Biocatalysis and Agricultural Biotechnology 18 (2019) 101007

Contents lists available at ScienceDirect

Biocatalysis and Agricultural Biotechnology

journal homepage: www.elsevier.com/locate/bab

Evaluation of biofilm formation, cell surface hydrophobicity and gelatinase activity in *Acinetobacter baumannii* strains isolated from patients of diabetic and non-diabetic foot ulcer infections

Diwan Mahmood Khan[a,b,c], Muhammed A.P. Manzoor[c,1], I. Venkatakrishna Rao[b], M.S. Moosabba[a,*]

[a] Department of General Surgery, Yenepoya Medical College, Yenepoya (Deemed to be University), Mangalore, Karnataka, 575018, India
[b] Department Microbiology, Yenepoya Medical College, Yenepoya (Deemed to be University), Mangalore, Karnataka, 575018, India
[c] Yenepoya Research Centre, Yenepoya (Deemed to be University), Mangalore, Karnataka, 575018, India

ARTICLE INFO

Keywords:
Diabetic foot infections
Acinetobacter baumanni
Biofilm
Cell surface hydrophobicity
Gelatinase production
Virulence factors

ABSTRACT

Diabetic foot ulcer infections (DFIs) are one of the most complicated diseases worldwide. DFIs are a significant public health concern and may lead to high rates of amputations. The complications of DFIs depend on clinical characteristics of the patients, drug susceptibility and virulence factors of bacteria. *Acinetobacter baumannii* is one of the virulent pathogens which causes DFIs. The present study was conducted to compare the virulence factors of *A. baumannii* strains isolated from diabetic and non-diabetic foot ulcer infections (non-DFIs). The virulence factors such as cell surface hydrophobicity (CSH), gelatinase activity and biofilm formation were tested. Confocal laser scanning microscopy (CLSM) was adopted to monitor the biofilm architecture. Out of 480 cases tested, 70 patients were infected with *A. baumannii*. Biofilm formation was detected in 31.43% of the isolates. We found a significant difference in the biofilm formation and CSH in isolates from the patients with DFIs and non-DFIs ($P < 0.05$). The study reveals that various virulence factors can cause *A. baumannii* induced DFIs, suggesting that these bacteria could be a potential biomarker for the frequent and endangering infections in patients with type 2 diabetes mellitus.

1. Introduction

Diabetes mellitus is a non-communicable disease worldwide and has an estimated prevalence of 7.7% cases by 2030 (Whiting et al., 2011). It is expected that almost 25% cases of type 2 diabetes mellitus patients experience diabetic foot ulcer infections (DFIs) during their lifetime (Sargen et al., 2013; Al-Rubeaan et al., 2015). Patients with diabetic foot ulcers have more than 10 fold risk of infection compared to non-DFIs (Lavery et al., 2006; Amin and Doupis, 2016). *Acinetobacter baumannii* is a rare colonizer in human skin and one of the most common causative agents of DFIs (El-Din et al., 2014). The prevalence of *A. baumannii* infection in DFIs varies from 11.1% to 33% (Col et al., 2004; McIntosh and Earnshaw, 2009; Bali et al., 2013). *A. baumannii* is one of the important human pathogens and a significant cause of hospital-acquired infection especially in immune-compromised patients (Almasaudi, 2018). The over usage of antibiotics in health care sectors resulted in emergence of the multidrug-resistant bacterial strains (Chandra et al., 2017); especially prevalence of drug resistant gene producing *A. baumannii* (El-Din et al., 2014).

The recent reports highlighted that patients with DFIs have a high risk of *A. baumannii* infection compared to non-DFIs (Karmaker et al., 2016; Khan et al., 2018a). The factors that helps in the survival of

* Corresponding author.
E-mail address: drmssonkal1@gmail.com (M.S. Moosabba).
[1] Current Address: ICAR–Indian Institute of Spices Research, Kozhikode, 673012, Kerala, India.

https://doi.org/10.1016/j.bcab.2019.01.045
Received 11 December 2018; Received in revised form 10 January 2019; Accepted 17 January 2019
Available online 28 January 2019
1878-8181/

Table 1
Correlation of cell surface hydrophobicity in *Acinetobacter baumannii strains* among DFIs and non-DFIs isolates.

Cell Surface Hydrophobicity	DFI (n = 35)	Non-DFI (n = 35)	
Hydrophobic			
> 70% Strong	32/35(91.43%)	07/35(20%)	*P*- value
> 30–70% Moderate	2/35(5.71%)	20/35(57%)	$\chi^2 = 36.19$,
Hydrophilic < 30%	1/35 (2.86%)	08/35(23%)	df = 2,
			$p < 0.00001$

Chi-square (χ^2) test: *P*-value = < 0.05, df = degree of freedom; DFI:Diabetic foot ulcer infections; Non-DFI:Non-diabetic foot ulcer infections.

Table 2
Comparison of biofilm formation, cell surface hydrophobicity and gelatinase production among DFI and non-DFIs isolates.

DFIs (n = 35)	Weak	Moderate	Strong
Biofilm Formation	05/35 (14.29%)	19/35 (54.28%)	11/35 (31.43%)
CSH	1/35 (2.86%)	2/35 (5.71%)	32/35 (91.43%)
Gelatinase Production	0	0	12 (34.29%)
Non-DFIs (n = 35)	**Weak**	**Moderate**	**Strong**
Biofilm Formation	10/35 (28.57%)	22/35 (62.86%)	03/35 (8.57%)
CSH	08/35(23%)	20/35 (57%)	07/35 (20%)
Gelatinase Production	0	1/35 (2.86%)	4/35 (11.43%)

CSH:cell surface hydrophobicity; DFI:Diabetic foot ulcer infections; Non-DFI: non-diabetic foot ulcer infections.

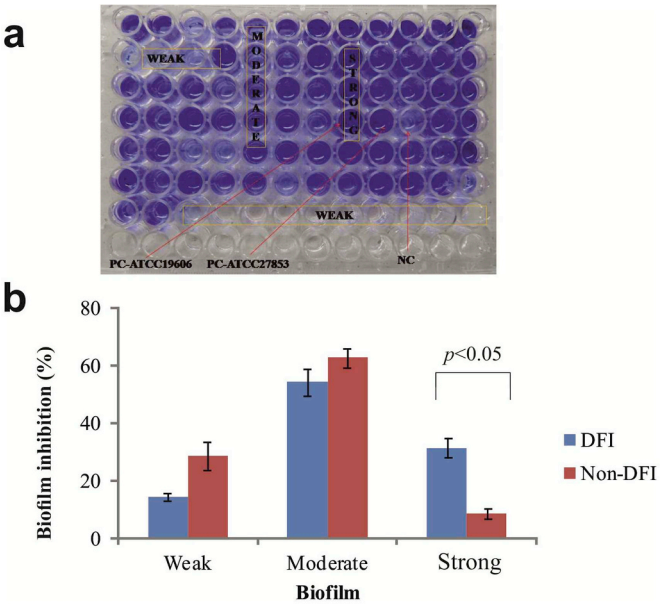

Fig 1. (a & b) Percentage of biofilm formation in clinical isolates of *Acinetobacter baumannii* strains isolated from diabetic and non-diabetic foot infections.

A. baumannii strains include biofilm formation, cell surface hydrophobicity, pellicle formation, serum bactericidal activity, siderophore and gelatinase production (Beceiro et al., 2013; Murali et al., 2014, Khan et al., 2018b). Among the various virulence factors, biofilm formation is the main cause of many chronic infections especially in DFIs (Banu et al., 2015), urinary tract infections and kidney stones (Manzoor et al., 2017, 2018a, 2018b, 2018c, 2019).

The biofilms may contribute to the ineffective penetration of antibiotics into the DFIs due to developing antiphagocytic properties within the matrix of biofilm which can facilitate the intercellular communication, altered gene expression and horizontal gene transfer in bacteria (Murali et al., 2014; Beceiro, et al., 2013). Gelatinase is a protease that is capable of hydrolyzing gelatin which may lead to necrotisis of tissue (Cevahir et al., 2008; Khan et al., 2018b). On the other hand, cell surface hydrophobicity (CSH) facilitates the bacterial adhesion of the host individuals (Costa et al., 2006). It has been hypothesized that patients with DFIs may have a high degree of variation in virulence factors which may contribute to non-healing ulcers and amputation. This study was conducted in order to understand the level of virulence factors (biofilm formation, CSH, and gelatinase production) among *A. baumannii* strains isolated from the patients with DFIs and non-DFIs.

2. Material and methods

A prospective study was conducted in the Department of General Surgery at a tertiary care hospital in Mangalore, India. The study was approved by the institutional Ethical Committee (Reg. No.-YU2016/172) of Yenepoya (Deemed to be University) and written informed consent was obtained from each patient. Among the 480-foot ulcer patients screened, 280 patients had DFIs and 200 patients were non-DFI. We selected 35 isolates from each group according to Meggit's Wagner grading (0 to I graded and age < 20 years patients excluded) (Oyibo et al., 2001), International world group of diabetic foot (IWGDF) grading (0–1 grade eliminated) or PEDIS grading (Lipsky et al., 2012) and inhabited with *A. baumannii* only.

2.1. Sample collection and bacterial identification

Pus and tissue samples were collected from the patients and processed as per CLSI guidelines (CLSI, 2016). The samples were then inoculated on Blood agar and MacConkey agar, and the plates were incubated at 37 °C overnight and the growth was monitored. Each isolates were identified by Gram staining followed by standard microbiological procedures. All of the strains were further identified by BD Phoenix 100 systems (Becton Dickinson, USA) (Prashanth and Badrinath, 2000; Shabeena et al., 2018; Manzoor et al., 2018d).

2.2. Biofilm formation

Biofilm formation was quantified using the method reported earlier with slight modification (Christensen et al., 1995; De Breij et al., 2009; Dheepa et al., 2011; Babapour et al., 2016; Khan et al., 2018c). Briefly, overnight cultures of *A. baumannii* were diluted to 1:20 with fresh trypticase soya broth (TSB). 200 µL of these mixtures was transferred to the 96 well microtiter plates (Hi-media, India). The plates were incubated overnight at 37 °C and wells with only TSB broth served as control. After incubation, the supernatant was discarded and washed with phosphate buffered saline (pH: 7.2). The plates were further dried and 200 µL of absolute ethanol was added to each well and kept for 15 min incubation at room temperature. After incubation, ethanol was discarded and plates were dried. 0.1% (w/v) of hucker's crystal violet solution was added to each well and incubated at room temperature for

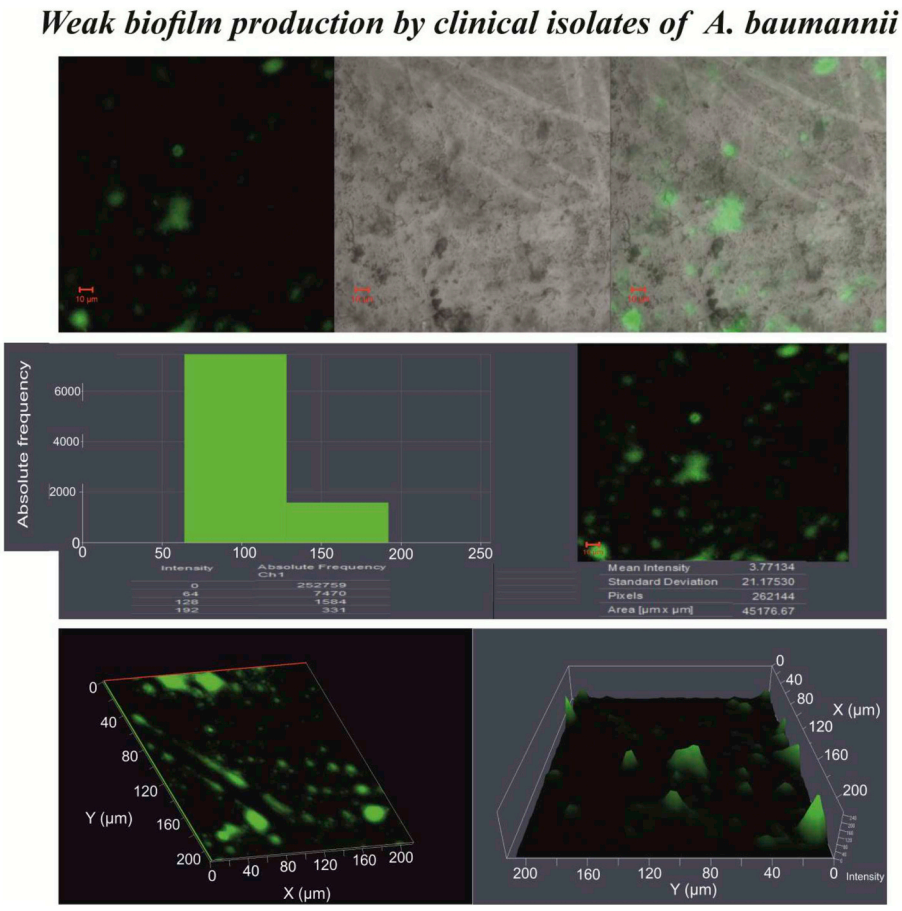

Fig. 2. CLSM showing weak biofilm formation in clinical isolates of *Acinetobacter baumannii* strains isolated from diabetic foot infection.

15 min. Subsequently, the plates were washed with PBS thrice and dried. Stain was reolubilize in 160 µL of 33% glacial acetic acid. Absorbance at 630 nm was measured after the addition of glacial acetic acid (initial) and after decanting (final) using a multimode microplate reader (FluoSTAR omega, BMG Labtech, Germany).

The qualitative analysis of the biofilm architecture was performed using Confocal laser scanning microscopy (CLSM) as described earlier (LewisOscar et al., 2015). Briefly, *A. baumannii* biofilms were allowed to grow on glass pieces (1 × 1 cm), placed in 6-well polystyrene plates (Greiner Bio-One), and incubated at 37 °C for 24 h. Following the incubation; the biofilms was observed under CLSM (Carl Zeiss, Germany).

2.3. Cell surface hydrophobicity

The CSH was quantified as described by Costa et al. (2006) with certain modification. Briefly, overnight cultures of *A. baumannii* in nutrient broth was washed with phosphate buffer (pH 7.4) and re-suspended at a turbidity of 0.4 (OD_{660} nm). Aliquots of 2.5 mL of cultures were mixed with 1 mL of xylene and vortexed thoroughly. The tubes were incubated for 20 min at room temperature to allow for the separation of mixtures into two phases. The aqueous phase was collected in a separate tube, and the turbidity was recorded at 660 nm. The hydrophobicity index (HI) was calculated using the following equation

$$HI = \left(\frac{A_{660}\ \text{control} - A_{660}\ \text{test}}{A_{660}\ \text{control}} \right) \times 100$$

Where,A_{660} control = optical density of the strains before xylene treatment.A_{660} test = optical density of the strains after xylene treatment.

The strains were considered as strongly hydrophobic when the hydrophobicity index was > 70% and hydrophilic when the hydrophobicity index was < 30%.

2.4. Gelatinase activity

The gelatinase activity of the isolates was calculated as described earlier (Cevahir et al., 2008; Khan et al., 2018b). Briefly, the isolates were cultured in Brain heart infusion broth and incubated at 37 °C for 18 h. After incubation, a loopful of young culture was inoculated onto Luria Bertani agar containing 3% gelatin (30 g/L) and incubated overnight at 37 °C. The plates were then cooled at 4°C for 5 h. The appearance of turbid halo (opacity around growth) considered as positive for gelatinase production. *Pseudomonas aeruginosa* (PAO1) and *A. baumannii* ATCC 19606 were used as positive and negative control respectively.

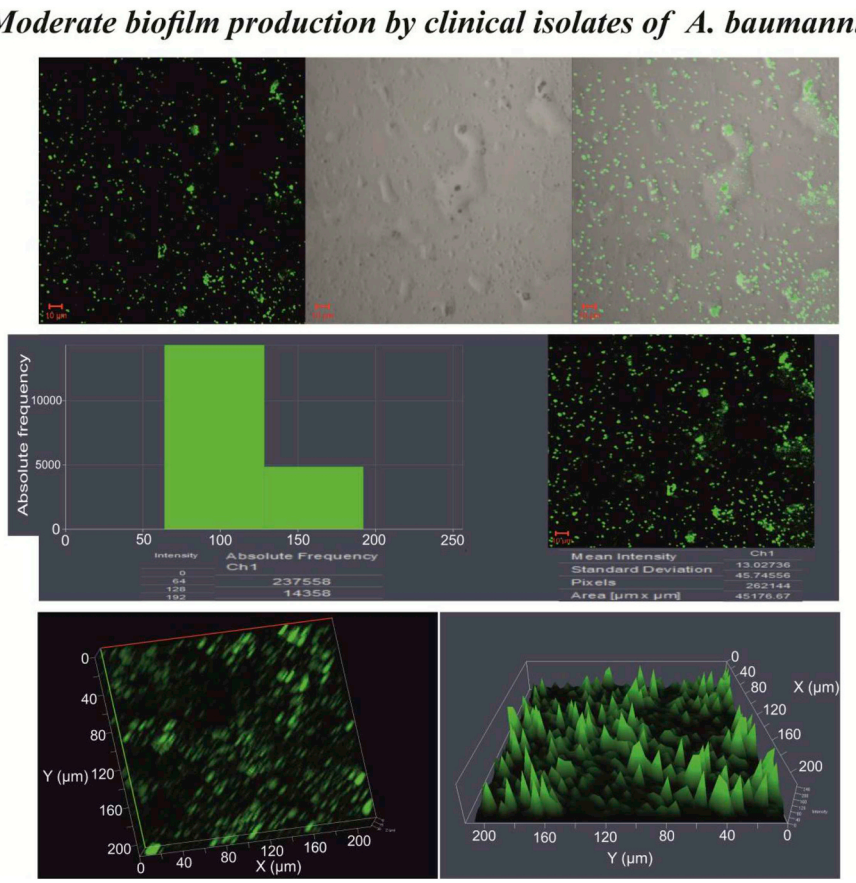

Fig. 3. CLSM showing moderate biofilm formation in clinical isolates of *Acinetobacter baumannii* strains isolated from diabetic foot infection.

2.5. Statistical analysis

Statistical analysis was performed using SPSS software version 23.0 (SPSS Inc., Chicago, IL). Data was expressed in frequency and percentage. Chi-Square test was used for comparing the virulence factors among DFIs and non-DFIs. $p < 0.05$ was considered as statistically significant.

3. Result and discussion

A total of 70 (14.58%) isolates of *A. baumannii* were obtained from 480-foot ulcer patients. There were 57 (81.4%) males and females 13 (18.6%) in the study, DFIs patients had comorbidities/associated diseases with hypertension, retinopathy, ischemic heart disease, and nephropathy. DFIs patients are immunodeficient during infection of *A. baumannii* which increases virulence factors such as biofilm formation which acts as an anti-phagocytosis barrier that prevents penetration of antibiotics (He et al., 2015; Qi et al., 2016). Host immune system does not respond after prolonged hospital stays Hence, *A. baumannii* produces extended spectrum β-lactamase, metallo-β-lactamase, and becomes multidrug-resistant compared to non-DFIs.

Based on the OD reading the isolates were classified as strong [OD > 0.350], moderate (OD between 0.200 - 0.350) or weak (OD 0.041 - 0.200) biofilm producers. Among the isolates from DFIs, 31.43% showed positive biofilm formation, 91.43% showed positive CSH and 34.29% showed positive for gelatinase activity. While 8.6%, 20%, and 14.3% from non-DFIs showed positive biofilm formation, cell surface hydrophobicity and gelatinase activity respectively (Tables 1 and 2). Murali et al. (2014) reported that biofilm formation was found in 39.13% in patients with DFIs which were in accordance with our reports. Another study reported by Zubair et al. (2011) showed that, in DFIs 40% of the isolates exhibited weak biofilm formation and 60% isolate showed strong biofilm formation. Similarly, in a recent report by Vatan et al (2018), 40.8% and 59.3% of isolates were weak and strong biofilm producers respectively. Chi-square test showed a significant difference between DFI and non-DFI infections ($p < 0.05$) (Fig. 1). The biofilm architecture among the weak, moderate and strong biofilm producers is depicted in Figs. 2–4. Most common factors which influence biofilm production are nutrient availability, outer membrane proteins adhesions, bacterial pili, and flagella (Gaddy and Actis, 2009). Biofilm formation may render leukocytes inefficient by developing anti-phagocytic activity (He et al., 2015).

In the present study, the isolates were classified as strong [hydrophobic > 70%], moderate [> 30–70%] and weak [hydrophilic < 30%] based on CSH activity. We found a significant difference in the CSH activity among DFI and non-DFI patients ($p < 0.05$). CSH among *A. baumannii* has been demonstrated to be one of the essential factors of bacterial adhesion (Almasaudi, 2018). The comparison of biofilm formation and CSH in DFIs and non-DFIs is indicated in Table 2. A study reported by Costa et al. (2006) showed that majority of the clinical isolates exhibited higher hydrophobicity. In addition, another report by Pour et al. (2011) suggested that strong hydrophobicity was found in 44% of the clinical isolates. In the present study, the strong CSH was found in patient with necrotizing fasciitis, abscess, osteomyelitis and gangrene. In non-DFIs, strong CSH isolates were found in patients with

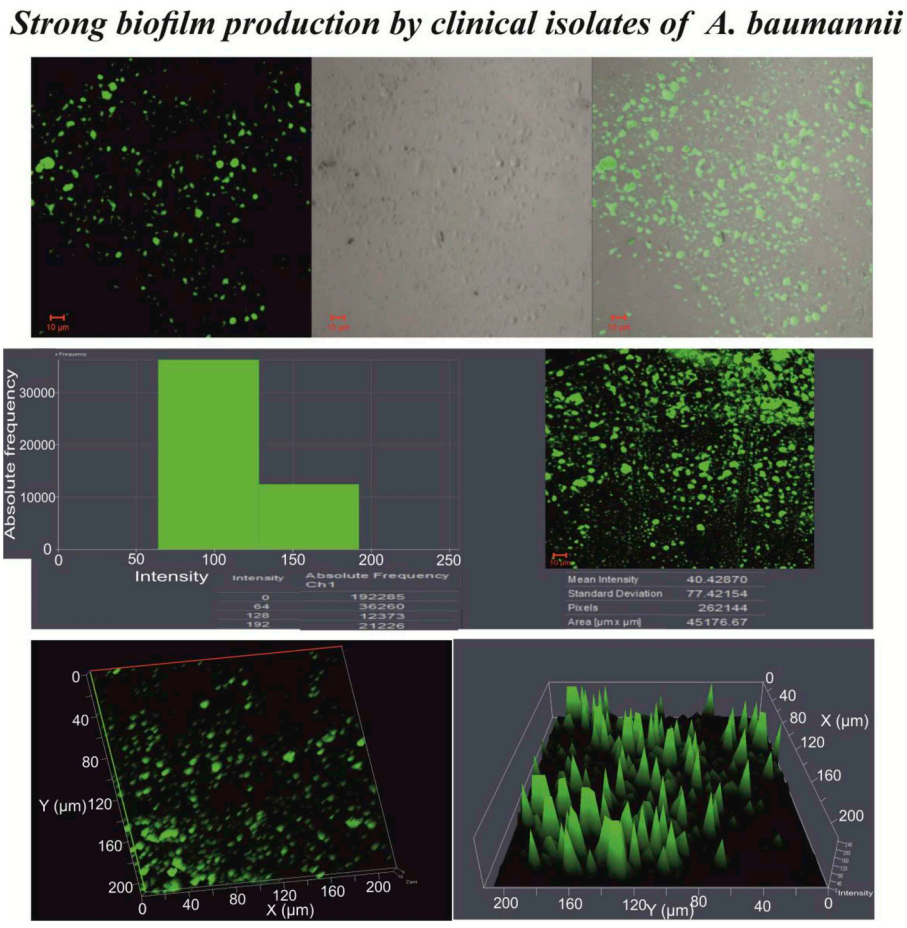

Fig. 4. CLSM showing strong biofilm formation in clinical isolates of *Acinetobacter baumannii* strains isolated from diabetic foot infection.

Table 3
Distribution of *A. baumannii* strains from different infection site in DFI isolates.

Infection site	Cell surface hydrophobicity			Biofilm formation			Gelatinase Activity
DFI(N = 35)	Weak	Moderate	Strong	Weak	Moderate	Strong	Production
Cellulitis	1	1	1	1	3	0	0
Abscess	0	1	9	1	9	4	5
Necrotizing fascitis	0	0	15	1	4	2	4
Gangrene	0	0	3	1	2	1	1
Osteomyelitis	0	0	4	1	1	4	2
Total	**1**	**2**	**32**	**5**	**19**	**11**	**12**

abscess and necrotizing fasciitis. However, to the best of our knowledge, this was the first study reporting the higher prevalence of virulence factors among DFIs isolates compared to non-DFIs patients.

Gelatinase production is also associated with inflammation, contributing to virulence in patients with DFIs (Cevahir et al., 2008). Gelatinase production facilitates to break down subcutaneous tissue in foot ulcer infections (Harrington, 1996). In the present study, gelatinase production was found in 34.29% DFIs isolates (Table 3). The appearance of a turbid halo (opacity around growth) was considered as positive for gelatinase production (Fig. 5). Our previous reports indicated that gelatinase activity was exhibited in 38.09% of ESBL isolates among DFIs (Khan et al., 2018b). Among non-DFIs isolates, 14.29% were gelatinase producers. Table 4 shows the distribution of *A. baumannii* from different infection site in non-DFI isolates. Cevahir et al. (2008) and Valli and Gopinath (2016) reported that 14% and 40% of gelatinase production was found among the patients with non-DFIs respectively.

4. Conclusion

DFIs are a significant public health concern worldwide due to the

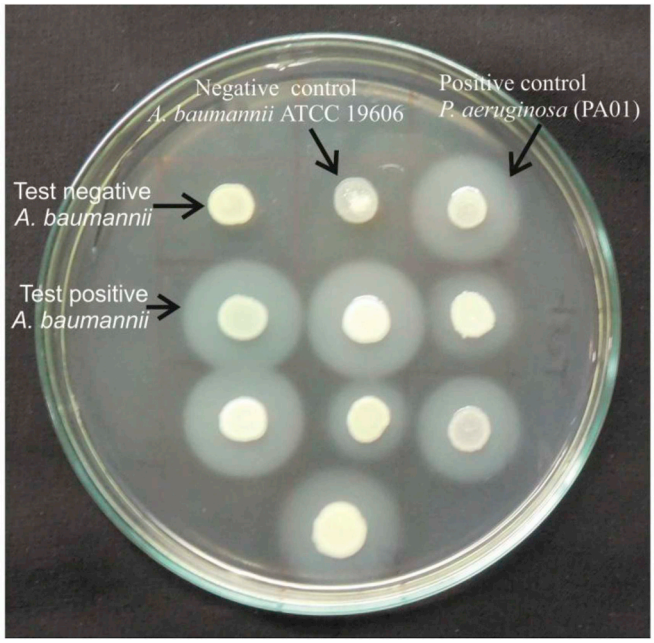

Fig. 5. The image showing the gelatinase production in Luria Bertani agar containing gelatin (3%). The appearance of a turbid halo (opacity around growth) was considered as positive for gelatinase production.

Table 4
Distribution of *A. baumannii* strains from different infection sites in non-DFIs isolates.

Infection site	Cell surface hydrophobicity			Biofilm formation			Gelatinase Activity
Non-DFI (N = 35)	Weak	Moderate	Strong	Weak	Moderate	Strong	Production
Cellulitis	1	1	1	3	2	0	0
Abscess	3	4	1	2	10	1	2
Necrotizing fascitis	1	10	4	2	5	2	3
Gangrene	2	3	1	3	4	0	0
Osteomyelitis	1	2	0	0	1	0	0
Total	**8**	**20**	**7**	**10**	**22**	**03**	**5**

socio-economic burden. Biofilm formation, CSH, and gelatinase production were found higher in isolates from patients with DFIs compared to non-DFIs. Virulence factors especially biofilm formation, CSH and gelatinase production play a vital role in the pathogenesis of *A. baumannii* infections. Combination of these virulence factors may facilitate necrotizing fasciitis, osteomyelitis, gangrene, and abscess in the site of infection. Furthermore, new experimental approaches are warranted to develop and evaluate novel therapeutic strategies for dealing with *A. baumannii* infections among DFIs and non-DFIs patients.

Acknowledgments

We would like to acknowledge the Confocal Laser Scanning Microscopy Workstation, Bharathidasan University, Tiruchirappalli, India (DSTPURSE- DST Sanction Order No.SR/FT/LS-113/2009) for providing us the CLSM facility and Mr. Sabarinathan for his valuable help. The authors Diwan Mahmood Khan and Muhammed A.P. Manzoor acknowledges Yenepoya (Deemed to be University) for Research Fellowship.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bcab.2019.01.045.

References

Almasaudi, S.B., 2018. *Acinetobacter* spp. as nosocomial pathogens: epidemiology and resistance features. Saudi J. Biol. Sci. 25, 586–596.
Al-Rubeaan, K., Al-Derwish, M., Ouizi, S., Youssef, A.M., Subhani, S.N., Ibrahim, H.M., Alamri, B.N., 2015. Diabetic foot complications and their risk factors from a large retrospective cohort study. PLoS One 10 0124446.
Amin, N., Doupis, J., 2016. Diabetic foot disease: from the evaluation of the "foot at risk" to the novel diabetic ulcer treatment modalities. World J. Diabetes 7, 153–164.
Babapour, E., Haddadi, A., Mirnejad, R., Angaji, S.A., Amirmozafari, N., 2016. Biofilm formation in clinical isolates of nosocomial *Acinetobacter baumannii* and its relationship with multidrug resistance. Asian Pac. J. Trop. Biomed. 6, 528–533.
Bali, N.K., Fomdam, B.A., Bashir, H., Zahoor, D., Lone, S., Koul, R.A., 2013. Emergence of carbapenem-resistant Acinetobacter in a temperate north Indian State. J. Biomed. Sci. 70, 156–160.
Banu, A., Hassan, M.M., Rajkumar, J., Srinivasa, S., 2015. Spectrum of bacteria associated with diabetic foot ulcer and biofilm formation: a prospective study. Australas. Med. J. 8, 280.
Beceiro, A., Tomás, M., Bou, G., 2013. Antimicrobial resistance and virulence: a successful or deleterious association in the bacterial world? Clin. Microbiol. Rev. 26, 185–230.
Cevahir, N., Demir, M., Kaleli, I., Gurbuz, M., Tikvesli, S., 2008. Evaluation of biofilm production, gelatinase activity, and mannose-resistant hemagglutination in *Acinetobacter baumannii* strains. J. Microbiol. Immunol. Infect. 41, 513–518.
Chandra, H.J., Rao, B.S., Manzoor, M.A.P., Arun, A.B., 2017. Characterization and antibiotic sensitivity profile of bacteria in orofacial abscesses of odontogenic origin. J. Maxillofac. Oral Surg. 16, 445–452.
Christensen, G.D., Simpson, W.A., Younger, J.J., Baddour, L.M., Barrett, F.F., Melton, D.M., Beachey, E.H., 1995. Adherence of coagulase negativestaphylococci to plastic tissue cultures: a quantitative model for the adherence of staphylococci to medical devices. J. Clin. Microbiol. 22, 996–1006.
Clinical and Laboratory Standards Institute (CLSI), 2016. Performance Standards for

Antimicrobial Susceptibility Testing, 26th edition. CLSI supplements M100S.
Col, K.L.L., Col, N.M.L., Col, S.P.L., 2004. *Acinetobacter* spp as nosocomial pathogen: clinical significance and antimicrobial sensitivity. Med. J. Armed Forces India 60, 7–10.
Costa, G.F.D.M., Tognim, M.C.B., Cardoso, C.L., Carrara-Marrone, F.E., Garcia, L.B., 2006. Preliminary evaluation of adherence on abiotic and cellular surfaces of *Acinetobacter baumannii* strains isolated from catheter tips. Braz. J. Infect. Dis. 10, 346–351.
De Breij, A., Gaddy, J., van der Meer, J., Koning, R., Koster, A., van den Broek, P., Actis, L., Nibbering, P., Dijkshoorn, L., 2009. CsuA/BABCDE-dependent pili are not involved in the adherence of *Acinetobacter baumannii* ATCC 19606T to human airway epithelial cells and their inflammatory response. Res. Microbiol. 160, 213–218.
Dheepa, M., Rashme, V.L., Appalaraju, B., 2011. Comparison of biofilm production and multiple drug resistance in clinical isolates of *Acinetobacter baumanii* from a tertiary care hospital in South India. Int. J. Pharma Bio Sci. 2, 103–107.
El-Din, R.A.A., El-Bassat, H., El-Bedewy, M., El-Mohamady, H., 2014. Prevalence of metallo–lactamases producers among carbapenem-resistant *Acinetobacter baumannii* strains isolated from diabetic foot ulcers. Afr. J. Microbiol. Res. 8, 1580–1585.
Gaddy, J.A., Actis, L.A., 2009. Regulation of *Acinetobacter baumannii* biofilm formation. Future Microbiol. 4, 273–278.
Harrington, D.J., 1996. Bacterial collagenases and collagen-degrading enzymes and their potential role in human disease. Infect. Immun. 64, 1885–1891.
He, X., Lu, F., Yuan, F., Jiang, D., Zhao, P., Zhu, J., Cheng, H., Cao, J., Lu, G., 2015. Biofilm formation caused by clinical *Acinetobacter baumannii* isolates is associated with over-expression of the AdeFGH efflux pump. Antimicrob. Agents Chemother. 59, 4817–4825.
Karmaker, M., Sanyal, S.K., Sultana, M., Hossain, M.A., 2016. Association of bacteria in diabetic and non-diabetic foot infection–An investigation in patients from Bangladesh. J. Infect. Pub. Health 9, 267–277.
Khan, D.M., Moosabba, M.S., Rao, I.V., 2018a. Changing Antibiogram profile of *Acinetobacter baumannii* in diabetic and non-diabetic foot ulcer Infections. J. Clin. Diagn. Res. 12, 12–16.
Khan, D.M., Rao, I.V., Moosabba, M.S., 2018b. Comparative study of gelatinase activity and pellicle formation among extended-spectrum beta-lactamase and non-extended-spectrum beta-lactamase producing *Acinetobacter baumannii* from diabetic foot ulcer infections. Asian J. Pharmaceut. Clin. Res. 11, 496–498.
Khan, M.H., Sneha, U., Ramalingam, K., 2018c. Bactericidal potential of silver-tolerant bacteria derived silver nanoparticles against Multi Drug Resistant ESKAPE pathogens. Biocatal. Agric. Biotechnol. https://doi.org/10.1016/j.bcab.2018.12.004.
Lavery, L.A., Armstrong, D.G., Wunderlich, R.P., Mohler, M.J., Wendel, C.S., Lipsky, B.A., 2006. Risk factors for foot infections in individuals with diabetes. Diabetes Care 29, 1288–1293.
LewisOscar, F., MubarakAli, D., Priyanka, R., Nithya, C., Gopinath, V., Naif Al-Harbi, S., Thajuddin, N., 2015. One pot synthesis and potential antibiofilm property of copper nanoparticles against clinically isolated strains of *Pseudomonas aeruginosa*. Biofouling 31, 379–391.
Lipsky, B.A., Berendt, A.R., Cornia, P.B., Pile, J.C., Peters, E.J., Armstrong, D.G., Deery, H.G., Embil, J.M., Joseph, W.S., Karchmer, A.W., Pinzur, M.S., 2012. 2012 Infectious Diseases Society of America clinical practice guideline for the diagnosis and treatment of diabetic foot infections. Clin. Infect. Dis. 54, 132–173.
McIntosh, J., Earnshaw, J.J., 2009. Antibiotic prophylaxis for the prevention of infection after major limb amputation. Eur. J. Vasc. Endovasc. Surg. 37, 696–703.
Manzoor, M.A.P., Mujeeburahiman, M., Rekha, P.D., 2017. Association of serum biochemical panel with mineralogical composition of kidney stone in India. Acta. Med. Int. 4, 26–30.
Manzoor, M.A.P., Duwal, S.R., Mujeeburahiman, M., Rekha, P.D., 2018a. Vitamin C inhibits crystallization of struvite from artificial urine in the presence of *Pseudomonas aeruginosa*. Int. Braz J. Urol. 44, 1234–1242.
Manzoor, M.A.P., Singh, B., Agrawal, A.K., Arun, A.B., Mujeeburahiman, M., Rekha, P.D., 2018b. Morphological and micro-tomographic study on evolution of struvite in synthetic urine infected with bacteria and investigation of its pathological biomineralization. PloS One 13 (8), e0202306.
Manzoor, M.A.P., Mujeeburahiman, M., Rekha, P.D., 2018c. Electron probe micro-analysis reveals the complexity of mineral deposition mechanisms in urinary stones. Urolithiasis 46, 1–12.
Manzoor, M.A.P., Shabeena, K.S., Mujeeburahiman, M., Khan, A., 2018d. In dwelling J ureteral stents associated asymptomatic bacteraemia caused by multidrug resistant strain of *Kocuria kristinae*. J. Clin. Diagn. Res. 12, 15–16.
Manzoor, M.A.P., Mujeeburahiman, M., Duwal, S.R., Rekha, P.D., 2019. Investigation on growth and morphology of *in vitro* generated struvite crystals. Biocatal. Agric. Biotechnol. 17, 566–570.
Murali, T.S., Kavitha, S., Spoorthi, J., Bhat, D.V., Prasad, A.S.B., Upton, Z., Ramachandra, L., Acharya, R.V., Satyamoorthy, K., 2014. Characteristics of microbial drug resistance and its correlates in chronic diabetic foot ulcer infections. J. Med. Microbiol. 63, 1377–1385.
Oyibo, S.O., Jude, E.B., Tarawneh, I., Nguyen, H.C., Harkless, L.B., Boulton, A.J., 2001. A comparison of two diabetic foot ulcer classification systems: the Wagner and the University of Texas wound classification systems. Diabetes Care 24, 84–88.
Pour, N.K., Dusane, D.H., Dhakephalkar, P.K., Zamin, F.R., Zinjarde, S.S., Chopade, B.A., 2011. Biofilm formation by *Acinetobacter baumannii* strains isolated from urinary tract infection and urinary catheters. FEMS Immunol. Med. Microbiol. 62, 328–338.
Prashanth, K., Badrinath, S., 2000. Simplified phenotypic tests for identification of *Acinetobacter* spp. and their antimicrobial susceptibility status. J. Med. Microbiol. 49, 773–778.
Qi, L., Li, H., Zhang, C., Liang, B., Li, J., Wang, L., Du, X., Liu, X., Qiu, S., Song, H., 2016. Relationship between antibiotic resistance, biofilm formation, and biofilm-specific resistance in *Acinetobacter baumannii*. Front. Microbiol. 7, 483.
Sargen, M.R., Hoffstad, O., Margolis, D.J., 2013. Geographic variation in Medicare spending and mortality for diabetic patients with foot ulcers and amputations. J. Diabetes Complicat. 27, 128–133.
Shabeena, K.S., Bhargava, R., Manzoor, M.A.P., Mujeeburahiman, M., 2018. Characteristics of bacterial colonization after indwelling double-J ureteral stents for different time duration. Urol. Ann. 10, 71–75.
Valli, B., Gopinath, P., 2016. Detection of gelatinase and lipase among clinical isolates of *Acinetobacter baumannii*. J. Chem. Pharmaceut. Sci. 9, 3245–3247.
Vatan, A., Saltoglu, N., Yemisen, M., Balkan, I.I., Surme, S., Demiray, T., Mete, B., Tabak, F., 2018. Association between biofilm and multi/extensive drug resistance in diabetic foot infection. Int. J. Clin. Pract. 72, 13060.
Whiting, D.R., Guariguata, L., Weil, C., Shaw, J., 2011. IDF diabetes atlas: global estimates of the prevalence of diabetes for 2011 and 2030. Diabetes Res. Clin. Pract. 94, 311–321.
Zubair, M., Malik, A., Ahmad, J., Rizvi, M., Farooqui, K.J., Rizvi, M.W., 2011. A study of biofilm production by gram-negative organisms isolated from diabetic foot ulcer patients. Biol. Med. 3, 147–157.