Infectious Diseases

ISSN: 2374-4235 (Print) 2374-4243 (Online) Journal homepage: http://www.tandfonline.com/loi/infd20

Acinetobacter baumannii in Southern Croatia: clonal lineages, biofilm formation, and resistance patterns

Vanja Kaliterna, Mariano Kaliterna, Jasna Hrenović, Zvonimir Barišić, Marija Tonkić & Ivana Goic-Barisic

To cite this article: Vanja Kaliterna, Mariano Kaliterna, Jasna Hrenović, Zvonimir Barišić, Marija Tonkić & Ivana Goic-Barisic (2015) Acinetobacter baumannii in Southern Croatia: clonal lineages, biofilm formation, and resistance patterns, Infectious Diseases, 47:12, 902-907

To link to this article: http://dx.doi.org/10.3109/23744235.2015.1078906

Published online: 18 Aug 2015.

Infectious Diseases, 2015; 47: 902–907

Taylor & Francis
Taylor & Francis Group

ORIGINAL ARTICLE

Acinetobacter baumannii in Southern Croatia: clonal lineages, biofilm formation, and resistance patterns

VANJA KALITERNA[1,4], MARIANO KALITERNA[2], JASNA HRENOVIĆ[3], ZVONIMIR BARIŠIĆ[1], MARIJA TONKIĆ[2,4] & IVANA GOIC-BARISIC[2,4]

From the [1]Public Health Institute of Split-Dalmatia County, Split, [2]University Hospital Centre Split, Split, [3]Faculty of Science, Division of Biology, University of Zagreb, Zagreb, and [4]University of Split School of Medicine, Split, Croatia

Abstract

Background: Acinetobacter baumannii is one of the most prevalent causes of severe hospital-acquired infections and is responsible for the dramatic increase in carbapenem resistance in Croatia in the last 5 years. Such data have encouraged multicenter research focused on the organism's ability to form biofilm, susceptibility to antibiotics, and particular genotype lineage. *Methods:* Biofilm formation in 109 unrelated clinical isolates of *A. baumannii* recovered in six cities of Southern Croatia was investigated. Genotyping was performed by pulsed-field gel electrophoresis and antibiotic profile was tested by applying the disc diffusion method and confirmed by determining the minimum inhibitory concentrations. The ability to form biofilm in vitro was determined from overnight cultures of the collected isolates on microtiter plates, after staining with crystal violet, and quantified at 570 nm after solubilization with ethanol. The statistical relevance was calculated in an appropriate program with level of statistical confidence. *Results:* There was no significant difference in biofilm formation due to the genotype lineage. Isolates collected from intensive care units (ICUs) and isolated from respiratory samples were more likely to create a biofilm compared with isolates from other departments and other samples. There was a significant difference in the ability to produce biofilm in relation to antibiotic resistance pattern. A large proportion of *A. baumannii* isolates that were resistant to ampicillin/sulbactam, carbapenems, and amikacin were found to be biofilm-negative. In contrast, isolates susceptible and intermediately susceptible to ampicillin/sulbactam, carbapenems, and amikacin were biofilm producers. *Conclusion:* Clinical isolates of A. baumannii from respiratory samples in ICUs with a particular susceptibility pattern are more prone to form biofilm.

Keywords: *Acinetobacter baumannii; genotyping; biofilm formation; resistance to antibiotics; Southern Croatia*

Introduction

Acinetobacter baumannii is known as a highly adaptable hospital pathogen because of its virulence factors. It has a great ability to survive on the skin of hospitalized patients and on hospital surfaces. In this way it is spread among patients, thereby contributing to the development and persistence of outbreaks in hospitals [1–6]. The ability of clinical isolates of *A. baumannii* to form biofilm may contribute to its persistence in hospital environments. The biofilm-forming *A. baumannii* clinical isolates are capable of surviving longer than non-biofilm-forming isolates (36 versus 15 days) in a desiccated environment [7]. The particular traits of biofilm infections include resistance to disinfectants, antibiotics, and the host's immune system, which make these infections persistent and difficult to treat [1,8–11].

Research on the epidemiology of *A. baumannii* as a major hospital pathogen in Croatia started in 2002 on isolates with reduced susceptibility to carbapenems at the University Hospital Centre Split (UHCS). In the period from 2002 to 2007, only the existence of international clonal lineage I (ICL I) was proved in UHCS, the second largest clinical hospital center in Croatia [12,13]. At the beginning of 2009, the ICL II clone spread in the neurosurgical intensive care

This study was partially presented as a poster presentation at the 25th European Congress of Clinical Microbiology and Infectious Diseases (ECCMID), Copenhagen, Denmark, 2015.

Correspondence: Ivana Goic-Barisic MD PhD, Clinical Department of Microbiology, University Hospital Centre Split and School of Medicine University of Split, Spinciceva 1, 21000 Split, Croatia. Tel: +385 21 556196. E-mail: igoic@kbsplit.hr.

(Received 29 April 2015; accepted 27 July 2015)

ISSN 2374-4235 print/ISSN 2374-4243 online
DOI: 10.3109/23744235.2015.1078906

unit (ICU), after transfer of a patient colonized by this clone from the General Hospital Mostar (Bosnia and Herzegovina) to the UHCS [14,15]. The published study also shows the existence of the ICL II clone in the largest University Hospital Centre Zagreb during 2009 and 2010 [16]. According to the information from the Croatian Committee for Antibiotic Resistance Surveillance (CARS) of the Croatian Academy of Medical Sciences (CAMS), in the past 5 years the resistance rates of *A. baumannii* to carbapenems has increased significantly, from 10% in 2008 to 78% in 2013 [17,18]. This observation was the driving force for a detailed study on clinical isolates of *A. baumannii* from different medical centers in Southern Croatia.

The scope of this multicenter study was to investigate if clinical isolates of *A. baumannii* from various medical institutions in Southern Croatia appertain to various genotypes, and if they had various abilities to form biofilm depending on the type of clinical sample, genotype (clone), and resistance to antibiotics.

Material and Methods

This study included 109 isolates of *A. baumannii* sampled in 6 cities of Southern Croatia, in different medical institutions/departments and from different types of clinical samples (respiratory samples, urine, blood culture, wound swabs), obtained during the 3-month monitoring period (2009–2010), in cooperation with CARS.

Identification of A. baumannii *isolates and antibiotic susceptibility testing*

After isolation of *A. baumannii* in local laboratories, the isolates were stored in nutrient agar and transported to the Department for Microbiology and Parasitology of the UHCS. Initial identification was made using commercial tests API 20NE and VITEK 2 (bioMerieux, Marcy l'Etiole, France). Isolates of *A. baumannii* were confirmed by the presence of an OXA-51-type β-lactamase, which is specific to *A. baumannii*, determined by polymerase chain reaction (PCR) [19]. Routine antimicrobial susceptibility testing was performed by the disk diffusion method for piperacillin/tazobactam, ceftazidime, cefepime, ampicillin/sulbactam, imipenem, meropenem, amikacin, gentamicin, and ciprofloxacin, confirmed with minimum inhibitory concentrations (MICs) by Vitek-2 automatic system (bioMerieux) and interpreted according to Clinical and Laboratory Standards Institute (CLSI) criteria [20]. The gradient strip microdilution test for colistin was performed and interpreted according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) criteria [21].

Genotyping of A. baumannii *isolates*

Grouping of *A. baumannii* to individual international clonal lineages (ICLs) was investigated by the pulsed field gel electrophoresis (PFGE) method by macrorestriction with *Apa*I enzyme performed with a CHEF-DRIII system (Bio-Rad, Marnes La Croquette, France), according to published protocols [22,23]. Representatives of major international clones I (RUH 2037) and II (RUH 134) were used as controls. The images were processed using Gel-Compar software, and the dendrogram was computed after band intensity correlation using global alignment with 1.5% optimization and unweighted pair group method with arithmetic mean (UPGMA) clustering.

Ability to form biofilm

The ability to form biofilm in vitro was tested for all *A. baumannii* isolates, according to published protocols [9,11]. Biofilm formation was determined in triplicate from overnight cultures diluted in Luria Bertoni (LB) medium to an optical density (OD) of 0.01 at 600 nm and deposited in 96-well plates. LB medium without inoculum was used as negative control. The covered plate was incubated at 37°C for 48 h without shaking. After incubation, the planktonic bacteria were removed and washed twice with distilled water. Biofilm was stained with 0.5% crystal violet (w/v) and quantified at 570 nm after solubilization with 95% ethanol for 15 min at room temperature. The OD_{570} corresponds to the amount of biofilm formed by bacteria. The interpretation for OD values was as follows: non-biofilm-forming isolate (<0.4), low biofilm-forming isolate (0.4–1.0), and strong biofilm-forming isolate (>1.0) [7]. We also observed if ability to form biofilm depended on the genotype, the isolate origin (departments from which samples are taken as well as types of samples), and the resistance to antibiotics.

Statistical analysis

Tests of statistical significance were made by Monte Carlo simulation (on 10 000 tables) and with the chi-squared ($\chi 2$) test. Statistical analysis of data was performed using SPSS 17.0 software (SPSS Inc., Chicago, IL, USA). Confidence intervals for proportions were calculated in the Statistics Calculator 3.0 (StatPac Inc., Bloomingtom, MN, USA). All statistical values were considered significant at the p level of 0.05.

Results

Genotyping

Of 109 isolates, according to results of PFGE analysis, 56 (51.4%) belonged to ICL I, 29 (26.6%) to ICL II, and 24 (22.0%) to other clones. ICL II clone was found only in one hospital (UHCS) in Southern Croatia and in that hospital it was the dominant clonal lineage in 82.9% (29/35) of all *A. baumannii* isolates.

Ability to form biofilm

Of the total number of 109 isolates, biofilm was not formed by 28 (25.7%) isolates, and 81 (74.3%) isolates were positive for biofilm production (47 showed a low ability to form biofilm, while 34 were strong biofilm producers). Although isolates of the ICL I genotype in the majority of isolates showed the ability to form biofilm (46.4% were low and 37.5% were strong biofilm producers), and isolates of the ICL II genotype were almost equally distributed across all biofilm-forming categories, no statistically relevant difference in the formation of biofilm was established, with respect to their association with individual genotype ($p = 0.111$).

Of 65 isolates recovered from ICU patients, the majority of them (52, 80%) were biofilm producers. Of 45 isolates obtained from clinical respiratory samples (tracheal and bronchial aspirates and bronchoalveolar lavage), 37 of them (82.2%) were biofilm producers, while of 28 isolates recovered from wound samples, 17 (60.7%) isolates showed the ability to form biofilm.

Resistance to antibiotics

Resistance rates of *A. baumannii* isolates to tested antibiotics are shown in Table I.

The majority of isolates resistant to ampicillin/sulbactam, imipenem, meropenem, and amikacin belonged to ICL II clone (Table II). The ability to form biofilm was compared with resistance to ampicillin/sulbactam, imipenem, meropenem, and amikacin. It was shown that the majority of isolates susceptible or intermediate susceptible to those antibiotics have the ability (low or strong) to form biofilm, but the majority of resistant isolates do not form any biofilm (Table III).

Table I. Resistance of *A. baumannii* isolates to tested antibiotics.

Antibiotic	Susceptible, *n* (%)	Intermediate, *n* (%)	Resistant, *n* (%)
Piperacillin/tazobactam	17 (15.6)	4 (3.7)	88 (80.7)
Ceftazidime	14 (12.8)	2 (1.8)	93 (85.3)
Cefepime	20 (18.3)	6 (5.5)	83 (76.1)
Ampicillin/sulbactam	94 (86.2)	1 (0.9)	14 (12.8)
Imipenem	44 (40.4)	30 (27.5)	35 (32.1)
Meropenem	31 (28.4)	43 (39.4)	35 (32.1)
Amikacin	53 (48.6)	20 (18.3)	36 (33.0)
Gentamicin	21 (19.3)	1 (0.9)	87 (79.8)
Ciprofloxacin	16 (14.7)	3 (2.8)	90 (82.6)
Colistin	109 (100)	0 (0.0)	0 (0.0)

Discussion

In this investigation of *A. baumannii* isolates from six cities in Southern Croatia, we have determined that more than half of the collected isolates belonged to ICL I clone, 26.6% to ICL II clone, and 22.0% to other clones. According to the published studies, the distribution of the ICL II genotype is in the range from 24 to 49% [6,24], which is in accordance with our results.

The ability to form biofilm is an important factor for bacterial virulence [7]. In the present study, we determined that of 109 isolates 74.3% of them formed biofilm, which is in accordance with the data published in various studies (42–80% of biofilm-positive isolates) [11,25–27]. In certain studies, it was determined that isolates of the ICL II genotype had a stronger ability to form biofilm [9,28]. Wroblewska and associates proved that there was no connection between genotypes and the ability to form biofilm [27]. In the present study, no significant difference in the biofilm formation was established regarding the genotypes.

It has been reported that the ability of *A. baumannii* isolates to form biofilm is not associated with the type of clinical sample [9,27]. On the other hand, some studies indicated that the type of the clinical sample has an impact on the isolate's ability to form biofilm [11,25,26]. In our research we have established that isolates obtained from respiratory and wound samples were more likely to create biofilm compared with isolates from other samples. Strong ability to form biofilm for respiratory samples was established by Rao and associates [26]. On the other hand, Rodriguez-Bano and associates determined that isolates from respiratory samples had a low ability to form biofilm [11]. Stronger ability of isolates obtained from wounds to form biofilm was also indicated by Rajamohan et al. and Rao et al. in their works [25,26]. However, Rodriguez-Bano et al. determined in their study that treatment in ICU is statistically more often associated with isolates that do not form biofilm [11]. In the present study, of the 65 isolates obtained from ICU patients, 80% of them had an ability to form biofilm. Stronger ability of respiratory isolates to form biofilm may explain the high incidence of ventilator-associated pneumonia in ICUs.

Table II. Resistance of *A. baumannii* isolates to tested antibiotics, with respect to their association with individual genotype.

Antibiotic	ICL I, *n* (%)	ICL II, *n* (%)	Other clones, *n* (%)	Total, *n* (%)	*p* value[a]
Ampicillin/sulbactam					
Susceptible	55 (58.5)	16 (17.0)	23 (24.5)	94 (100)	<0.001
Intermediate	0 (0.0)	1 (100)	0 (0.0)	1 (100)	
Resistant	1 (7.1)	**12 (85.7)**	1 (7.1)	14 (100)	
Imipenem					
Susceptible	22 (50.0)	3 (6.8)	19 (43.2)	44 (100)	<0.001
Intermediate	29 (96.7)	0 (0.0)	1 (3.3)	30 (100)	
Resistant	5 (14.3)	**26 (74.3)**	4 (11.4)	35 (100)	
Meropenem					
Susceptible	14 (45.2)	3 (9.7)	14 (45.2)	31 (100)	<0.001
Intermediate	37 (86.0)	0 (0.0)	6 (14.0)	43 (100)	
Resistant	5 (14.3)	**26 (74.3)**	4 (11.4)	35 (100)	
Amikacin					
Susceptible	31 (58.5)	2 (3.8)	20 (37.7)	53 (100)	<0.001
Intermediate	18 (90.0)	0 (0.0)	2 (10.0)	20 (100)	
Resistant	7 (19.4)	**27 (75.0)**	2 (5.6)	36 (100)	

[a]*p* value = Monte Carlo simulation on N_{tables} = 10.000, level of statistical significance; probability of type I error.

Within the biofilm bacteria have a greater ability to survive because they are protected against environmental stress, such as desiccation and exposure to disinfectants and antibiotics, which makes biofilm infections persistent and difficult to treat. Today, *A. baumannii* isolates have become resistant to most antibiotics [29]. According to information from the Croatian Committee for Antibiotic Resistance Surveillance of the CAMS, in the past 5 years the resistance of acinetobacter to carbapenems has increased significantly. At the UHCS, in 2013, carbapenem resistance was marked at the rate of 87% [18]. The growing resistance to carbapenems has been indicated lately also by other authors [24]. The resistance of acinetobacter to carbapenems, mostly to imipenem, has been the topic of various papers, and the published data on the resistance rates to imipenem have reported from 21% up to 100% of the tested isolates of *A. baumannii* [13,26,30–34]. Among the European countries the resistance rates to carbapenems are

Table III. Resistance of *A. baumannii* isolates to tested antibiotics, with respect to their ability to form biofilm.

Antibiotic	Non-biofilm forming (*n* = 28), *n* (%)	Low biofilm forming (*n* = 47), *n* (%)	Strong biofilm forming (*n* = 34), *n* (%)	Total, *n* (%)	*p* value[a]
Ampicillin/sulbactam					
Susceptible	20 (21.3)	43 (45.7)	31 (33.0)	94 (100)	0.014
Intermediate	0 (0.0)	0 (0.0)	0 (0.0)	1 (100)	
Resistant	8 (57.1)	4 (28.6)	2 (14.3)	14 (100)	
Imipenem					
Susceptible	11 (25.0)	23 (52.3)	10 (22.7)	44 (100)	0.003
Intermediate	2 (6.7)	13 (43.3)	15 (50.0)	30 (100)	
Resistant	15 (42.9)	11 (31.4)	9 (25.7)	35 (100)	
Meropenem					
Susceptible	9 (29.0)	17 (54.8)	5 (16.1)	31 (100)	0.011
Intermediate	6 (14.0)	16 (37.2)	21 (48.8)	43 (100)	
Resistant	13 (37.1)	14 (40.0)	8 (22.9)	35 (100)	
Amikacin					
Susceptible	13 (24.5)	29 (54.7)	11 (20.8)	53 (100)	<0.001
Intermediate	1 (5.0)	4 (20.0)	15 (75.0)	20 (100)	
Resistant	14 (38.9)	14 (38.9)	8 (22.2)	36 (100)	

[a]*p* value = Monte Carlo simulation on N_{tables} = 10.000, level of statistical significance; probability of type I error.

highest in the countries in southern Europe, such as Greece and Croatia [18,34]. Although it was not the subject of this study, we also explored the molecular basis of carbapenem resistance among isolates belonging to ILC I and ILC II. In the majority of isolates belonging to ILC I the presence of insertion sequences IS*Aba*1 upstream to coding fragment (*bla*OXA-51-like genes) was the main mechanism of carbapenem resistance, in contrast to the carbapenem-resistant strains belonging to the ILC II with acquired OXA-72 (an OXA-40-type enzyme), which matches the published data in Croatia [13–16]. According to the available literature, it can be concluded that these significantly varying data on the resistance to carbapenem depend on: genotype, origin of isolates (departments as well as types of samples), and the ability of isolates to form biofilm. In our research we have also demonstrated that there are significant differences in the resistance of tested isolates to antibiotics (including carbapenem), depending on the above-mentioned factors.

Some published studies indicate that the ICL II clone was more frequently resistant to carbapenems than other clones [6,24,28,32]. Also the present study showed that the majority of isolates resistant to ampicillin/sulbactam, imipenem, meropenem, and amikacin belonged to ICL II clone. There are various observations in the literature concerning the antibiotic resistance of *A. baumannii* isolates and its ability to form biofilm. It is a well-known fact that bacterial biofilm protects bacteria from the effect of antibiotics, reducing penetration of antibiotics through biofilm [10,25]. Therefore, some authors have stated that isolates with the ability to form biofilm show more resistance to antibiotics [25,26,28,35,36]. In their investigations, de Breij et al. and Wroblewska et al. have indicated that the ability to form biofilm is not associated with increased resistance to antibiotics [9,27]. Some authors indicated that biofilm-forming strains are less frequently resistant to antibiotics than non-biofilm-forming strains [7,11,37]. In the present study it was determined that the majority of isolates susceptible or intermediately susceptible to ampicillin/sulbactam, imipenem, meropenem, and amikacin have a low or strong ability to form biofilm, but the majority of isolates resistant to the listed antibiotics do not form any biofilm. Such observations allow us to conclude that isolates of the *A. baumannii* strain that are susceptible or intermediately susceptible to antibiotics are more prone to form biofilm in order to use that biofilm to protect themselves from antibiotics, as opposed to isolates resistant to antibiotics that are less prone to form biofilm, because they have already developed some different type of defense mechanism against antibiotics. Knowledge of the phenotype of resistance in clinical isolates of *A. baumannii*, especially from respiratory samples in patients on mechanical ventilation, can be supported by biofilm formation. Therefore we have to think about the antimicrobial and antibiofilm potential of new treatment options, as indicated by recent published data [38,39]. The clinical relevance of results obtained using the in vitro biofilm assay on abiotic surfaces remains somewhat unclear. Theoretically, it seems logical that there would be a correlation between laboratory results and the clinical findings. Furthermore, these data may suggest that biofilm formation on abiotic surfaces from respiratory samples in ICU is critical for the clinical success of *A. baumannii*. The results of this multicenter study represent a step forward in understanding the epidemiology and virulence factors of this important hospital pathogen and may open up possibilities for using biosurfactants as an alternative therapeutic approach for the prevention and/or treatment of hospital-acquired infections.

Acknowledgments

We thank all the Croatian collaborative centers of the CAMS for providing clinical isolates of *A. baumannii* for this multicenter investigation. We would also like to thank Dr Kevin Towner (Nottingham, UK) for help and suggestions in preparing this manuscript.

Declaration of interest: This work was supported by the University of Zagreb (project no. 202649-202710) and in part by the Croatian Science Foundation (project no. 5656). The authors report no conflicts of interest.

References

[1] Roca I, Espinal P, Vila-Farres X, Vila J. The *Acinetobacter baumannii* oxymoron: commensal hospital dweller turned pan-drug-resistant menace. Front Microbiol 2012;3:1–30.

[2] de Breij A. Towards an explanation for the success of *Acinetobacter baumannii* in the human host. Leiden, Netherland: Faculty of Medicine Leiden University; 2012.

[3] Peleg AY, Seifert H, Paterson DL. *Acinetobacter baumannii*: emergence of a successful pathogen. Clin Microbiol Rev 2008;21:538–82.

[4] Perez F, Hujer AM, Hujer KM, Decker BK, Rather PN, Bonomo RA. Global challenge of multidrug-resistant *Acinetobacter baumannii*. Antimicrob Agents Chemother 2007;51:3471–84.

[5] de Breij A, Haisma E, Rietveld M, El Ghalbzouri A, van den Broek P, Dijkshoorn L, et al. Three-dimensional human skin equivalent as a tool to study *Acinetobacter baumannii* colonization. Antimicrob Agents Chemother 2012;56:2459–64.

[6] Perez F, Ponce-Terashima R, Adams MD, Bonomo RA. Are we closing in on an "elusive enemy"? The current status of our battle with *Acinetobacter baumannii*. Virulence 2011; 2:86–90.

[7] Espinal P, Marti S, Vila J. Effect of biofilm formation on the survival of *Acinetobacter baumannii* on dry surfaces. J Hosp Infect 2012;80:56–60.
[8] Marti S, Rodriguez-Bano J, Catel-Ferreira M, Jouenne T, Vila J, Seifert H, et al. Biofilm formation at the solid-liquid and air-liquid interfaces by *Acinetobacter* species. BMC Res Notes 2011;4:5.
[9] De Breij A, Dijkshoorn L, Lagendijk E, van der Meer J, Koster A, Bloemberg G, et al. Do biofilm formation and interactions with human cells explain the clinical success of *Acinetobacter baumannii*? PloS One 2010;5:e10732.
[10] Donlan RM, Costerton JW. Biofilm: survival mechanisms of clinically relevant microorganisms. Clin Microbiol Rev 2002;15:167–93.
[11] Rodriguez-Bano J, Marti S, Soto S, Fernandez-Cuenca F, Cisneros JM, Pachon J, et al. Biofilm formation in *Acinetobacter baumannii*: associated features and clinical implications. Clin Microbiol Infect 2008;14:276–8.
[12] Goić-Barišić I, Bedenić B, Tonkić M, Katić S, Kalenić S, Punda-Polić V. First report of molecular characterization of carbapenem-resistant *Acinetobacter baumannii* in different intensive care units in University hospital Split, Croatia. J Chemother 2007;19:416–18.
[13] Goić-Barišić I, Bedenić B, Tonkić M, Novak A, Katić S, Kalenić S, et al. Occurrence of OXA-107 and ISAba1 in carbapenem-resistant isolates of *Acinetobacter baumannii* from Croatia. J Clin Microbiol 2009;47:3348–9.
[14] Goić-Barišić I, Towner KJ, Kovačić A, Šiško-Kraljević, Tonkić M, Novak A, et al. Outbreak in Croatia caused by a new carbapenem-resistant clone of *Acinetobacter baumannii* producing OXA-72 carbapenemase. J Hosp Infect 2011; 77:368–9.
[15] Goić-Barišić I, Kaliterna V. Multidrug-resistant *Acinetobacter baumannii* – the pathogen with no borders? Med Glas (Zenica) 2011;8:312–13.
[16] Franolić-Kukina I, Bedenic B, Budimir A, Herljević Z, Vraneš J, Higgins PG. Clonal spread of carbapenem-resistant OXA-72-positive *Acinetobacter baumannii* in a Croatian university hospital. Int J Inf Dis 2011;15:706–9.
[17] Croatian Committee for Antibiotic Resistance Surveillance. Antibiotic resistance in Croatia in 2008. Zagreb: Croatian Academy of Medical Sciences (CAMS); 2008.
[18] Croatian Committee for Antibiotic Resistance Surveillance. Antibiotic resistance in Croatia in 2013. Zagreb: Croatian Academy of Medical Sciences (CAMS); 2014.
[19] Evans BA, Hamouda A, Towner KJ, Amyes SGB. OXA-51-like β-lactamases and their association with particular epidemic lineages of *Acinetobacter baumannii*. Clin Microbiol Infect 2008;14:268–75.
[20] Clinical and Laboratory Standards Institute (CLSI). Performance standards for antimicrobial disk susceptibility tests – Approved standard (M2-A9), 17th informational supplement. CLSI document M100-S17. Wayne, PA: CLSI; 2007.
[21] European Committee on Antimicrobial Susceptibility Testing (EUCAST). Breakpoint tables for interpretation of MICs and zone diameters, version 1.1, 2010. Available from: http://www.eucast.org/clinical_breakpoints
[22] Seifert H, Dolzani L, Bressan R, van der Reijden, van Strijen B, Stefanik D, et al. Standardization and interlaboratory reproducibility assessment of pulsed-field gel electrophoresis-generated fingerprints of *Acinetobacter baumannii*. J Clin Microbiol 2005;43:4328–35.
[23] PFGE typing protocol recommended by ARPAC for *Acinetobacter baumannii*. Available from: www.abdn.ac.uk/arpac/
[24] Schleicher X, Higgins PG, Wisplinghoff H, Korber-Irrgang B, Kresken M, Seifert H. Molecular epidemiology of *Acinetobacter baumannii* and *Acinetobacter nosocomialis* in Germany over a 5-year period (2005-2009). Clin Microbiol Infect 2013;19:737–42.
[25] Rajamohan G, Srinivasan VB, Gebreyes WA. Biocide-tolerant multidrug-resistant *Acinetobacter baumannii* clinical strains are associated with higher biofilm formation. J Hosp Infect 2009;73:287–9.
[26] Rao RS, Karthika RU, Singh SP, Shashikala P, Kanungo R, Jayachandran S, et al. Correlation between biofilm production and multiple drug resistance in imipenem resistant clinical isolates of *Acinetobacter baumannii*. Indian J Med Microbiol 2008;26:333–7.
[27] Wroblewska MM, Sawicka-Grzelak A, Marchel H, Luczak M, Sivian A. Biofilm production by clinical strains of *Acinetobacter baumannii* isolated from patients hospitalized in two tertiary care hospitals. FEMS Immunol Med Microbiol 2008;53:140–4.
[28] Kaliterna V, Goić-Barišić I. The ability of biofilm formation in clinical isolates of *Acinetobacter baumannii* belonging to two different European clones causing outbreaks in the Split University Hospital, Croatia. J Chemother 2013;25:60–2.
[29] Munoz-Price LS, Weinstein RA. *Acinetobacter* infection. N Engl J Med 2008;358:1271– 81.
[30] Rodriguez-Bano J, Cisneros JM, Fernandez-Cuenca F, Ribera A, Vila J, Pascual A, et al. Clinical features and epidemiology of *Acinetobacter baumannii* colonization and infection in Spanish hospitals. Infect Control Hosp Epidemiol 2004;25:819–24.
[31] Higgins PG, Dammhayn C, Hackel M, Seifert H. Global spread of carbapenem-resistant *Acinetobacter baumannii*. J Antimicrob Chemother 2010;65:233–8.
[32] Nemec A, Krizova L, Maixnerova M, Diancourt L, van der Reijden TJK, Berisse S, et al. Emergence of carbapenem resistance in *Acinetobacter baumannii* in the Czech Republic is associated with the spread of multidrug-resistant strains of European clone II. J Antimicrob Chemother 2008; 62:484–9.
[33] Longo B, Pantosi A, Luzzi I, Tarasi A, Di Sora F, Gallo S, et al. Molecular findings and antibiotic-resistance in an outbreak of *Acinetobacter baumannii* in an intensive care unit. Ann Ist Super Sanita 2007;43:83–8.
[34] Gogou V, Pournaras S, Giannouli M, Voulgari E, Piperaki ET, Zarrilli R, et al. Evolution of multidrug-resistant *Acinetobacter baumannii* clonal lineages: a 10 year study in Greece (2000-09). J Antimicrob Chemother 2011;66: 2767–72.
[35] Lee HW, Koh YM, Kim J, Lee JC, Lee YC, Seol SY, et al. Capacity of multidrug-resistant clinical isolates of *Acinetobacter baumannii* to form biofilm and adhere to epithelial cell surfaces. Clin Microbiol Infect 2008;14: 49–54.
[36] Davey ME, O'toole GA. Microbial biofilms: from ecology to molecular genetics. Microbiol Mol Biol Rev 2000;64: 847–67.
[37] Perez LR. *Acinetobacter baumannii* displays inverse relationship between meropenem resistance and biofilm production. J Chemother 2015;27:13–16.
[38] Nait Chabane Y, Mlouka MB, Alexandre S, Nicol M, Marti S, Pestel-Caron M, et al. Virstatin inhibits biofilm formation and motility of *Acinetobacter baumannii*. BMC Microbiol 2014;14:62.
[39] Sambanthamoorthy K, Feng X, Patel R, Patel S, Paranavitana C. Antimicrobial and antibiofilm potential of biosurfactants isolated from lactobacilli against multi-drug-resistant pathogens. BMC Microbiol 2014;14:197.