RESEARCH NOTE

Biofilm formation in *Acinetobacter baumannii*: associated features and clinical implications

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ABSTRACT

Biofilm formation in 92 unrelated strains of *Acinetobacter baumannii* isolated in a multicentre cohort study was investigated using a microtitre plate assay. Fifty-six (63%) isolates formed biofilm. These isolates were less frequently resistant to imipenem or ciprofloxacin than were non-biofilm-forming isolates (25% vs. 47%, p 0.04; and 66% vs. 94%, p 0.004, respectively). All catheter-related urinary or bloodstream infections and the sole case of shunt-related meningitis were caused by biofilm-forming strains. Multivariate analysis revealed that treatment in an intensive care unit, ciprofloxacin resistance and isolation from a respiratory sample were associated with non-biofilm-forming isolates, while previous aminoglycoside use was associated with biofilm-forming isolates.

Keywords *Acinetobacter baumannii*, biofilm formation, ciprofloxacin resistance, imipenem resistance, infections, risk-factors

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Acinetobacter baumannii is a significant worldwide nosocomial pathogen with a particular ability to develop antimicrobial resistance and cause nosocomial outbreaks of infection [1]. This organism frequently causes infections associated with medical devices, e.g., vascular catheters, cerebrospinal fluid shunts or Foley catheters [1–3]. Biofilm formation is a well-known pathogenic mechanism in such infections [4]. In addition, the environmental survival of some microorganisms may be facilitated by biofilm formation on abiotic surfaces. Little is known concerning biofilm formation in *A. baumannii* [5–8]. Therefore, the present study investigated the frequency of biofilm formation and the associated clinical correlations and variables for 92 clonally unrelated isolates selected from among 221 isolates of *A. baumannii* collected during the GEIH-Ab 2000 project [2], which was a multicentre prospective cohort study performed in 28 Spanish hospitals. The methods and general clinical, epidemiological and microbiological results of this study have been reported in detail elsewhere [2,9,10]. For the purpose of the present analysis, if an isolate included in this study was clonally related to at least one other isolate from the original collection, it was considered to be epidemic [2]. The study was approved by the local ethics committees of the participating hospitals.

Biofilm formation was determined in the Hospital Clinic, Barcelona, Spain, using an overnight culture, diluted 1:100 in fresh Luria–Bertoni broth in 96-well plates and incubated without shaking at 37°C for 48 h. Of the 96 wells, four were left uninoculated and used as negative controls. Biofilm was stained with crystal violet 1% w/v and quantified at 570 nm after solubilisation with ethanol–acetone. The experiment was performed in duplicate in two 96-well plates. Isolates were classified as biofilm-forming if they yielded OD_{570} values that were at least twice those of the negative controls. When an isolate was clearly positive for biofilm formation in the assay and the duplicate assay was borderline, the isolate was considered to be biofilm-positive. When an isolate was clearly positive in the first assay and the duplicate assay was clearly negative, the isolate was considered to be non-evaluable and was excluded. Susceptibility to antimicrobial agents was determined by microdilution according to CLSI recommendations [11].

The epidemiological and clinical features of patients colonised or infected with biofilm-

forming and non-biofilm-forming *A. baumannii* isolates were compared. Continuous variables were compared using the Mann–Whitney *U*-test and categorical variables were compared using the chi-square test (Fisher's exact test, if required). Multivariate analysis was performed by logistic regression analysis. Statistical analyses were performed using SPSS v.12.0 (SPSS Inc., Chicago, IL, USA).

Of the 92 isolates studied, 56 (63%) formed biofilm *in vitro*, 33 (36%) did not form biofilm, and three (3%) were non-evaluable. Thus, 89 isolates were used in the final analysis. Although one representative isolate of each pulsed-field gel electrophoresis type was initially analysed, the results for biofilm formation always agreed with the representative isolate when other isolates belonging to the same pulsed-field gel electrophoresis type from the original collection ('epidemic strains') were tested. Biofilm-forming isolates were less frequently imipenem-resistant (25% vs. 47%, p 0.04), ciprofloxacin-resistant (66% vs. 94%, p 0.004) and epidemic (31% vs. 53%, p 0.04) than were non-biofilm-forming isolates. No significant differences in susceptibility to doxycycline (65% vs. 60%), ceftazidime (73% vs. 83%), sulbactam (39% vs. 27%), gentamicin (80% vs. 77%), tobramycin (76% vs. 73%) or rifampicin (0 vs. 3%) were observed (p >0.1).

Complete epidemiological and clinical data were available for 78 patients and were included in the analysis of factors associated with biofilm formation. Univariate analyses are shown in Table 1. ORs (95% CI) for the variables selected in multivariate analysis were: treatment in an intensive care unit, 0.1 (0.004–0.8); respiratory tract sample, 0.2 (0.005–0.4); ciprofloxacin resistance, 0.06 (0.009–0.4); and previous receipt of aminoglycosides, 13.1 (2.3–74.9). When CDC criteria were used [12], the frequencies of infection caused by biofilm-forming and non-biofilm-forming isolates were similar (20/49 (41%) vs. 13/29 (45%), p 0.1). Types of infections are shown in Table 2. Infections caused by non-biofilm-forming isolates showed a non-significant trend toward the presence of sepsis and a higher mortality rate when compared with infections caused by biofilm-forming isolates (92% vs. 70%, p 0.1, and 23% vs. 14%, p 0.6, respectively).

There is very limited information concerning the ability of *A. baumannii* to form biofilm [5–7]. In a collection of clinical isolates of *A. baumannii*, Sechi *et al.* [8] found that 16 (80%) of 20 isolates formed biofilm, perhaps because of a dominant *A. baumannii* clone. In the present study, 63% of 92 clonally unrelated *A. baumannii* clinical isolates formed biofilm. Interestingly, all clonally related isolates shared either an ability or an inability to form a biofilm, which suggests that this is a clone-specific feature and that its expression does not vary substantially under different conditions; however, further studies are needed to investigate this hypothesis.

Table 1. Univariate analysis of factors associated with biofilm-forming isolates of *Acinetobacter baumannii* (data expressed as a percentage of cases unless otherwise specified)

	Biofilm-forming (*n* = 49)	Non-biofilm-forming (*n* = 29)	OR (95% CI)	p value[a]
Mean age, years (SD)[b]	55 (21)	62 (14)	–	0.08[c]
Male gender	72	78	0.7 (0.2–2.0)	0.5
Underlying disease				
Non-fatal	74	62	–	0.5
Ultimately fatal	24	32		
Rapidly fatal	4	6		
Diabetes mellitus	10	22	0.4 (0.1–1.4)	0.1
Neoplasia	17	28	0.5 (0.1–1.5)	0.2
Chronic pulmonary disease	15	28	0.5 (0.1–1.5)	0.2
ICU treatment	26	53	0.3 (0.1–0.7)	0.01
Mean days of hospital stay (SD)	29 (37)	22 (25)	–	0.3[c]
Central venous catheter	58	61	0.8 (0.3–2.2)	0.7
Mechanical ventilation	44	52	0.7 (0.2–1.8)	0.4
Urinary catheter	77	77	0.9 (0.3–2.8)	0.9
Previous antimicrobial agents	86	84	1.3 (0.3–4.8)	0.7
Aminoglycosides	43	20	3.0 (0.9–10.3)	0.06
Fluoroquinolones	21	10	2.4 (0.5–12.3)	0.2
Cephalosporins	46	27	2.3 (0.8–6.2)	0.09
Carbapenems	13	17	0.7 (0.1–3.2)	0.1
Type of sample				
Respiratory tract	25	53	0.3 (0.1–0.8)	0.01
Blood	10	0	–	0.07[d]
Urine	32	14	3.0 (0.9–10.1)	0.06
Wound	27	27	0.9 (0.3–3.0)	0.8
Others	6	6	0.9 (0.1–8.1)	0.8[d]

ICU, intensive care unit; SD, standard deviation.
[a]Chi-square test except where specified.
[b]There were only three paediatric patients, all of whom yielded a biofilm-forming isolate.
[c]Mann–Whitney *U*-test.
[d]Fisher's exact test.

Table 2. Types of infections caused by biofilm-forming and non-biofilm-forming isolates of *Acinetobacter baumannii* (data expressed as absolute numbers of infections)

	Biofilm-forming (*n* = 20)	Non-biofilm forming (*n* = 13)
IV catheter-related infection	3	0
Foley-related UTI	6	0
CSF shunt infection	1	0
VA respiratory tract infection	5	8
Non-VA respiratory tract infection	1	0
Skin and soft-tissue infection	4	5

IV, intravascular; UTI, urinary tract infection; CSF, cerebrospinal fluid; VA, ventilator-associated.

Although limited by the low number of cases, the present results suggest that biofilm plays a role in the pathogenesis of some device-associated *A. baumannii* infections (e.g., those involving Foley catheters, venous catheters and cerebrospinal fluid shunts); in contrast, ventilator-associated pneumonia was not caused predominantly by biofilm-forming isolates. These results suggest the hypothesis that infections caused by biofilm-forming isolates might be associated with a diminished frequency of systemic response or mortality; however, this association was not statistically significant and further studies would be necessary to investigate this possibility.

Biofilm-forming isolates were less frequently resistant to imipenem and ciprofloxacin, and seemed to be less epidemic. A possible explanation is that biofilm-forming isolates are not as dependent as their non-biofilm-forming counterparts on antimicrobial resistance and epidemic characteristics to survive in the hospital environment. Sechi *et al.* [8] have previously reported no relationship between biofilm formation and the production of PER-1 β-lactamase. However, patients who had previously received aminoglycosides were at an increased risk of being colonised or infected by biofilm-forming *A. baumannii*. Previous aminoglycoside use may exert a different selection pressure on biofilm formation, irrespective of the in-vitro susceptibility.

In summary, >60% of unrelated *A. baumannii* isolates from clinical samples formed biofilm, and these isolates were associated mainly with device-associated infections. These isolates were less frequently resistant to imipenem and ciprofloxacin.

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