Technical Report

Testing for antimicrobial activity against multi-resistant *Acinetobacter baumannii*

For

Forbo Flooring B.V.

Final Report

Work Carried Out By

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Global Surface Coatings Covered

Final Report

PRA Ref. Number	75221-244
Date Received	04 September 2012
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Client	Forbo Flooring B.V. Industrieweg 12 1566 JP Assendelft Netherlands FAO: Jose Jak
Work Requested	Testing for antimicrobial activity against multi-resistant *Acinetobacter baumannii*
Samples Submitted	Replicate samples of linoleum floor covering

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1 Materials Submitted For Testing

Replicate samples of a linoleum floor covering were submitted to be tested for antimicrobial activity against a multi-resistant strain of *Acinetobacter baumannii*, using a procedure based on ISO 22196: 2011 (Plastics – Measurement of antibacterial activity on plastics and other non-porous surfaces).

2 Test Organism

A culture of *Acinetobacter baumannii* strain NCTC 13420 was obtained from the HPA Culture Collection. This organism is described as being multi-resistant and is detailed in the following paper

A prevalent, multi-resistant clone of *Acinetobacter baumannii* in Southeast England. J Hosp Infect. 2004 Nov;58(3):170-9. Coelho JM, Turton JF, Kaufmann ME, Glover J, Woodford N, Warner M, Palepou MF, Pike R, Pitt TL, Patel BC, Livermore DM - Specialist and Reference Microbiology Division, Laboratory of Healthcare Associated Infection, Health Protection Agency, 61 Colindale Avenue, London NW9 5HT, UK.

Abstract:

A multi-resistant clone of *Acinetobacter baumannii* was identified in 24 hospitals in the UK, predominantly in the London area, over a period of three years. Isolates were characterized by distinctive ApaI macrorestriction profiles, as resolved by pulsed-field gel electrophoresis (PFGE), which all clustered within 80% similarity using a 1% band position tolerance setting. The first isolates identified were received by the reference laboratories in April 2000, and by June 2003, a total of 375 isolates with similar PFGE profiles from 310 patients from 24 hospitals had been received. The isolates originated mainly from sputum and wound specimens, with the majority from patients in intensive care units. Amplified fragment length polymorphism analysis of a subset of isolates showed that they clustered closely, supporting the PFGE results. All the isolates tested were highly resistant to ampicillin, piperacillin, piperacillin/tazobactam, ceftazidime, cefotaxime, gentamicin and ciprofloxacin, and most isolates were carbapenem resistant. Amikacin sensitivity varied from susceptible [minimum inhibitory concentration (MIC) <or= 4 mg/L] to highly resistant (MIC >256 mg/L)

3 Test Procedure

Testing was carried out using a procedure based on ISO 22196: 2011 (formerly JIS Z 2801: 2000).

0.1ml of a suspension containing approx. 5 x 10^5 cells of the test organism was placed on the upper surface of triplicate test samples (60mm x 60mm) and on triplicate samples of polystyrene sheet (used as the PRA control and known to have no antimicrobial activity).

The suspension was held in intimate contact with the test and control surfaces using a polyethylene film rectangle, 20mm x 20mm in size.

To provide a time zero inoculation level, an additional triplicate set of PRA control samples (polypropylene film) were similarly inoculated, and the inoculum then immediately recovered from the surface using the method described below. The remaining replicates were incubated at 21°C and relative humidity of not less than 90%. After 24 hours incubation the inoculum was removed from the test surfaces (again using the method described below) and bacterial counts determined.

Owing to the porosity of the underlying surface of the test sample a modified procedure developed by the International Biodeterioration Research Group (IBRG) was used to recover the inoculum from the surfaces of the control pieces (at time zero and after 24 hours) and the test pieces (after 24 hours).

The polyethylene film covering the inoculum was removed with sterile forceps and placed in 10 ml of sterile neutralising medium. The surface which had been covered by the film was then thoroughly cleaned using a sterile cotton swab, and the untouched portion of this broken off into the neutralising medium. After vigorous agitation bacterial counts were determined on the washings.

4 Results and Observations

The microbial counts obtained (shown as a geometric mean), together with the antimicrobial activity (shown as a Log_{10} reduction) and the % kill, are given in the Table. The antimicrobial activity was calculated as follows:

R = [log (B/A) - log (C/A) = [log (B/C)]

where, R = antimicrobial activity

A = mean microbial count on PRA control sample at time zero

B = mean microbial count on PRA control sample after 24 hours

C = mean microbial count on test piece after 24 hours

Table : Antimicrobial activity against *Acinetobacter baumannii* NCTC 13420

Test Sample	Mean Count		Antimicrobial Activity	% Kill
	Initial count	24 hr count		
PRA Control	6.1×10^5	5.4×10^5	-	-
Linoleum Floor Covering	-	<10	>4.7	>99.9

5 Conclusion

The ISO standard 22196: 2011 specifies a method of evaluating the antimicrobial activity of antimicrobial-treated materials.

The predecessor to this ISO standard, JIS Z 2801: 2000, stated that for a coating to demonstrate antimicrobial efficacy the value of the antimicrobial activity shall not be less than 2.0. The ISO standard provides a means of quantifying the antimicrobial effectiveness of a surface in terms of antimicrobial activity, but no longer specifies a value for determining antimicrobial efficacy.

As a pass/ fail criterion is not defined in the current standard, PRA uses the following criterion to comment on the level of activity determined.

Antibacterial Activity	% Kill	Comment
<1.5	<96.8	poor
1.5 – 2.0	96.8 - 99.0	borderline
2.0 - 3.0	>99.0 - 99.9	good
>3.0	>99.9	excellent

Referring to the Table, the linoleum floor covering demonstrated excellent antimicrobial activity against the multi-resistant strain of *Acinetobacter baumannii*.

End of Report

TJG

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