Antibacterial and antibiofilm activity of cinnamaldehyde against carbapenem-resistant *Acinetobacter baumannii* in Egypt: *In vitro* study

Sara H. Mohamed¹, Dalia Salem²*, Magda Azmy³, Nevine S. Fam²

¹Department of Microbiology, National Organization for Drug Control and Research, Giza, Egypt.  
²Department of Microbiology, Theodor Bilharz Research Institute (TBRI), Giza, Egypt.  
³Department of Clinical Pathology and Electron Microscopy, Theodor Bilharz Research Institute (TBRI), Giza, Egypt.

**ARTICLE INFO**
Received on: 25/09/2018  
Accepted on: 21/10/2018  
Available online: 30/11/2018  

**Key words:**  
*Acinetobacter baumannii*, biofilm, carbapenem-resistant, cinnamaldehyde, SEM.

**ABSTRACT**

*Acinetobacter baumannii* has emerged as a problematic nosocomial pathogen due to its antibiotic resistance as well as its ability to colonize and cause serious infection among patients. This study aimed to evaluate the ability of *A. baumannii* to form biofilms as well as to investigate the antibacterial activity of cinnamaldehyde against carbapenem-resistant strains of *A. baumannii*. A total of 23 *A. baumannii* clinical strains were screened for their ability to form a biofilm using the tissue culture plate method. Cinnamaldehyde antibacterial activity was investigated on planktonic cells and its biofilm inhibition ability was tested. Scanning electron microscopy (SEM) was applied to confirm the antibiofilm effect of cinnamaldehyde. Biofilm formers (86.95%) were categorized into strong (17.39%), moderate (52.17%), and weak (17.39%). Cinnamaldehyde showed a strong antimicrobial activity against planktonic cells of *A. baumannii* at low concentrations. The best antibiofilm activity was achieved at ½ minimum inhibitory concentration (MIC) and ¼ MIC causing inhibition percentages ranging from 49.5% to 71.2% and 18.5% to 29.6%, respectively. Cinnamaldehyde exerted strong antimicrobial and antibiofilm properties indicating their potential therapeutic value that can be used as an option for treating biofilm associated clinical problems caused by *A. baumannii*.

**INTRODUCTION**

*Acinetobacter baumannii* (*A. baumannii*) is a well-known pathogen which is responsible for hospital-acquired infections due to its remarkable ability to survive in the hospital environment as well as its rapidly acquire resistance determinants agents (Bogdan et al., 2017). Some of the difficulties in the treatment of the infections caused by this pathogen is its remarkable widespread resistance to different antibiotics and its ability to persist in nosocomial environments and medical devices (Abdulhasan et al., 2016).

One of the important mechanisms in the development of a multi-drug resistant (MDR) *A. baumannii* is the potential ability to form biofilms, which could explain its outstanding antibiotic resistance, survival properties, and owing protection from disinfectants and/or desiccation on the abiotic surfaces (Ivanković et al., 2017; Ryu et al., 2017). The adhesion ability of *Acinetobacter* strains is an important mechanism in its pathogenicity process, which is determined by specific and non-specific factors that vary among the strains (Imane et al., 2014).

Treatment options for MDR *A. baumannii* are extremely limited as a result for its resistant to most antimicrobial agents including carbapenems (Bae et al., 2016; Hu et al., 2017). Many natural compounds, such as plant extracts and secondary metabolites have been evaluated for antimicrobial activity (Nowotarska et al., 2017), and within this group, essential oils (EOs) are the most important members. In the recent years, many studies have done on a wide range of bacterial species showed the strong activity of EOs main components (Budzyńska et al., 2011; Kim et al., 2016; Mohamed et al., 2018a). Cinnamaldehyde,
a major component of cinnamon EOs, occurs naturally in parts of cinnamon trees of the genus *Cinnamomum*. It has been proven to have antibacterial activities against a number of Gram-positive and Gram-negative bacteria (Jia et al., 2011; Khan et al., 2017). Therefore, our study was designed to evaluate the ability of *A. baumannii* to form biofilms as well as investigate the antimicrobial and antibiofilm activities of the cinnamaldehyde on clinical isolates of carbapenem-resistant *A. baumannii*.

**MATERIALS AND METHODS**

**Bacterial strains and antimicrobial susceptibility**

In this study, a total of 23 carbapenem-resistant *A. baumannii* clinical strains were collected from the different sources of Theodor Bilharz Research Institute (TBRI) during the period from November 2014 to December 2015 (Fam et al., 2017). All strains were previously identified by API 20E (bioMérieux, France) and confirmed using VITEK 2 compact system (bioMérieux, France). Antimicrobial susceptibility testing was done by using the disc diffusion method, and then minimum inhibitory concentration (MIC) was detected by VITEK2 compact system (bioMérieux, France).

**Quantitative biofilm production assay**

Colonies from 23 strains of fresh *A. baumannii* cultures (18 hours) were grown overnight at 37°C in brain heart infusion (BHI) broth medium for 24 hours. Biofilm formation was tested by adding 100 µl of this standardized cell suspension to wells of microtiter plates that contained 100 µl of fresh BHI media and incubating them at 37°C for 48 hours (Song et al., 2015). Thereafter, the medium was removed and planktonic cells were removed by washing the biofilms in phosphate buffered saline (PBS). After staining plates with 2% crystal violet for 20 m, excess stain was removed using water. The plates were air dried and then the dye was resolubilized with absolute ethanol. The optical density (OD) of each well was calculated using the equation: average OD of negative control + (3*SD of negative control). Samples were considered positive when OD was > 0.228.

**Antibacterial activity of cinnamaldehyde**

Nearly, 100 µl (0.5 McFarland) of the bacterial culture was inoculated into each well of a 96-well microtiter plate containing 100 µl trans-cinnamaldehyde (Sigma Aldrich, St. Louis, MO) at different concentrations (0.027–28 mg/ml). Wells without cinnamaldehyde were used as a positive control while those without bacteria were considered as negative controls. After 24 hours incubation at 37°C, the wells were visually inspected for the growth. The MIC was considered as the lowest concentration of cinnamaldehyde that inhibits the bacterial growth. Nearly, 100 µl of culture from each clear well in the MIC assay was placed on a Mueller–Hinton agar plate and incubated at 37°C for 24 hours. The lowest concentration of cinnamaldehyde that inhibits the bacterial growth on an agar plate was considered as the minimum bactericidal concentration (MBC) (Jia et al., 2011).

**Antibiofilm activity of cinnamaldehyde**

This test was performed on four strains that showed strong biofilm formation ability in the biofilm production assay. The effect of different concentrations of cinnamaldehyde to inhibit the ability of *A. baumannii* cells to form a biofilm was assessed using the TCP method adopted by Adukwu et al. (2012) with minor modifications. Nearly, 100 µl of 0.5 McFarland bacterial culture was dispensed into each well of 96-well polystyrene microtiter plates in the presence of 100 µl of cinnamaldehyde different concentrations, and plates were incubated at 37°C for 48 hours. Antimicrobial agent free wells served as positive controls for the biofilm growth. After incubation, the medium and non-adherent cells were removed and wells were washed three times with sterile PBS. The plates were air dried and then the dye was resolubilized with absolute ethanol. The OD of each well was measured at 570 nm using ELISA reader (Stat Fax 2100). Each assay was performed in triplicates.

**Scanning electron microscopy (SEM)**

SEM was used to investigate the effect of cinnamaldehyde on *Acinetobacter* biofilm. Sections of polystyrene tubes that coated with bacterial biofilm were processed similarly with the method described by Mohamed et al. (2018a). Then examined with Philips XL30 scanning electron microscope (Eindhoven, Netherlands) operated at 20 kV.

**Statistical analysis**

Quantitative variables were expressed as mean ± standard deviation (SD), and data were analyzed by SPSS statistical software, version 12.0. One-way analysis of variance (ANOVA) with post hoc multiple comparisons (Duncan test) were used to compare the effect of different concentrations of cinnamaldehyde on *Acinetobacter* strains. Statistically, the values were considered significant at \( p < 0.05 \).

**RESULTS**

All strains were found to be resistant to carbapenems when tested by E-test strips including doripenem showing MIC >32 µg/ml. Most of the strains also showed resistance to multiple classes of antibiotics including; ß-lactams, aminoglycosides, quinolones, and trimethoprim/sulphamethoxazole (Fam et al., 2017).

In this study, biofilm formation assay was performed for 23 *A. baumannii* MDR strains, and the biofilm formation ability was determined by the estimation of OD570 values. Out of 23 *Acinetobacter* strains, 20 (86.95%) strains were able to form biofilms (Table 1). The formation ability of biofilm was categorized as follows: 4 isolates as strong formers (17.39%), 12 isolates as moderate formers (52.17%), 4 isolates as weak formers (17.39%), and 3 isolates as non-biofilm formers (13.04%).

The four strong biofilm formers were chosen to test the antimicrobial and antibiofilm activity of Cinnamaldehyde. Our results showed a strong antimicrobial activity against planktonic cells of *A. baumannii*, at which MIC and MBC were detected at 0.875 and 1.75 mg/ml, respectively, for the four strains.

The antibiofilm activity of cinnamaldehyde was measured at different sub-inhibitory concentrations (½ MIC, ¼ MIC, ⅛ MIC, and ⅛¼ MIC). The best antibiofilm activity was achieved at ½ MIC which caused inhibition percentages ranging from 49.452% to 71.158%. Following ½ MIC concentration, ¼ MIC, ⅛ MIC, and ⅛¼ MIC caused inhibition percentages ranging from 18.540% to 29.606%, 11.749% to 13.337%, and 5.841% to 12.120%, respectively.

**Table 1**

<table>
<thead>
<tr>
<th>Strain</th>
<th>MIC (µg/ml)</th>
<th>MBC (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.875</td>
<td>1.75</td>
</tr>
<tr>
<td>2</td>
<td>0.875</td>
<td>1.75</td>
</tr>
<tr>
<td>3</td>
<td>0.875</td>
<td>1.75</td>
</tr>
<tr>
<td>4</td>
<td>0.875</td>
<td>1.75</td>
</tr>
</tbody>
</table>
Table 1. Screening of biofilm formation ability among Acinetobacter baumannii strains at 570 nm.

<table>
<thead>
<tr>
<th>Strains</th>
<th>OD570 Ex. 1</th>
<th>OD570 Ex. 2</th>
<th>OD570 Ex. 3</th>
<th>Average ± SD</th>
<th>Biofilm formation ability</th>
</tr>
</thead>
<tbody>
<tr>
<td>R2</td>
<td>0.297</td>
<td>0.435</td>
<td>0.649</td>
<td>0.460 ± 0.177</td>
<td>Weak biofilm</td>
</tr>
<tr>
<td>R7</td>
<td>0.219</td>
<td>0.245</td>
<td>0.214</td>
<td>0.226 ± 0.016</td>
<td>No biofilm</td>
</tr>
<tr>
<td>R8</td>
<td>0.78</td>
<td>0.71</td>
<td>0.724</td>
<td>0.738 ± 0.037</td>
<td>Moderate biofilm</td>
</tr>
<tr>
<td>R9</td>
<td>1.66</td>
<td>1.378</td>
<td>1.433</td>
<td>1.490 ± 0.149</td>
<td>Strong biofilm</td>
</tr>
<tr>
<td>R11</td>
<td>0.804</td>
<td>0.804</td>
<td>0.899</td>
<td>0.835 ± 0.054</td>
<td>Moderate biofilm</td>
</tr>
<tr>
<td>R13</td>
<td>0.322</td>
<td>0.315</td>
<td>0.57</td>
<td>0.402 ± 0.145</td>
<td>Weak biofilm</td>
</tr>
<tr>
<td>R14</td>
<td>1.258</td>
<td>1.706</td>
<td>1.602</td>
<td>1.522 ± 0.234</td>
<td>Strong biofilm</td>
</tr>
<tr>
<td>R18</td>
<td>0.279</td>
<td>0.389</td>
<td>0.383</td>
<td>0.350 ± 0.061</td>
<td>Weak biofilm</td>
</tr>
<tr>
<td>R24</td>
<td>0.716</td>
<td>0.562</td>
<td>0.464</td>
<td>0.581 ± 0.127</td>
<td>Moderate biofilm</td>
</tr>
<tr>
<td>R28</td>
<td>0.53</td>
<td>0.524</td>
<td>0.391</td>
<td>0.482 ± 0.078</td>
<td>Moderate biofilm</td>
</tr>
<tr>
<td>R31</td>
<td>0.6</td>
<td>0.84</td>
<td>0.331</td>
<td>0.590 ± 0.254</td>
<td>Moderate biofilm</td>
</tr>
<tr>
<td>R34</td>
<td>2.103</td>
<td>2.366</td>
<td>2.103</td>
<td>2.191 ± 0.151</td>
<td>Strong biofilm</td>
</tr>
<tr>
<td>R36</td>
<td>1.273</td>
<td>1.095</td>
<td>1.466</td>
<td>1.278 ± 0.185</td>
<td>Strong biofilm</td>
</tr>
<tr>
<td>R41</td>
<td>0.531</td>
<td>0.601</td>
<td>0.578</td>
<td>0.57 ± 0.0356</td>
<td>Moderate biofilm</td>
</tr>
<tr>
<td>R45</td>
<td>0.689</td>
<td>0.677</td>
<td>0.747</td>
<td>0.704 ± 0.037</td>
<td>Moderate biofilm</td>
</tr>
<tr>
<td>R51</td>
<td>0.703</td>
<td>0.493</td>
<td>0.621</td>
<td>0.606 ± 0.105</td>
<td>Moderate biofilm</td>
</tr>
<tr>
<td>R58</td>
<td>0.217</td>
<td>0.252</td>
<td>0.21</td>
<td>0.226 ± 0.022</td>
<td>No biofilm</td>
</tr>
<tr>
<td>R71</td>
<td>0.796</td>
<td>0.743</td>
<td>0.697</td>
<td>0.745 ± 0.049</td>
<td>Moderate biofilm</td>
</tr>
<tr>
<td>R73</td>
<td>0.758</td>
<td>0.684</td>
<td>0.467</td>
<td>0.636 ± 0.151</td>
<td>Moderate biofilm</td>
</tr>
<tr>
<td>R74</td>
<td>0.796</td>
<td>0.897</td>
<td>0.824</td>
<td>0.839 ± 0.052</td>
<td>Moderate biofilm</td>
</tr>
<tr>
<td>R76</td>
<td>0.651</td>
<td>0.725</td>
<td>0.752</td>
<td>0.709 ± 0.052</td>
<td>Moderate biofilm</td>
</tr>
<tr>
<td>R77</td>
<td>0.344</td>
<td>0.34</td>
<td>0.539</td>
<td>0.408 ± 0.113</td>
<td>Weak biofilm</td>
</tr>
<tr>
<td>R80</td>
<td>0.207</td>
<td>0.229</td>
<td>0.228</td>
<td>0.221 ± 0.012</td>
<td>No biofilm</td>
</tr>
<tr>
<td>C</td>
<td>0.202</td>
<td>0.204</td>
<td>0.215</td>
<td>0.207 ± 0.007</td>
<td>-</td>
</tr>
</tbody>
</table>

OD = optical density; SD = standard deviation; C = control.

Sub-inhibitory concentrations of cinnamaldehyde (½ MIC and ¼ MIC) were found to cause inhibition percentage, which is not significantly different when ¼ MIC was used but was significantly different for strain R36 when ½ MIC was used among different Acinetobacter strains (Fig. 1).

SEM was employed to confirm the effect of cinnamaldehyde on Acinetobacter biofilm at the concentrations of ½ MIC and ¼ MIC (Fig. 2). Colonized cells were detected in the untreated biofilm (control) (Fig. 2A and a), while for biofilm treated with ¼ MIC cinnamaldehyde concentration, lower number of colonized cells was shown (Fig. 2B and b). On the other hand, noticeable inhibition of A. baumannii biofilm formation was detected in ½ MIC cinnamaldehyde concentration treated biofilm (Fig. 2C and c) which confirmed the results obtained phenotypically.

**DISCUSSION**

Nowadays, A. baumannii has emerged as a pathogen that related to outbreaks of colonization and infection of critically ill patients (Imane et al., 2014). Resistant strains of A. baumannii are known to be one of the most difficult treating (Almaghrabi et al., 2018; Ramanathan et al., 2017). The ability of resistant A. baumannii strains to spread widely seems to depend on some virulence factors that allow bacterial colonization such as biofilm formation ability (Reena et al., 2017), which plays an effective role in medical device-associated infections (Longo et al., 2014).

In our study, 86.95% of A. baumannii strains were found to have the ability to form a biofilm, which considered as a high percentage when compared with other recent studies reporting 70% in Brazil (de Campos et al., 2016), 72.91% in Iran (Dehbalaei et al., 2017), 76.92% in India (Anish et al., 2017), 85.6% in Lebanon (Dahdouh et al., 2016), but lower than that was reported in Iraq (91.25%) (Authman et al., 2017). While in our country Egypt, several studies reported the biofilm formation among A. baumannii by the percentages of 60%, 62.5%, and 84% (Elhabibi and Ramzy, 2017; El-Kady, 2015; Mansour and Rhman, 2012). This high percentage of biofilm formation among our isolates is a serious concern. It is known that bacterial cells acquire more resistance to multiple drugs within the biofilm mode than the planktonic one. Thus, function the biofilm formed by A. baumannii encompasses its ability to resist antimicrobial therapies as well as to protect bacterial cells from external stresses such as dehydration and limited availability of nutrients (Badave and Dhananjay, 2015).

Another serious concern about A. baumannii infections is the detection of an increasing rate of resistance to multiple antibiotics and in some cases, resistant to all available antibiotics (Bialvaei et al., 2017). The wide spread antibiotic resistance observed is now posing a serious public health concern and warning of a return to the pre-antibiotic era. Recently, an interest was given to natural compounds which have antimicrobial and anti-adherence activities (Gupta and Birdi, 2017; Mohamed et al., 2018b).
Mansour and Rahman (2012) indicated the importance of controlled antibiotic usage and appliance of proper hospital infection control measures. While recently Bogdan et al. (2017) stated that there are no published extensive studies about the effect of sub MICs of antimicrobials on the virulence factors of \textit{A. baumannii}, to face the growing global problem of \textit{A. baumannii} multi-resistance to antimicrobial drugs.

Our study showed a strong antibacterial and antibiofilm activity at low concentrations of cinnamaldehyde against strong biofilm producing strains of \textit{A. baumannii} clinical strains at which MIC and MBC were detected at 0.875 and 1.75 mg/ml, respectively. Anish et al. (2017) tried to use chemical compounds in the war against biofilm production and antimicrobial resistance of certain bacteria, stating that 55\textendash}75\% reduction of biofilm was seen by using Ethylene Diamine Tetraacetic Acid EDTA. Other studies tried to use natural compounds such as Cinnamaldehyde against different bacterial species, concluded that Cinnamaldehyde represent potential antimicrobial agents for treating wound infections and suggesting that it may represent an alternative strategy to control bacterial infections as it presents the ability to inhibit bacterial growth without promoting an adaptive phenotype (Ferro et al., 2016; Jia et al., 2011).

This antimicrobial activity of cinnamaldehyde was studied recently and mechanisms were suggested such as (a) interacting with the cell membrane rapid inhibition of energy metabolism (Friedman, 2017); (b) its hydrophobicity enables it to enter and disturb the lipid bilayer of the cell membrane and cause increased permeability to protons and the exit of critical molecules and ions from bacterial cells ultimately leading to bacterial cell death (Jia et al., 2011); and (c) by affecting membrane integrity, energy generation, and extended-spectrum beta-lactamase protein dependent drug resistance (Thakre et al., 2016).

Concerning its antibiofilm activity, extensive research exploring alternative strategies for microbial biofilm control...
has highlighted the efficacy of plant-derived antimicrobials in controlling biofilm formation of human pathogens. Extracellular polymeric substances (EPS) in biofilms are responsible for binding cells and other particulate materials to each other’s and to the surface, as well as for the development of biofilm structure and maturation (Azizun et al., 2013; Thummeepak et al., 2016). Cinnamaldehyde, at a certain concentration significantly reduces quorum sensing (QS) signaling, which results in reduction or prevention of EPS formation, and this may explain its strong antibiofilm activity (Niu et al., 2006; Bai A and Vittal, 2014). Our results strongly support such findings as both tissue culture plate method and scanning electron microscope studies showed a marked inhibition of A. baumannii biofilm formation in 1/2 MIC cinnamaldehyde concentration treated biofilms.

CONCLUSION

Cinnamaldehyde exerts strong antimicrobial and antibiotic activities against carbapenem-resistant A. baumannii strains which can be used as an option for treating biofilm associated clinical problems caused by this pathogen.

CONFLICT OF INTEREST

Authors declare that there is no conflict of interest exists.

FUNDING

This work was supported by Theodor Bilharz Research Institute (TIRI) as a part of an ongoing internal project No. 96 D.

REFERENCES


How to cite this article: