.53	1.08–5.93
Metronidazole	0.045	1.79	1.01–3.19	0.008	3.65	1.44–9.25
Vancomycin	0.045	1.78	1.01–3.13	–	NS	–
Imipenem	0.611	1.19	0.60–2.36	–	NS	–
Cefepime	0.017	1.91	1.12–3.26	–	NS	–

OR, odds ratio; CI, confidence interval; NS, non-significant.
Risk factors adjusted for age, number of invasive procedures and localisation of the patient in the hospital.
[a] Likelihood = 226.485; 6 df; $P \leq 0.001$.

approach, we showed that the previous use of metronidazole and clindamycin were independent risk factors for acquisition of MDRPA.

Prevention of the emergence of multidrug-resistant strains requires changes in infection control measures and antibiotic policies, since *P. aeruginosa* is frequently endemic in ICUs.[5] Here, for the first time, clindamycin and metronidazole were identified as independent risk factors for acquisition of MDRPA, which has implications for antibiotic policies.

Conflict of interest statement

None declared.

Funding sources

This work was supported by Oswaldo Cruz Institute and Federal University of Maranhão Grants.

References

1. Cipriano R, Vieira VV, Fonseca EL, Rangel K, Freitas FS, Vicente ACP. Coexistence of epidemic colistin-only-sensitive clones of *Pseudomonas aeruginosa*, including the blaSPM clone, spread in hospitals in a Brazilian Amazon City. *Microb Drug Resist* 2007;**13**:142–146.
2. Brook I. Treatment of anaerobic infection. *Expert Rev Anti Infect Ther* 2007;**5**:991–1006.
3. Paramythiotou E, Lucet JC, Timsit JF, *et al*. Acquisition of multidrug-resistant *Pseudomonas aeruginosa* in patients in intensive care units: role of antibiotics with antipseudomonal activity. *Clin Infect Dis* 2004;**38**:670–677.
4. Talon D, Mulin B, Rouget C, Bailly P, Thouverez M, Viel JF. Risks and routs for ventilator-associated pneumonia with *Pseudomonas aeruginosa*. *Am J Respir Crit Care Med* 1998; **157**:978–984.
5. Cao B, Wang H, Sun H, Zhu Y, Chen M. Risk factors and clinical outcomes of nosocomial multi-drug resistant *Pseudomonas aeruginosa* infections. *J Hosp Infect* 2004;**57**: 112–118.

R. Cipriano Souza[a,*]
A.C. Vicente[b]
V.V. Vieira[b]
S.G. Marques[a]
M. das Graças A. Soares[a]
M.C.L. Moura[a]
R.J. Koifman[b]
[a]*Federal University of Maranhão, Brazil*
[b]*Oswaldo Cruz Institute (FIOCRUZ), Brazil*
E-mail address: rosacip@ioc.fiocruz.br

Available online 3 June 2008

* Corresponding author. Address: Federal University of Maranhão, Sao Marcos Street, 65071-590 São Luís, Maranhão, Brazil. Tel.: +55 02138658168; fax: +55 02122604282.

© 2008 The Hospital Infection Society. Published by Elsevier Ltd. All rights reserved.

doi:10.1016/j.jhin.2008.03.012

Use of three molecular methods for investigations of Legionnaires' disease in a hospital in Milan

Madam,

Molecular typing, associated with epidemiological surveying, can be of particular importance in identifying sources of infection, demonstrating the link between clinical and environmental isolates.[1] Various techniques have been developed in recent years for the molecular typing of microorganisms; those most used for analysing strains of *Legionella pneumophila* include:[2] pulsed-field gel electrophoresis (PFGE),[3,4] amplified fragment length polymorphism (AFLP)[1,5] and sequence-based typing (SBT).[6] However, because of the low incidence of legionellosis, there are relatively

few isolates from both clinical cases and associated environmental strains with which to work.

In this study, a search was conducted for clinical and environmental strains, isolated at the same time or during routine assessments, in a hospital in Milan (2003—2005).

We investigated a total of eight isolates of *L. pneumophila* serogroup 1, with AFLP [according to the European Working Group for Legionella Infections (EWGLI) protocol][5] and with PFGE (by using *Sfi*I as described by Kool *et al*. and with a modified protocol):[3,7] three isolated from patients with probable nosocomial infection, one of whom died, five environmental samples isolated from shower water, in the corresponding wards and following the appearance of the clinical cases. Comparison was made with control strain Philadelphia 1 (Figure 1). Our main findings were as follows:

— It was possible to correlate a clinical case with only one of the environmental isolates taken from the places frequented by patients, demonstrating the nosocomial origin of the case and identifying the source of infection as the shower water (Clinical 1 and Environmental 1N).

— Two clinical samples from patients admitted to different wards presented an identical profile, which suggests nosocomial spread, even without having isolated an associated environmental strain due to the absence of samples taken during the period under consideration (Clinical 2 and 3).

— Finally, the isolated environmental strains were heterogeneous and not related to each other, although they belong to the same serogroup, having profiles clearly different from one another regarding number and position of bands (Environmental 2 and 4).

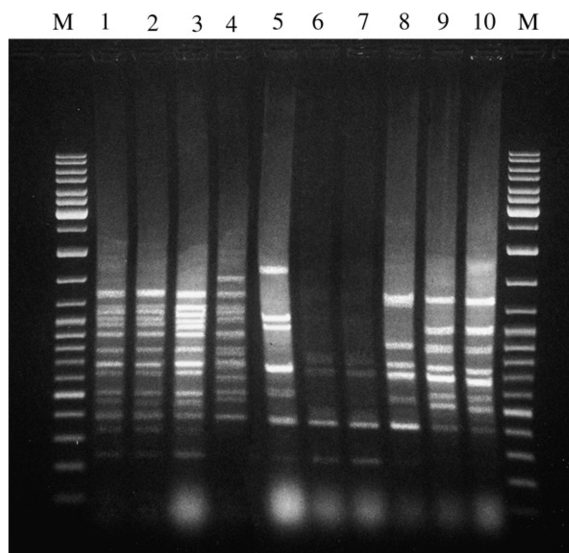

Figure 1 Amplified fragment length polymorphism profiles of genomic DNA extracted from *L. pneumophila* clinical (lanes 1 and 2: Clinical 1; lane 9: Clinical 2; lane 10: Clinical 3) and environmental isolates (lane 3: Environmental 1N; lane 4: Environmental 1C; lanes 6, 7, 8: Environmental 2, 3, 4). Lane 5 corresponds to *L. pneumophila* American Type Culture Collection 33152. M: markers correspond to λ ladder.

In addition, the four samples that were related to each other in pairs were also analysed using the SBT sequencing amplicons of the six genes *asd*, *flaA*, *Mip*, *mompS*, *pilE*, *proA*, as proposed by Gaia *et al*. in order to assess the molecular biology techniques to attempt to establish a gold standard and compare the results with the European EWGLI database (currently we are performing the amplification for the seventh gene *neuA* according to the new protocol).[6]

The SBT subtyping confirmed the results previously obtained; the comparison of allelic profiles of the analysed strains with those provided by the EWGLI on the European database showed that while the profile derived from the two clinical strains (Clinical 2 and 3: 1, 4, 3, 1, 1, 1) is very common in Europe, the profile belonging to clinical/environmental pair (Clinical 1 and Environmental 1N: 2, 10, 18, 10, 1, 1) had never been isolated and typed previously and was new for the database.

The comparison of genomic profiles obtained by AFLP has sometimes revealed problems of discontinuity in the reproducibility and especially in the interpretation of results, such as the presence of very thick or closely spaced bands, constituted by one or more unresolved fragments.

The SBT has proved a better technique for practicality, reproducibility and the interpretation of results than the PFGE and AFLP. This is particularly important in the case of single or epidemic events occurring over a period of time; the comparison of allelic profiles can be performed with the same effectiveness. Furthermore, the method also offers the possibility of comparing the results of typing, accessing data in the database and avoiding sending the isolated strains to different countries for further work.

Conflict of interest statement

None declared.

Funding sources

None.

References

1. Valsangiacomo C, Baggi F, Gaia V, Balmelli T, Peduzzi R, Piffaretti JC. Use of amplified fragment length polymorphism in molecular typing of *Legionella pneumophila* and application to epidemiological studies. *J Clin Microbiol* 1995;**33**:1716–1719.
2. Fields BS, Benson RF, Besser RE. Legionella and legionnaires' disease: 25 years of investigation. *Clin Microbiol Rev* 2002; **15**:516–517.
3. Kool JL, Buchholz U, Pterson C, *et al*. Strengths and limitation of molecular subtyping in a community outbreak of Legionnaires' disease. *Epidemiol Infect* 2000;**125**:599–608.
4. Thouverez M, Godard C, Leprat R, Talon D. Is pulse-field gel electrophoresis a valuable tool to identify nosocomial cases of *Legionella pneumophila* disease? *J Hosp Infect* 2003;**55**:254–259.
5. Fry NK, Bangsborg JM, Bergmans A, *et al*. Designation of European Working Group on Legionella Infections amplified fragment length polymorphism types of *Legionella pneumophila* serogroup 1 and results of intercentre proficiency testing using a standard protocol. *Eur J Clin Microbiol Infect Dis* 2002;**21**:722–728.
6. Gaia V, Fry NK, Afshar B, *et al*. Consensus sequence-based scheme for epidemiological typing of clinical and environmental isolates of *Legionella pneumophila*. *J Clin Microbiol* 2005;**43**:2047–2052.
7. Doleans A, Aurell H, Reyrolle M, *et al*. Clinical and environmental distributions of Legionella strains in France are different. *J Clin Microbiol* 2004;**42**:458–460.

M.G. Galli*
A. Bianchi
A. Raimondi
M. Tesauro
M. Consonni
Department of Public Health, Microbiology and Virology, Università degli Studi di Milano, Italy
E-mail address: maria.galli@unimi.it

Available online 9 June 2008

* Corresponding author. Address: Department of Public Health, Microbiology and Virology, Università degli Studi di Milano, via Pascal 36, 20133 Milano, Italy. Tel.: +039 02 50315111; fax: +039 02 50315100.

© 2008 The Hospital Infection Society. Published by Elsevier Ltd. All rights reserved.

doi:10.1016/j.jhin.2008.04.004

Luminol-based forensic detection of latent blood; an approach to rapid wide-area screening combined with Glo-Germ™ oil simulant studies

Madam,

Bergervoet *et al*. describe the use of luminol-based kits for the forensic detection of latent blood residues.[1] Used to monitor cleaning and disinfection procedures in clinical areas, and as a training aid to raise the awareness of healthcare workers to the possibility of contamination with blood, luminol-based products show considerable promise as an infection control training and evaluation tool. However, the technique may be cumbersome in use since it requires low-light or near-blackout conditions for effective visualisation, particularly if photography is contemplated, and necessitates spraying of surfaces with an aqueous solution that may be inappropriate for use on electrical and electronic devices.

In-situ detection of latent blood residues is widely used by forensic science services. Luminol-based products permit rapid visualisation of blood residues with high sensitivity and specificity, and the opportunity for subsequent DNA fingerprinting of recovered blood residues. Since it is unlikely that this degree of sophistication would be required in hygiene and infection control studies, a much simplified approach may be appropriate. We are presently using a commercial luminol-based product to evaluate the risk of contamination with blood splashes during handling of clinical waste containers.[2] In circumstances where low-light conditions for in-situ visualisation of chemiluminescence cannot be achieved, or would be intrusive or inconvenient, we have devised a modification of the standard forensic approach described by Bergervoet *et al*. For this, we used 100 cm^2 plain cotton dressing pads lightly moistened with sterile water. Using a gloved hand, these swabs are rubbed vigorously over areas to be tested and then examined in a convenient darkroom using the luminol reagent. All materials were first tested for cross-reactivity, with uniformly negative results. Areas in excess of 2 m^2 can be sampled quickly and economically using a single swab. This approach allows screening of large areas with minimal disruption, without the need for blackout, and with no risk from wetting of electrical or electronic equipment with the luminol reagent spray. Overall, our findings compare favourably with in-situ examination, with only the smallest and lightest of trace blood residues not detected by this indirect approach to testing. Heavy soiling of the sample swabs does not interfere with the test reaction, or diminish the intensity of chemiluminescence. To maximise the use of the luminol reagents that have a 12-h shelf life once activated, we have additionally compared the chemiluminescence of fresh moistened swabs examined immediately after sample collection with duplicate swabs stored at room temperature for up to three days with no apparent variation in sensitivity.

We are additionally using absorbent cotton or paper 'targets' fixed to the front of uniforms and