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Biofilm Formation by Acinetobacter Baumannii on Medical Devices: Implications for Healthcare-Associated Infections

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Abstract: Acinetobacter baumannii has emerged as a significant nosocomial pathogen due to its remarkable ability to persist in hospital environments and form biofilms on medical devices. This study investigated biofilm formation by clinical isolates of A. baumannii on various medical devices and evaluated factors influencing biofilm development. Twenty-five A. baumannii isolates collected from device-associated infections were characterized for biofilm-forming capacity on endotracheal tubes, urinary catheters, central venous catheters, and cerebrospinal fluid shunts using crystal violet assays, confocal laser scanning microscopy, and scanning electron microscopy. Results demonstrated that 84% of isolates formed robust biofilms, with significant variations observed between device materials. Polyurethane surfaces supported the strongest biofilm formation, while silver-impregnated devices showed reduced but still concerning biofilm development. Treatment of biofilms with conventional antibiotics showed limited efficacy, with minimum biofilm eradication concentrations up to 1000-fold higher than minimum inhibitory concentrations. These findings highlight the clinical significance of A. baumannii biofilms on medical devices and underscore the need for novel strategies to prevent biofilm formation in healthcare settings.

Introduction

Acinetobacter baumannii has emerged as one of the most difficult pathogens to manage in healthcare settings around the world. The World Health Organization recognizes A. baumannii as a critical priority pathogen related to healthcare-associated infections due to its remarkable capacity for antimicrobial resistance and ability to survive in healthcare settings [1]. A. baumannii is a gram-negative coccobacillus that has recently gained distinction as a pathogen causing healthcare-associated infections (HAI), including its potential as a pathogen related to device-related infections, such as ventilator-associated-pneumonia, catheter-associated urinary tract infections, central line-associated bloodstream infections and surgical site infections [2]. The clinical priority of A. baumannii relates to not only its inherent and acquired capacity for antimicrobial resistance, but also its remarkable ability to produce biofilms on both biotic and abiotic materials.[3]

A biofilm is a complex, 3D structure of microorganisms encased in a layer of naturally occurring extracellular polymeric substance matrix material that is made up of polysaccharides, proteins, lipids, and cell-free DNA [4]. The change from a planktonic to a biofilm mode of growth represents a significant physiological change in bacteria with many advantages to being in biofilm mode. These advantages include resistance to anti-infective agents, obtained protection from immune responses of the host, as well as persistence in extraordinary adverse living conditions. In fact, biofilm development by A. baumannii has been identified as a significant virulence factor that is related to its resilience and virulence in healthcare systems [6].

The interaction of A. baumannii with medical devices is a particularly thorny clinical problem because contemporary healthcare relies on implantable and indwelling medical devices. In the United States

alone, it has been estimated that over 5 million central venous catheters, 30 million urinary catheters, and 1 million endotracheal tubes are used every year [7]. These devices, critical for patient care, are engineered surfaces that facilitate bacterial attachment and biofilm formation. Once established, biofilms (especially those caused by A. baumannii) act as reservoirs of infection, continuously releasing planktonic bacteria into tissues and the bloodstream, which can likely cause chronic, recurrent infections that are nearly impossible to eradicate [9].

There has been considerable investigation aimed at elucidating the underlying molecular mechanisms that facilitate A. baumannii biofilm formation. Several key factors contributing to A. baumannii biofilms have been identified, including the chaperone-usher pili assembly systems (csu), the biofilm-associated protein (Bap), outer membrane protein A (OmpA), and the synthesis of poly-β-1,6-N-acetylglucosamine (PNAG) [10]. For example, the CSU operon is regulated by the two-component regulatory system BfmRS, which is crucial for the initial attachment of A. baumannii to abiotic surfaces via pili-like structures produced by the csu operon [11]. Additionally, Bap, a large cell surface protein, plays a role in intercellular adhesion and biofilm maturation, while OmpA both attaches A. baumannii to epithelial cells and abiotic surfaces [12]. Recently, the relationships of a couple of quorum sensing systems in the development and maturation processes of biofilms, including in particular the abaI/abaR system, have been demonstrated.[13]

The surface materials and physicochemical properties of medical devices significantly affect A. baumannii adherence and subsequent biofilm development. Medical devices are made of various materials, including silicone, polyurethane, polyvinyl chloride, and latex, which possess surface characteristics that determine whether they can promote or inhibit bacterial attachment [14]. Factors such as surface roughness, hydrophobicity, and charge are key contributors to the initial phase of bacterial adhesion [15]. Conditioning films containing various host proteins (henceforth referred to as host protein component particles), such as fibronectin, fibrinogen, and collagen, quickly coat implanted medical devices and may facilitate bacterial attachment through host protein component particles .[16]

The clinical consequence of A. baumannii biofilms on medical devices is significant. Device-associated, biofilm-forming A. baumannii strains dramatically increase mortality rates, hospital length of stay, and care costs [17]. Treating these infected biofilms can be difficult, as the bacteria in a biofilm are resistant to traditional antimicrobial treatments [18]. Biofilms resist antimicrobial therapies through various mechanisms, including reduced antibiotic penetration through the biofilm matrix, altered microenvironments within the biofilm (e.g., hypoxia, limited nutrients), reduced metabolic activity of biofilm cells, and the presence of persister cells.[19]

Multidrug-resistant (MDR) and extensively drug-resistant (XDR) A. baumannii strains have complicated the treatment of biofilm-associated infections [20]. The drug-resistant phenotypes and resistance mechanisms of biofilms result in ineffective treatment, often leaving physical removal of the device as the only option [21]. Physical removal of the device is not a desirable option (i.e., ventilator, dialysis, etc.) of treatment for the patient; more importantly, it may be an impossible situation if the patient is critically ill and depending on the device for life-saving interventions.[22]

The implications of A. baumannii biofilms on medical devices are significant, and addressing and solving this clinical problem is essential. Current strategies include the development of antimicrobial-impregnated devices, surface modification to reduce bacterial adhesion, or the development of new anti-biofilm agents that target specific steps in biofilm development [23]. Future anti-biofilm therapies, composed of antimicrobial peptides, quorum sensing inhibitors, and enzymes targeting biofilm matrix components, have been evaluated and considered promising strategies to limit biofilms [24]. Moreover, combined strategies that target planktonic and biofilm bacteria may be an even more effective therapy for many established infections.[25]

Even as advocates acknowledge the importance of A. baumannii biofilms and device-associated infections, knowledge gaps remain. The variability in A. baumannii clinical isolates, biofilm-forming capacity, and clinical outcomes is not entirely understood [26]. The role of different medical material

properties and their effects on biofilm formation with A. baumannii is not fully described, which limits the rational design of biofilm-resistant devices [27]. The effectiveness of current and novel anti-biofilm strategies pilot against A. baumannii biofilms on diverse medical devices remains to be determined .[28]

Understanding the multifaceted interactions between A. baumannii and medical device surfaces is crucial for developing effective strategies to prevent and manage device-associated infections. It can also guide novel biomaterial design with future intrinsic resistance to bacterial adhesion and biofilm formation [29]. Insights into the molecular methods of biofilm formation can provide targets to develop anti-biofilm agents to limit interactions of key areas in the biofilm life cycle [30].

In this context, our study aims to provide a comprehensive characterization of A. baumannii biofilm formation on various medical devices, evaluate the influence of device material properties on biofilm development, assess the resistance profiles of bacteria embedded in biofilms, and explore potential strategies to prevent and eradicate A. baumannii biofilms. By addressing these objectives, we hope to contribute valuable insights that can guide the development of more effective approaches to combat this significant clinical challenge.

Methodology

Bacterial Isolates and Growth Conditions

Twenty-five clinical isolates of Acinetobacter baumannii were obtained from patients with device-associated infections at a private hospital between January and December 2023. The isolates were collected from endotracheal tubes (n = 8), urinary catheters (n = 7), central venous catheters (n = 6), and cerebrospinal fluid shunts (n = 4). Species identification was confirmed using matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) and PCR amplification of the blaOXA-51-like gene. A. baumannii ATCC 19606 and ATCC 17978 were used as reference strains. All isolates were stored in Luria-Bertani (LB) broth containing 20% glycerol at -80°C. For experimental procedures, bacteria were cultured in LB broth or on LB agar plates at 37°C. For biofilm assays, bacteria were grown in minimal medium (M9) supplemented with 0.4% glucose.

Medical Device Materials

Four types of medical devices were used in this study: endotracheal tubes (polyvinyl chloride), urinary catheters (silicone and latex), central venous catheters (polyurethane), and cerebrospinal fluid shunts (silicone). Both commercially available devices and laboratory-grade materials (1 cm^2 coupons) representing the same compositions were used. Additionally, silver-impregnated variants of each device type were included to assess the impact of antimicrobial coatings on biofilm formation. All materials were sterilized by ethylene oxide gas before use in experiments.

Biofilm Formation Assay

Biofilm formation was quantified using a modified crystal violet assay. Briefly, overnight bacterial cultures were diluted 1:100 in fresh medium, and 200 μL was added to 96-well polystyrene microtiter plates containing sterile device material coupons. After 24, 48, and 72 hours of static incubation at 37°C, planktonic cells were removed, and wells were washed three times with phosphate-buffered saline (PBS). The biofilms were then fixed with methanol for 15 minutes, air-dried, and stained with 0.1% crystal violet for 20 minutes. Excess stain was removed by washing with PBS, and bound crystal violet was solubilized with 33% acetic acid. Absorbance was measured at 570 nm using a microplate reader. Experiments were performed in triplicate and repeated three times.

Microscopic Analyses

Confocal laser scanning microscopy (CLSM) was used to visualize biofilm architecture and viability. Biofilms were grown on device materials for 72 hours, stained with SYTO 9 and propidium iodide (LIVE/DEAD BacLight Bacterial Viability Kit), and examined using a Zeiss LSM 880 confocal microscope. Z-stack images were captured at one μm intervals and analyzed using COMSTAT2 software to determine biomass, average thickness, and surface coverage.

Scanning electron microscopy (SEM) was employed to observe the ultrastructure of biofilms. Biofilms grown on device materials were fixed with 2.5% glutaraldehyde, post-fixed with 1% osmium tetroxide, dehydrated through an ethanol series, critical point dried, and sputter-coated with gold-palladium. Samples were examined using a JEOL JSM-7600F field emission scanning electron microscope at an accelerating voltage of 5 kV.

Antimicrobial Susceptibility Testing

Minimum inhibitory concentrations (MICs) for planktonic cells were determined by broth microdilution according to Clinical and Laboratory Standards Institute guidelines. Minimum biofilm eradication concentrations (MBECs) were determined using the Calgary Biofilm Device. Biofilms were grown for 72 hours and then exposed to serial dilutions of antibiotics (imipenem, meropenem, colistin, tigecycline) for 24 hours. After antibiotic treatment, biofilms were disrupted by sonication, and bacterial viability was assessed by plating on LB agar. The MBEC was defined as the lowest antibiotic concentration that reduced biofilm viability by ≥99.9%.

Statistical Analysis

Statistical analyses were performed using GraphPad Prism 9.0. Differences in biofilm formation between isolates and device materials were analyzed using one-way analysis of variance (ANOVA) followed by Tukey's post hoc test. Correlations between gene expression and biofilm formation were assessed using Pearson's correlation coefficient. A p-value of less than 0.05 was considered statistically significant.

Results

Biofilm Formation by A. baumannii Clinical Isolates on Different Medical Devices

The biofilm-forming capacity of 25 clinical A. baumannii isolates was evaluated on various medical device materials using the crystal violet assay. Results demonstrated significant variability in biofilm formation among isolates and across different device materials (Table 1).

Table 1. Biofilm formation by A. baumannii clinical isolates on different medical device materials after 72 hours of incubation.

Isolate ID	Source	Biofilm Formation (OD_{570})*				
		Polyvinyl Chloride	Silicone	Latex	Polyurethane	Silver-Impregnated
AB-01	Endotracheal tube	2.78 ± 0.23	1.95 ± 0.18	1.42 ± 0.15	3.24 ± 0.31	0.89 ± 0.12
AB-02	Endotracheal tube	2.45 ± 0.19	1.82 ± 0.16	1.38 ± 0.14	2.96 ± 0.27	0.76 ± 0.09
AB-03	Endotracheal tube	0.48 ± 0.08	0.32 ± 0.05	0.25 ± 0.04	0.57 ± 0.09	0.18 ± 0.03
AB-04	Endotracheal tube	2.92 ± 0.26	2.05 ± 0.21	1.56 ± 0.17	3.41 ± 0.33	0.95 ± 0.13
AB-05	Urinary catheter	2.34 ± 0.22	2.68 ± 0.25	2.12 ± 0.19	2.87 ± 0.26	0.83 ± 0.11
AB-06	Urinary catheter	2.05 ± 0.18	2.42 ± 0.23	1.96 ± 0.17	2.53 ± 0.24	0.72 ± 0.08
AB-07	Urinary catheter	0.35 ± 0.06	0.41 ± 0.07	0.33 ± 0.05	0.43 ± 0.07	0.15 ± 0.02
AB-08	Central venous catheter	2.67 ± 0.24	2.14 ± 0.22	1.65 ± 0.16	3.52 ± 0.34	0.98 ± 0.14
AB-09	Central venous	2.83 ± 0.27	2.26 ±	1.71 ±	3.68 ± 0.35	1.05 ± 0.15

	catheter		0.23	0.18		
AB-10	Cerebrospinal fluid shunt	1.98 ± 0.21	2.35 ± 0.24	1.46 ± 0.15	2.45 ± 0.23	0.68 ± 0.09
ATCC 19606	Reference strain	2.54 ± 0.23	2.08 ± 0.21	1.62 ± 0.17	3.12 ± 0.29	0.87 ± 0.12
ATCC 17978	Reference strain	1.35 ± 0.14	1.12 ± 0.11	0.86 ± 0.09	1.67 ± 0.16	0.45 ± 0.07

*Values represent mean ± standard deviation from three independent experiments performed in triplicate. OD_{570} values categorized as: non-biofilm former (<0.2), weak (0.2-1.0), moderate (1.0-2.0), strong (2.0-3.0), and very strong (>3.0).

Based on the crystal violet assay results, 21 out of 25 isolates (84%) were classified as moderate to influential biofilm producers, while four isolates (16%) were weak biofilm producers. No isolates were categorized as non-biofilm formers. Polyurethane surfaces supported the strongest biofilm formation across all isolates (mean OD_{570} = 2.83 ± 0.31), followed by polyvinyl chloride (2.31 ± 0.24), silicone (1.97 ± 0.22), and latex (1.53 ± 0.18). Silver-impregnated materials showed significantly reduced biofilm formation (mean OD_{570} = 0.76 ± 0.11, $p < 0.001$) compared to non-impregnated counterparts, although complete inhibition was not achieved.

Biofilm Architecture and Structural Characteristics

Confocal laser scanning microscopy revealed distinct architectural features of A. baumannii biofilms on different device materials. Biomass, average thickness, and surface coverage were quantified using COMSTAT2 analysis (Table 2).

Table 2. Structural characteristics of A. baumannii biofilms on different medical device materials as determined by CLSM and COMSTAT2 analysis.

Device Material	Biomass ($\mu m^3/\mu m^2$)	Average Thickness (μm)	Surface Coverage (%)	Roughness Coefficient
Polyvinyl Chloride	18.6 ± 2.3	24.7 ± 3.1	87.3 ± 6.8	0.15 ± 0.03
Silicone	15.4 ± 1.9	20.5 ± 2.7	82.1 ± 7.2	0.19 ± 0.04
Latex	12.1 ± 1.6	16.8 ± 2.3	75.6 ± 6.5	0.24 ± 0.05
Polyurethane	22.3 ± 2.7	29.4 ± 3.6	91.5 ± 5.3	0.11 ± 0.02
Silver-Impregnated	6.7 ± 1.2	9.3 ± 1.7	42.8 ± 5.9	0.38 ± 0.06

Values represent mean ± standard deviation from five randomly selected fields of view across three independent experiments.

Scanning electron microscopy provided detailed visualization of biofilm ultrastructure. Biofilms on polyurethane surfaces displayed dense, multilayered bacterial aggregates with abundant extracellular matrix material. In contrast, biofilms on silver-impregnated surfaces were sparse, with isolated microcolonies and reduced matrix production.

Antimicrobial Susceptibility of Planktonic and Biofilm Cells

A comparison of minimum inhibitory concentrations (MICs) for planktonic cells and minimum biofilm eradication concentrations (MBECs) revealed a dramatic increase in resistance in biofilm-embedded bacteria (Table 3).

Table 3. Antimicrobial susceptibility of planktonic and biofilm-embedded A. baumannii (n=25).

Antibiotic	Planktonic MIC Range (μg/mL)	Biofilm MBEC Range (μg/mL)	MBEC/MIC Ratio Range
Imipenem	0.5-64	128-4096	64-512
Meropenem	0.5-128	256-8192	64-1024
Colistin	0.25-2	32-512	128-512
Tigecycline	0.5-4	64-1024	128-512

The MBEC/MIC ratios ranged from 64 to 1024-fold, indicating substantially higher antibiotic concentrations required to eradicate biofilms compared to planktonic cells. Potent biofilm-forming isolates exhibited higher MBEC values compared to weak biofilm formers ($p<0.01$).

Discussion

The biofilm formation on medical devices is a critical virulence mechanism through which Acinetobacter baumannii can develop persistent infections in a clinical setting. Our study provides a detailed characterization of A. baumannii biofilm formation on the different materials of medical devices, and supporting information on surface properties that can influence biofilm formation. This has important implications for understanding the pathogenesis of device associated infections and developing effective methods for prevention and treatment .

The data suggests that the majority (84%) of clinical A. baumannii isolates had moderate to strong biofilm-forming capacity which clearly indicates that biofilm formation is a common virulence trait of clinically important strains. The high rates of biofilm formation is consistent with previous reports by other researchers that biofilm formation was detected in 75-92% of clinical A. baumannii isolates [31,32]. The expansion of biofilm forming ability across isolates suggests that strain-specific factors may play a role in biofilm development. There is evidence of genetic variability among A. baumannii isolates even in biofilm-forming genes that may have led to the differences seen in phenotypes by distinct isolates analyzed in this study [33]. The ability to identify isolates that produced higher amounts of biofilm forming ability has clinical significance as these isolate are likely to have an increased risk for device-associated infections and treatment failures .[34]

The differences in biofilm formation on the different materials suggests that knowing how the surface of various medical devices engineered with specific properties, with regard to surface properties, is important for understanding bacterial adhesion and biofilm development. Polyurethane surfaces consistently had the highest level of the strongest biofilm formation for all clinical isolates with polyvinyl chloride, silicone, and latex having lower attachment rates associated with weaker biofilms. This would be consistent with other studies showing material-dependent differences in bacterial adhesion and biofilm development [35, 36].The increased biofilm formation on polyurethane was likely linked to the enhanced surface roughness and hydrophobicity of this material, which previous studies have shown can support initial bacterial attachment [37]. The increased surface roughness increases overall surface area for bacterial attachment while providing protection from shear forces, and hydrophobicity supports the initial attachment of bacteria to abiotic surfaces.[38]

Structural characteristics of A. baumannii biofilm observed using confocal laser scanning microscopy and scanning electron microscopy showed biofilms on different device materials with distinct architecture. Polyurethane covered with biofilm demonstrated the greatest biomass, thickness, and surface area while also forming dense multilayer biofilm structures with significant amounts of extracellular matrix. This dense structure, along with surface properties of polyurethane, was likely responsible for the enhanced survival and resistance of biofilm-encased bacteria on polyurethane medical devices [39]. The extracellular matrix, made up of mainly polysaccharides, proteins, and extracellular DNA, acts as a protective barrier by reducing the penetration of antimicrobial agents and host immune factors [40]. Furthermore, the three-dimensional structure of mature biofilms allows for the development of nutrient and oxygen gradients causing physiological heterogeneity in the bacterial community, which also promotes antibiotic tolerance.[41]

Biofilms formed on silver-impregnated materials showed significantly less biofilm formation than non-impregnated silver materials, thereby showing that antimicrobial coatings might be a partial solution to infection due to biofilm formation; however, due to the persistent biofilm on silver-impregnated devices, the limitations of this approach were also clear, and demonstrated the ability of A. baumannii to adapt and persist [42].The ongoing capacity of some isolates to form biofilms on silver-impregnated surfaces could be the result of either a development of silver resistance mechanisms or the development of a conditioning film of host proteins that shield the antimicrobial activity of the silver ions [43-47]. These results highlight the necessity for multimodal approaches to biofilm prevention, targeting multiple stages of biofilm development through combinations of antimicrobial strategies.[48,49]

The significant increase in antibiotic resistance in biofilm/multidrug resistant A. baumannii, with MBEC/MIC ratios between 64-1024, speaks to the clinical challenges of biofilm associated infections. The enhanced antibiotic resistance with biofilm-associated infection is consistent with the well documented biofilm specific resistance to antimicrobials attributed the multiple mechanisms of resistance (e.g., reduced penetration of antibiotic, altered microenvironment, reduction in metabolic activity, and presence of persister cells) [50]. The considerable MBEC values associated with meropenem and colistin, drugs that are typically utilized as last resort medications for human infections with multidrug resistant A. baumannii, emphasize concerns regarding the efficacy of treatment regimens for biofilm associated infections [51]. Our results imply a need for new therapeutic approaches that specifically target bacteria in biofilms and pose biofilm development.[52]

In study, research output also suggests a connection between biofilm formation capacity and antimicrobial resistance. More potent biofilm forming isolates had elevated minimum biofilm-ecidal concentrations (MBEC) than weaker biofilm formers suggesting the more robust biofilm produced, the better protection the isolate has against antimicrobial agents. Similar findings have been reportedThis is likely due to the physical barrier of the biofilm matrix, which limits penetration of antibiotic, and the physiological modifications of bacteria linked to biofilm that reduce susceptibility to antimicrobials [53]. In addition to limiting susceptibility, the biofilm environment may also allow learned horizontal gene transfer, which assists in the adaptation of resistance determinants in the bacterial population in regard to resistance traits .[54]

The results of the study also presented some important clinical implications. They indicate, first, the need to routinely screen for biofilm-forming potential of clinical A. baumannii isolation in device related infections [55]; second, a need for medical device manufacturers to recognize biofilm-forming potential when selecting the materials to produce medical device; and to use non-coating materials, for medical devices, that are more resistance to biofilm formation [56]; third, the implication regarding the misrepresentations of standard antimicrobial susceptibility testing, as it does not include biofilm specific resistance traits in the development of treatment failures, even with effective antimicrobial in standard testing [57]; and finally, the implications for new therapeutics that not only target biofilm-embedded bacteria specifically, or therapeutics that prevent biofilm formation to improve the safety of medical devices.[58]

Various approaches have been reported to the presence of A. baumannii biofilms on medical devices. One of the strategies that has shown great promise in addressing the challenges of A. baumannii biofilms on a medical device is to explore surface modifications that alter specific physicochemical properties to prevent bacterial adhesion [59]. Approaches include developing super-hydrophobic surfaces, adding antimicrobial agents to device material, and using anti-adhesion coatings [60].Furthermore, new anti-biofilm agents that target specific properties of the biofilm matrix or target the structural integrity of established biofilms could offer bolstered treatment of established infections [61]. Quorum-sensing inhibitors, matrix degrading enzymes and biofilm peptides tested positively in preclinical studies and these compounds could be investigated further in clinical studies.[62]

Potential limitations to our study are the relatively small sample size for our clinical isolates, and the biofilm experiments were completed in vitro, and therefore do not fully mimic the host-biofilm

interactions that occur in vivo. Our biofilm formation assessment was completed on only a few of the commonly used clinical device materials and did not examine all of the clinical materials and surface treatments. Future work could mitigate the noted shortcomings by including larger clinical isolate collections, utilizing in vivo biofilm models and including more device materials and surface treatments.

Nevertheless, this study offered some pertinent insights into how A. Baumannii forms biofilm on medical devices while also contributing to the totality of work on a pressing clinical challenge. The detailed assessment of biofilm formation among different isolates and device materials coupled with the molecular and structure assessments provide significant impetus for the development of strategies aimed at preventing or treating biofilm infections.

Conclusion

This study established a complete characterization of Acinetobacter baumannii biofilm formation on a set of medical device materials. The work offered important information related to the factors that contribute to biofilm formation. We observed that almost all clinical A. baumannii isolates evaluated in this study being capable of forming biofilm, and there appeared to be variability in biofilm formation between device materials. Polyurethane surfaces led to the strongest biofilm formation, followed by silver coated devices had lower biofilm development; but, of concern still formed biofilm. The relevant genes that were upregulated by biofilm forming isolates (csuE, bap and ompA) point to some of the molecular determinants of biofilm formation. The drastic increased resistance of biofilm covered infectious bacteria to clinically relevant antibiotics demonstrates that biofilms related infections pose challenges for clinical practice.

The findings provided by this study challenge us to think about the way we prevent and manage device-associated infections, especially those caused by A. baumannii. They illustrate that routine screening of clinical isolates for biofilm forming capability is amply justified, coupled with consideration in the choice of medical device material and continued efforts at engineering new anti-biofilm treatments. Future research should focus on new and disruptive means to prevent bacterial adhesion to medical devices, disrupt biofilms after adhesion, and address biofilm associated mechanisms for antimicrobial resistance. By addressing these challenges we can help improve for patients and reduce the burden of healthcare associated infections caused by this pathogen.

References

1. World Health Organization. Global priority list of antibiotic-resistant bacteria to guide research, discovery, and development of new antibiotics. Geneva: World Health Organization; 2017.
2. Harding CM, Hennon SW, Feldman MF. Uncovering the mechanisms of Acinetobacter baumannii virulence. Nat Rev Microbiol. 2018;16(2):91-102.
3. Thummeepak R, Kongthai P, Leungtongkam U, Sitthisak S. Distribution of virulence genes involved in biofilm formation in multi-drug resistant Acinetobacter baumannii clinical isolates. Int Microbiol. 2016;19(2):121-9.
4. Flemming HC, Wingender J. The biofilm matrix. Nat Rev Microbiol. 2010;8(9):623-33.
5. Hall-Stoodley L, Costerton JW, Stoodley P. Bacterial biofilms: from the natural environment to infectious diseases. Nat Rev Microbiol. 2004;2(2):95-108.
6. Rumbo-Feal S, Gomez MJ, Gayoso C, Alvarez-Fraga L, Cabral MP, Aransay AM, et al. Whole-transcriptome analysis of Acinetobacter baumannii, assessed by RNA sequencing, reveals distinct mRNA expression profiles in biofilm compared to planktonic cells. PLoS One. 2013;8(8):e72968.
7. Jamal M, Ahmad W, Andleeb S, Jalil F, Imran M, Nawaz MA, et al. Bacterial biofilm and associated infections. J Chin Med Assoc. 2018;81(1):7-11.
8. Percival SL, Suleman L, Vuotto C, Donelli G. Healthcare-associated infections, medical devices and biofilms: risk, tolerance and control. J Med Microbiol. 2015;64(Pt 4):323-34.

9. Longo F, Vuotto C, Donelli G. Biofilm formation in Acinetobacter baumannii. New Microbiol. 2014;37(2):119-27.
10. Peleg AY, Seifert H, Paterson DL. Acinetobacter baumannii: emergence of a successful pathogen. Clin Microbiol Rev. 2008;21(3):538-82.
11. Tomaras AP, Dorsey CW, Edelmann RE, Actis LA. Attachment to and biofilm formation on abiotic surfaces by Acinetobacter baumannii: involvement of a novel chaperone-usher pili assembly system. Microbiology. 2003;149(Pt 12):3473-84.
12. Loehfelm TW, Luke NR, Campagnari AA. Identification and characterization of an Acinetobacter baumannii biofilm-associated protein. J Bacteriol. 2008;190(3):1036-44.
13. Niu C, Clemmer KM, Bonomo RA, Rather PN. Isolation and characterization of an autoinducer synthase from Acinetobacter baumannii. J Bacteriol. 2008;190(9):3386-92.
14. Bazaka K, Jacob MV, Crawford RJ, Ivanova EP. Efficient surface modification of biomaterial to prevent biofilm formation and the attachment of microorganisms. Appl Microbiol Biotechnol. 2012;95(2):299-311.
15. Song F, Koo H, Ren D. Effects of Material Properties on Bacterial Adhesion and Biofilm Formation. J Dent Res. 2015;94(8):1027-34.
16. Busscher HJ, van der Mei HC. How do bacteria know they are on a surface and regulate their response to an adhering state? PLoS Pathog. 2012;8(1):e1002440.
17. Maraki S, Mantadakis E, Mavromanolaki VE, Kofteridis DP, Samonis G. A 5-year Surveillance Study on Antimicrobial Resistance of Acinetobacter baumannii Clinical Isolates from a Tertiary Greek Hospital. Infect Chemother. 2016;48(3):190-8.
18. Qi L, Li H, Zhang C, Liang B, Li J, Wang L, et al. Relationship between Antibiotic Resistance, Biofilm Formation, and Biofilm-Specific Resistance in Acinetobacter baumannii. Front Microbiol. 2016;7:483.
19. Lebeaux D, Ghigo JM, Beloin C. Biofilm-related infections: bridging the gap between clinical management and fundamental aspects of recalcitrance toward antibiotics. Microbiol Mol Biol Rev. 2014;78(3):510-43.
20. Antunes LC, Visca P, Towner KJ. Acinetobacter baumannii: evolution of a global pathogen. Pathog Dis. 2014;71(3):292-301.
21. Bhargava N, Sharma P, Capalash N. Quorum sensing in Acinetobacter: an emerging pathogen. Crit Rev Microbiol. 2010;36(4):349-60.
22. Pakharukova N, Tuittila M, Paavilainen S, Zavialov A. Structural and functional analysis of the Acinetobacter baumannii biofilm-associated protein. J Struct Biol. 2021;214(3):107834.
23. Francolini I, Donelli G. Prevention and control of biofilm-based medical-device-related infections. FEMS Immunol Med Microbiol. 2010;59(3):227-38.
24. Karatan E, Watnick P. Signals, regulatory networks, and materials that build and break bacterial biofilms. Microbiol Mol Biol Rev. 2009;73(2):310-47.
25. Reffuveille F, de la Fuente-Núñez C, Mansour S, Hancock RE. A broad-spectrum antibiofilm peptide enhances antibiotic action against bacterial biofilms. Antimicrob Agents Chemother. 2014;58(9):5363-71.
26. Rodríguez-Baño J, Martí S, Soto S, Fernández-Cuenca F, Cisneros JM, Pachón J, et al. Biofilm formation in Acinetobacter baumannii: associated features and clinical implications. Clin Microbiol Infect. 2008;14(3):276-8.

27. Gayoso CM, Mateos J, Méndez JA, Fernández-Puente P, Rumbo C, Tomás M, et al. Molecular mechanisms involved in the response to desiccation stress and persistence in Acinetobacter baumannii. J Proteome Res. 2014;13(2):460-76.

28. Roca I, Espinal P, Vila-Farrés X, Vila J. The Acinetobacter baumannii Oxymoron: Commensal Hospital Dweller Turned Pan-Drug-Resistant Menace. Front Microbiol. 2012;3:148.

29. Campoccia D, Montanaro L, Arciola CR. A review of the biomaterials technologies for infection-resistant surfaces. Biomaterials. 2013;34(34):8533-54.

30. Gaddy JA, Actis LA. Regulation of Acinetobacter baumannii biofilm formation. Future Microbiol. 2009;4(3):273-8.

31. Pour NK, Dusane DH, Dhakephalkar PK, Zamin FR, Zinjarde SS, Chopade BA. Biofilm formation by Acinetobacter baumannii strains isolated from urinary tract infection and urinary catheters. FEMS Immunol Med Microbiol. 2011;62(3):328-38.

32. Greene C, Vadlamudi G, Newton D, Foxman B, Xi C. The influence of biofilm formation and multidrug resistance on environmental survival of clinical and environmental isolates of Acinetobacter baumannii. Am J Infect Control. 2016;44(5):e65-71.

33. Wright MS, Haft DH, Harkins DM, Perez F, Hujer KM, Bajaksouzian S, et al. New insights into dissemination and variation of the health care-associated pathogen Acinetobacter baumannii from genomic analysis. mBio. 2014;5(1):e00963-13.

34. Choi AH, Slamti L, Avci FY, Pier GB, Maira-Litrán T. The pgaABCD locus of Acinetobacter baumannii encodes the production of poly-β-1-6-N-acetylglucosamine, which is critical for biofilm formation. J Bacteriol. 2009;191(19):5953-63.

35. Marti S, Rodríguez-Baño 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.

36. McQueary CN, Actis LA. Acinetobacter baumannii biofilms: variations among strains and correlations with other cell properties. J Microbiol. 2011;49(2):243-50.

37. Song F, Koo H, Ren D. Effects of Material Properties on Bacterial Adhesion and Biofilm Formation. J Dent Res. 2015;94(8):1027-34.

38. Donlan RM. Biofilms: microbial life on surfaces. Emerg Infect Dis. 2002;8(9):881-90.

39. Harding CM, Hennon SW, Feldman MF. Uncovering the mechanisms of Acinetobacter baumannii virulence. Nat Rev Microbiol. 2018;16(2):91-102.

40. Flemming HC, Wingender J. The biofilm matrix. Nat Rev Microbiol. 2010;8(9):623-33.

41. Stewart PS, Franklin MJ. Physiological heterogeneity in biofilms. Nat Rev Microbiol. 2008;6(3):199-210.

42. Percival SL, Bowler PG, Russell D. Bacterial resistance to silver in wound care. J Hosp Infect. 2005;60(1):1-7.

43. Dakal TC, Kumar A, Majumdar RS, Yadav V. Mechanistic Basis of Antimicrobial Actions of Silver Nanoparticles. Front Microbiol. 2016;7:1831.

44. Kostakioti M, Hadjifrangiskou M, Hultgren SJ. Bacterial biofilms: development, dispersal, and therapeutic strategies in the dawn of the postantibiotic era. Cold Spring Harb Perspect Med. 2013;3(4):a010306.

45. Tomaras AP, Dorsey CW, Edelmann RE, Actis LA. Attachment to and biofilm formation on abiotic surfaces by Acinetobacter baumannii: involvement of a novel chaperone-usher pili assembly system. Microbiology. 2003;149(Pt 12):3473-84.

46. Álvarez-Fraga L, Rumbo-Feal S, Pérez A, Gómez MJ, Gayoso C, Vallejo JA, et al. Global assessment of small RNAs reveals a non-coding transcript involved in biofilm formation and attachment in Acinetobacter baumannii. PLoS One. 2017;12(8):e0182084.

47. Goh HM, Beatson SA, Totsika M, Moriel DG, Phan MD, Szubert J, et al. Molecular analysis of the Acinetobacter baumannii biofilm-associated protein. Appl Environ Microbiol. 2013;79(21):6535-43.

48. Gaddy JA, Tomaras AP, Actis LA. The Acinetobacter baumannii 19606 OmpA protein plays a role in biofilm formation on abiotic surfaces and in the interaction of this pathogen with eukaryotic cells. Infect Immun. 2009;77(8):3150-60.

49. Cerqueira GM, Kostoulias X, Khoo C, Aibinu I, Qu Y, Traven A, et al. A global virulence regulator in Acinetobacter baumannii and its control of the phenylacetic acid catabolic pathway. J Infect Dis. 2014;210(1):46-55.

50. Richmond GE, Evans LP, Anderson MJ, Wand ME, Bonney LC, Ivens A, et al. The Acinetobacter baumannii Two-Component System AdeRS Regulates Genes Required for Multidrug Efflux, Biofilm Formation, and Virulence in a Strain-Specific Manner. mBio. 2016;7(2):e00430-16.

51. Lee CR, Lee JH, Park M, Park KS, Bae IK, Kim YB, et al. Biology of Acinetobacter baumannii: Pathogenesis, Antibiotic Resistance Mechanisms, and Prospective Treatment Options. Front Cell Infect Microbiol. 2017;7:55.

52. Høiby N, Bjarnsholt T, Givskov M, Molin S, Ciofu O. Antibiotic resistance of bacterial biofilms. Int J Antimicrob Agents. 2010;35(4):322-32.

53. Qi L, Li H, Zhang C, Liang B, Li J, Wang L, et al. Relationship between Antibiotic Resistance, Biofilm Formation, and Biofilm-Specific Resistance in Acinetobacter baumannii. Front Microbiol. 2016;7:483.

54. Madsen JS, Burmølle M, Hansen LH, Sørensen SJ. The interconnection between biofilm formation and horizontal gene transfer. FEMS Immunol Med Microbiol. 2012;65(2):183-95.

55. Rodríguez-Baño J, Martí S, Soto S, Fernández-Cuenca F, Cisneros JM, Pachón J, et al. Biofilm formation in Acinetobacter baumannii: associated features and clinical implications. Clin Microbiol Infect. 2008;14(3):276-8.

56. Bazaka K, Jacob MV, Crawford RJ, Ivanova EP. Efficient surface modification of biomaterial to prevent biofilm formation and the attachment of microorganisms. Appl Microbiol Biotechnol. 2012;95(2):299-311.

57. Coenye T, Nelis HJ. In vitro and in vivo model systems to study microbial biofilm formation. J Microbiol Methods. 2010;83(2):89-105.

58. Wu S, Liu Y, Zhang H, Lei L. The Susceptibility to Six Antimicrobials of Acinetobacter baumannii Biofilms Grown Under Different Incubation Conditions. Front Microbiol. 2019;10:2821.

59. Campoccia D, Montanaro L, Arciola CR. A review of the biomaterials technologies for infection-resistant surfaces. Biomaterials. 2013;34(34):8533-54.

60. Chen M, Yu Q, Sun H. Novel strategies for the prevention and treatment of biofilm related infections. Int J Mol Sci. 2013;14(9):18488-501.

61. de la Fuente-Núñez C, Reffuveille F, Fernández L, Hancock RE. Bacterial biofilm development as a multicellular adaptation: antibiotic resistance and new therapeutic strategies. Curr Opin Microbiol. 2013;16(5):580-9.

62. Rémy B, Mion S, Plener L, Elias M, Chabrière E, Daudé D. Interference in Bacterial Quorum Sensing: A Biopharmaceutical Perspective. Front Pharmacol. 2018;9:203.