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Effect of Aqueous garlic extract on biofilm formation and antibiotic susceptibility of multidrug-resistant uropathogenic *Escherichia coli* clinical isolates in Togo

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Keywords

Garlic,
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Adhesion,
Biofilm formation

Abstract

Uropathogenic *Escherichia coli* (UPEC) are main cause of Urinary tract infections (UTIs). UPEC Biofilm-producer have a high level of resistance to antibiotics and this leads to recurrent episodes of UTIs. This study tested the effects of aqueous garlic extract (AGE) on biofilm formation and antibiotics susceptibility of multidrug resistant (MDR) UPEC. The ability for *in vitro* biofilm formation was detected in 35 MDR UPEC isolates in the absence and presence of sub-MICs of AGE (15, 30 and 60 mg/ml). The minimum inhibitory concentrations (MIC) of the AGE were determined by broth microdilution method. The biofilm formation by strong and moderate biofilm producers was evaluated by measuring the DO_{595}. we observed high resistance of UPEC (n=35) against the penicillin group, cephalosporin, Nalidixic acid, fluoroquinolone and tetracycline group In contrast, they were less resistance to Amikacin (17.15%), Imipenem (11.42%) and Gentamicin (5.71%) but None of the UPEC was completely sensitive to all the tested antibiotics. UPEC isolates were susceptible to AGE in the range of 29±2 - 32±4.5 mm and 24±1 - 25±1 mm of Diameter of zone inhibition respectively at 1000 and 500mg/ml. The MICs values were in the range of 62.5 and 100mg/ml. Sub-MICs AGE inhibited bacteria adhesion at 45.8, 78.8, and 81.1%, respectively. It also inhibited the biofilm formation and dispersal. The use of AGE in parenteral preparations to treat UTIs could greatly improve the clinical outcome. There is a continuous need for the development of new strategies for treatment of UTIs and recurrent episodes.

Introduction

Garlic has been used to treat bacterial related diseases such as pile, cough and rheumatism; and to alleviate tumor, cardiovascular diseases and ageing (Kallel *et al.*, 2015). Many decades now, garlic has been known for his antibacterial, antifungal, and antiviral properties (Frank *et al.*, 2014). According to several studies carried, it contains aromatic Sulphur, allicin, based compounds which contributes to its odor and taste (Chen *et al.*, 2018; Fratianni *et al.*, 2016). Garlic has been shown to have a wide spectrum antibacterial activity, including effects on *Escherichia*, *Salmonella*, *Staphylococcus*, *Streptococcus*, *Klebsiella*, *Proteus*, *Clostridium*, *Mycobacterium*, and *Helicobacter* species (Venâncio, *et al.*, 2017; Fratianni*et al.*, 2016).

In recent years, many bacteria have developed drug resistance; these include *Enterobacteriaceae*, such as *E. coli* (Wiedemann *et al*, 2014). Antimicrobial resistance in *E. coli* has been reported worldwide and increasing rates of resistance among *E. coli* is a growing concern in both developed and developing countries (Sabtu, *et al*, 2015; Rasamiravaka *et al.*, 2015). The frequency of antibiotic resistant infections has been raised permanently around the world and has been attributed to a combination of the selective pressure of antimicrobial use, microbial characteristics, social changes and increased use of antibiotics, and mistakes in infection control programs leading to incremented transmission of resistant microorganisms (Petty *et al.*, 2014;Wiedemann *et al.*, 2014). Multidrug resistant, poses treatment problems resulting in high morbidity, high mortality, and increased health care costs (Neupane *et al.*, 2016). The multidrug resistant (MDR) UPEC strains are major public threat worldwide (Nadembega *et al.*, 2017; Tängdén and Giske, 2015) and are highly prevalent in Togo (Toudji *et al.*, 2017).

Uropathogenic *Escherichia coli* (UPEC) is responsible for more than 80% of UTI in healthy people and are the most common isolates in patients with UTI (Toudji *et al.*, 2017; Nadembega *et al.*, 2017). It can caused a recurrent infection by the way of the biofilm production (Sara and Soto, 2014). Biofilm production is a mechanism exhibited by several microbes to survive in unfavorable conditions (Sara and Soto, 2014). The bacterial biofilm is a structured community of cells enclosed in polymeric matrix and adherent to a surface (Paterson, 2017; Soto, 2014). Biofilm producing uropathogenic bacteria may be responsible for many recurrent UTIs (Sarkar *et al.*, 2016) and highly resistant to antibiotic treatment (Neupane *et al.*, 2016). To establish a biofilm, the first step of UPEC is to adhere (Nicolle, 2014). The adherence of UPEC to uroepithelial cells establishes the initial occurrence of the pathogenesis of a urinary tract infection (UTI) (Nicolle, 2014). Therefore, UPEC biofilm makes UTIs difficult to eradicate. There is a significant interest in the development of other antimicrobial agents for infection control. Therefore, the objective of this study aimed to evaluate antimicrobial properties of aqueous garlic extract (AGE) on multidrug resistance, on adherence, on biofilm formation and dispersal and it could be an alternative to the antibiotics that can represent an opportunity to develop innovative treatment of UTIs as well as recurrent UTIs caused by resistant bacteria.

Materials and Methods

Plant materials

Fresh bulbs of *Allium sativum* L., commonly known as garlic were purchase from “ADWLATO” market, the famous spices market of Lomé, capital city of Togo and stored at Laboratory temperature 24±°C.

Biochemical characterization and preparation of garlic extract

Garlic peel was analyze for moisture, protein, carbohydrate, and ash in triplicate using standard methods (AOAC, 2000). Crushed garlic paste of 100 g was mix with 100 ml of double standard distilled water in a glass container to obtain a homogenous mix by stirring it occasionally for 4 days at 3-5°C. The mixture was filtered and centrifuged at 10 000 rpm for 20 min. The supernatant was filter through 0.22 mm filter (Wattman) to remove any impurities. This aqueous garlic extract (AGE) obtained was used for antimicrobial assay (Shenawy *et al.*, 2008).

Bacterial strains growth and identification

The study was perform on 35 MDR UPEC from inpatients and outpatients. All isolates were collect from Center of Regional Hospital of Lomé (Togo) from September 2017 to March 2018. All MDR UPEC isolates, as well as *E. coli* standard strain (ATCC 25922), were grown overnight in Trypticase Soy Broth at 37°C. Identification of the isolates was done by Gram staining and isolation on Uriselect agar (Oxoid, UK) and Brilliance E.coli (Oxoid, UK) media. API 20E (BioMerieux, France) was used to identify *Escherichia coli* strains.

The antibiotic susceptibility testing of 35 MDR UPEC isolates was perform by using double disk diffusion methods (Cockerill *et al.,* 2012). Tested antibiotics: amikacin (30 µg), amoxicillin/clavulanic acid (30 µg), ampicillin (10 µg), cefoxitime (30 µg), cefotaxime (30 µg), ceftazidime (30 µg), ceftriaxone (30 µg), ciprofloxacin (5 µg), gentamicin (15 µg), nalidixic acid (30 µg), imipenem (10 µg) and tetracycline (30 µg) were supply from (Oxoid, UK). *E. coli* strain ATCC 25922 was use as a reference strain and the result was interpret according to Clinical and Laboratory Standards Institute guidelines (EUCAST, 1993). The isolates were classify as MDR according to Magiorakos *et al.* (2012).

Determination of MIC

MDR UPEC, as well as *E. coli* standard strain (ATCC 25922), were grow with different concentrations of AGE. Controls were constituted with AGE without germs. All experiments were performing in triplicate (CLSI, 2012). The MIC of AGE was defined as the lowest concentration that inhibited visible bacterial growth.

Antibacterial activity of AGE

After adjusting the inoculum to a 0.5 McFarland unit turbidity standard, a sterile cotton swab was dip into the inoculum and rotated against the wall of the tube above the liquid to remove excess inoculum. Entire surface of plate count agar was swab three times, rotating plates approximately 60° between streaking to ensure even distribution. The inoculated plate was allow to stand for at least 3 min but no longer than 15 min before punching the wells in the agar plate. A hollow tube of 5mm diameter was take and heated. It was press on the inoculated agar plate and removed immediately after making a well in the plate. Likewise, four wells were make on each plate. 50µl of AGE (500mg/ml and at stock solution) were added into the respective wells on each plate. The plates were incubate within 15 min of compound application for 18-24 h at 37°C. The plates were read only if the lawn of growth was confluent or nearly confluent. The diameter of the inhibition zone was measure to nearest whole millimeter (CLSI, 2012).

Effect of AGE on curli production and *in vitro* biofilm formation

Curli fibers were determined using Luria-Bertani agar (L.B.) (Difco Laboratories, U.S.A) containing 40 mg/L congo red dye without salts (Aldrich Chemical Co. Ltd. England) (Hammar *et al.,* 1995). The morphotypes of each strain was determine by the morphology of the colonies after incubation for 24 h at 37°C. All plates were visually examine and the morphotypes were categorize as: Red, dry and rough (rdar) indicating expression of curli fimbriae and cellulose, brown (bdar), indicating expression of fimbriae but not cellulose, pink (pdar), indicating expression of cellulose but not fimbriae, smooth and white (saw), indicating expression of neither cellulose nor fimbriae. The effect of AGE on curli production by curli positive strains was tested using sub-MICs of AGE (15, 30 and 60 mg/ml).

The ability of *in vitro* biofilm formation was determined using the microtiter plate assay (Saroj Golia *et al.,* 2012) in a 96-well microtiter plate (Greiner Bio-one, Stuttgart, Germany), in the absence and presence of AGE (15, 30 and 60 mg/ml), in triplicates. The optical density was measured at 595 nm with ELISA reader (BioTek®, MQX 200, USA) and the degree of biofilm formation was estimated (Saroj Golia *et al.,* 2012).

Effect of garlic extract on Uropathogenic growth and Biofilm dispersal

To perform biofilm assay, two strains of MDR UPEC (UPEC moderate and strong biofilm producer) were use. UPEC moderate (Ec16) and strong biofilm producer (Ec33) were grow in LB broth overnight (37°C, 170 rpm) and the culture was dilute to a concentration of $2x10^7$ CFU/mL in LB broth with sub-MICs of AGE or in LB broth only. Then, 200 µL of aliquots were loaded into a 96-well polystyrene microtiter plate and the plate was incubated for 24 h at 37 °C to allow biofilm formation. After the incubation, the wells were wash twice with PBS to remove non-adherent bacteria. As described elsewhere, the biofilm formation was quantified using the crystal violet assay (Stepanović*et al.,* 2000). Briefly, wells were gently wash with deionized (DI) water and air dried for 10 min. Then, 200µL of 0.1% crystal violet in PBS was added to each well and the plate was incubated at 37°C for 15 min. Wells were washed again with DI water, air dry and 200 µL of 95% ethanol was added. The crystal violet bound to the biofilm was solubilize in the ethanol solution and the OD at 595 nm was measure to estimate the biofilm formed. In order to decrease potential bias, biofilm level was normalize to the level of bacterial growth (determined previously). Biofilm dispersal assay was also perform.

As described above, UPEC were grown in LB broth overnight, diluted in fresh LB broth, and inoculated into a 96-well plate. After the biofilm formed, sub-MICs of AGE in LB broth or fresh LB broth only was added and the plate was incubated in 37°C for another 1 h. The biofilm was also quantified using the crystal violet assay. The absorbance of control was ODA and the absorbance of AGE treated biofilm was ODB. The biofilm dispersal level was defined as $(OD_A - OD_B)/OD_A$.

Data Analysis

All analyses were carried out in triplicate. The data analysis was done using the Microsoft excel and Minitab 16. The effects of AGE at various concentrations were quantitatively evaluated by One-way analysis of variance (ANOVA). The anti-adhesion against UPEC was compared under aqueous garlic extract treated and untreated conditions using student's pair *t*-test. Statistical significance was accepted at the $P < 0.05$ level.

Results

Biochemical analysis of Garlic

The results of the proximate composition of garlic are show on Table 1. Garlic has high moisture (62±2%) and 24±1.50% carbohydrate. The oil content is 2.8±1% while the total ash content and the protein content are 0.20±0.06% and 11±2% respectively.

Table 1: Proximate composition of 100g Garlic

Component	Percentage
Oil content	2.8±1
Carbohydrate	24±1.50
Protein	11±2.00
Total ash content	0.20±0.06
%Moisture	62±2

Values are mean ±SD of three replicates

Bacterial strains, identification and antibiotic susceptibility

35 MDR UPEC isolates presumptively identified using the conventional culture methods and API 20 E test, are include in the study.

The antibiotic susceptibility profiles of the isolates against 12 antimicrobial agents UPEC are represent in figure 1 and table 2.

Table 2: Antibiotics susceptibility of Uropathogenic *E. coli*

Antibiotics (code and Concentration µg/ml)	**Interpretive break points**	UPEC (n=35)	
	R< - S>	**S (%)**	**R (%)**
Amikacine (AK 30)	13 - 16	29/35 (82.85)	6/35 (17.15)
Gentamicine (CN 15)	14 - 17	33/35 (94.29)	2/35 (5.71
Amoxicillin+ clavulanic acid (AMC 30)	16 - 19	0/35 (0)	35/35 (100)
Ampicilline (AM 10)		7/35(20)	28/35 (80)
Ceftriazone (CRO 30)	20 - 23	15/35(42.86)	20/35(57.14)
Cefotaxime (CTX 5)	17 - 20	15/35(42.86)	20/35 (57.14)
Ceftazidime (CAZ 30)	19 - 22	15/35 (42.86)	20/35 (57.14)
Cefoxitine (FOX 30)	15 - 19	10/35(28.57)	25/35(71.43)
Imipenème (IMP 10)	16 - 22	31/35(88.57)	4/35 (11.42)
Tetracycline (TE 30)	17 - 19	6/35(17.14)	29/35 (82.85)
Nalidixic acid (NA 30)	15 - 20	10/35(28.57)	25/35(71.43)
Ciprofloxacine (CIP 5)	22 - 25	14/35(40)	21/35(60)

S: susceptible; R: resistance

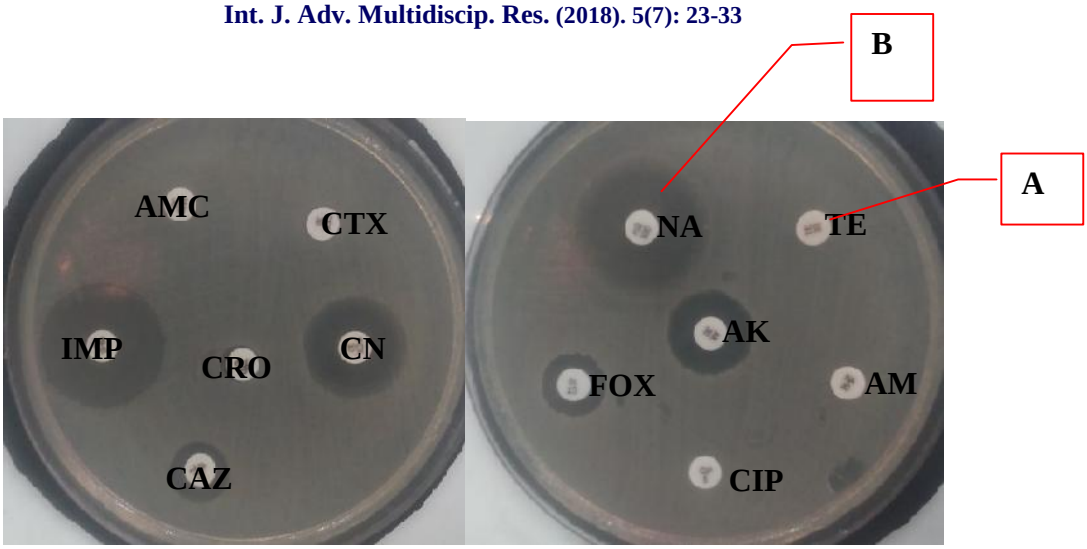

Figure 1 : Antibiotic susceptibility of Uropathogenic *E. coli*
Legend: A: antibiotic disc; B: zone inhibition. The absence of an inhibition zone around the antibiotic disc means that the *E. coli* strain is 100% resistant to this antibiotic. For example the case of antibiotics Tetracycline (TE), Ciprofloxacin (CIP), Cefotaxime (CTX), Ceftriaxone (CRO)

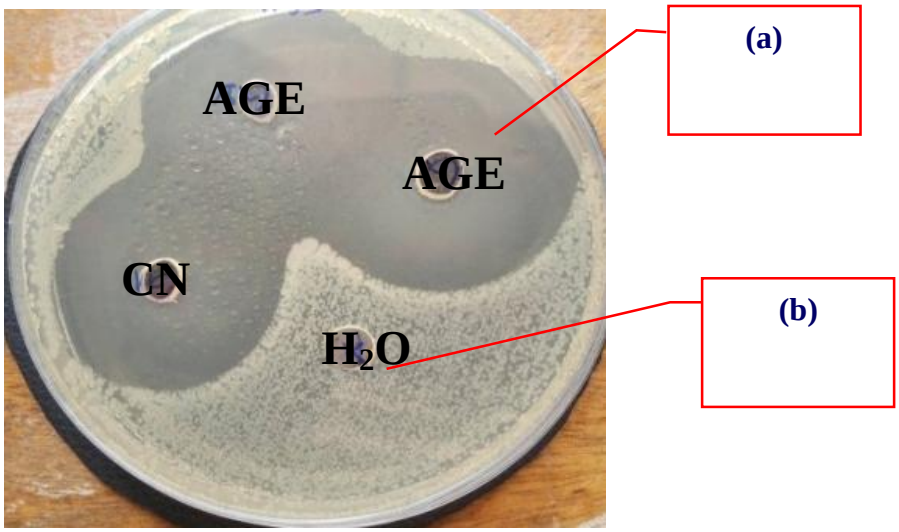

Figure 2: Inhibition of *E. coli* by Aqueous Garlic Extract at 500mg/ml.
Legend: H_2O: negative control; CN (Gentamicin): Positive control; AGE: aqueous garlic extract. (a): zone inhibition; (b): no inhibition zone around de whole hole.

In supplementary, in table 2 we observe high resistance of UPEC (n=35) against the penicillin group, cephalosporin, Nalidixic acid, fluoroquinolone and tetracycline group while the less resistance is observe against the Carbapenem and Glycopeptide groups. None of the isolates bacteria strains is completely sensitive to all the tested antibiotics. Resistance to amoxicillin/clavulanic acid, tetracycline, ampicillin, cefoxitin, nalidixic acid and ciprofloxacin are 100%, 82.85%, 80%, 71.82%, 71.43% and 60% respectively. In contrast, they are less resistance to Amikacin (17.15%), Imipenem (11.42%) and Gentamicin (5.71%).

Antibacterial activity of garlic extracts

The garlic antibacterial activity is represent in Figure 3 and table 3. The sizes of inhibition zones are inversely proportional to the increase in dilution of AGE. The diameter zone of inhibition of stock solution (1000mg/ml) and 500mg/ml AGE (1/2 dilution) various respectively to 29±2 - 32±4.5 mm and 24±1 - 25±1 mm on MDR UPEC. The MIC value of AGE on UPEC MDR are in the range of 62.5 – 100 mg/ml and the MBC of AGE are 125mg/ml (Table 3).

Table 3: Diameter of zone inhibition (mm) of AGE and MICs value on Multidrug resistance Uropathogenic *E. coli*

Strains	Diameter of zone inhibition (mm)		MIC	MBC
	1000(mg/ml)	500(mg/ml)		
E. coli ATCC 25922	34±1 - 36±2	26±1 - 28±1	62,5	62,5
MDR UPEC (n=35)	29±2 - 32±4.5	24±1 - 25±1	62,5 - 100	125

Effect of AGE on *in vitro* biofilm formation and curli production

The effect of AGE on *in vitro* biofilm formation and curli production are show on figure 3a and 3b. The degree of biofilm formation in the tested MDR UPEC clinical isolates reveal on microplate titer that 71.43 (25/35), 20 (7/35) and 8.52% (3/35) of the isolates are strong, moderate and weak biofilm producers, respectively.

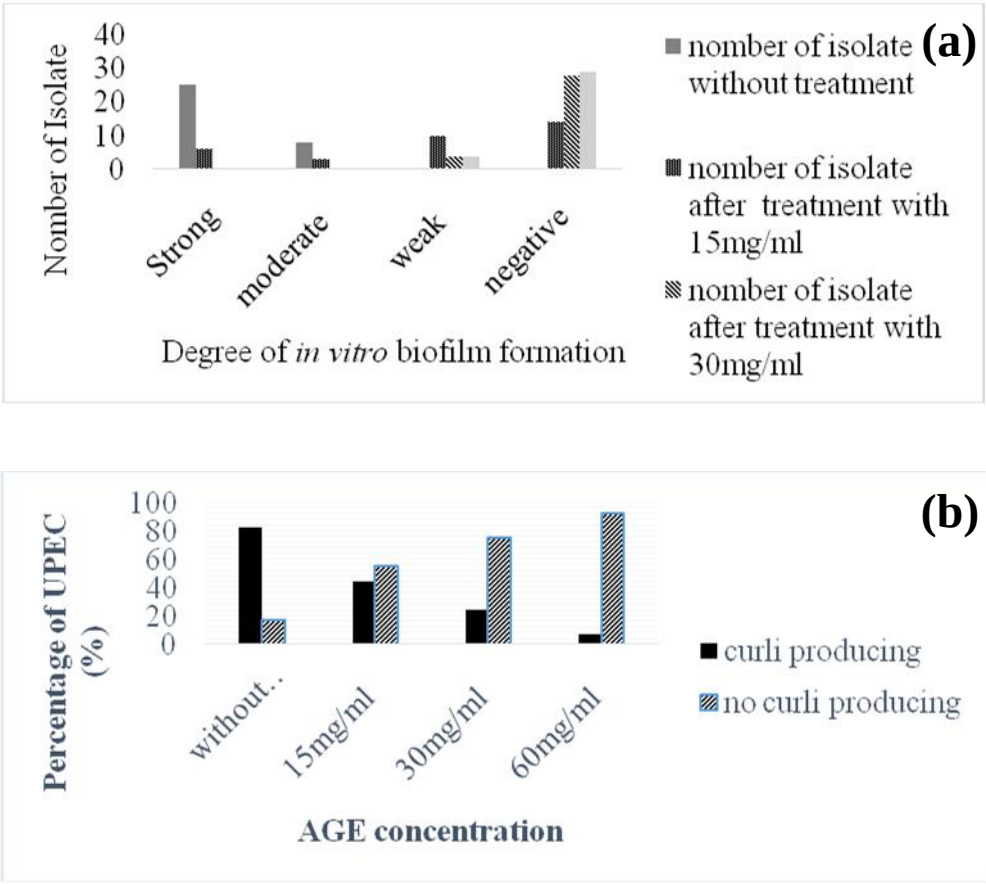

Figure 3: The effect of addition of 15, 30 and 60mg/ml AGE on biofilm formation.
Legend: effect of sub-MICs on biofilm formation by MDR UPEC strong and moderate biofilm producer (a) and on curli production (b)

The degree of *in vitro* biofilm formation is determine for strong and moderate biofilm producers (32 isolates) in the presence of sub-MICs of AGE (15, 30 and 60 mg/ml). The ability of *in vitro* biofilm formation decrease with the increase in AGE concentration as shown in Figure 3; after the addition of 15 mg/ml AGE, the result where 43.75 (14/32), 28.12 (9/32), 9.37 (3/32) and 18.75% (6/32) arerender negative, weak, moderate and still strong (no effect) biofilm-producers, respectively. Also, 84.38 (27/32), 12.5 (4/32) and 3.12% (1/32) were rendered negative, weak and still strong (no effect) biofilm producers, respectively after the addition of 30 mg/ml AGE. The addition of 60 mg/ml AGE are rendered negative and weak biofilm producers respectively 87.5 (28/33) and 12.5 (4/33).

The curli production is detect in 82.85% (29/35) of teste isolates. The ability for curli production is teste in the presence of sub-MICs of AGE (15, 30 and 60 mg/ml), where the ability of curli production decrease by increasing the concentration of AGE; 55.17% (16/29), 75.86% (22/29) and 93.10% (27/29) of curli producing isolates are negative producers after the addition of 15, 30 and 60 mg/ml AGE, respectively.

The Effect of garlic extract on Uropathogenic *Escherichia coli* growth and biofilm dispersal.

Garlic effect on MDR UPEC moderate and strong are present in Figure 4 and 5.

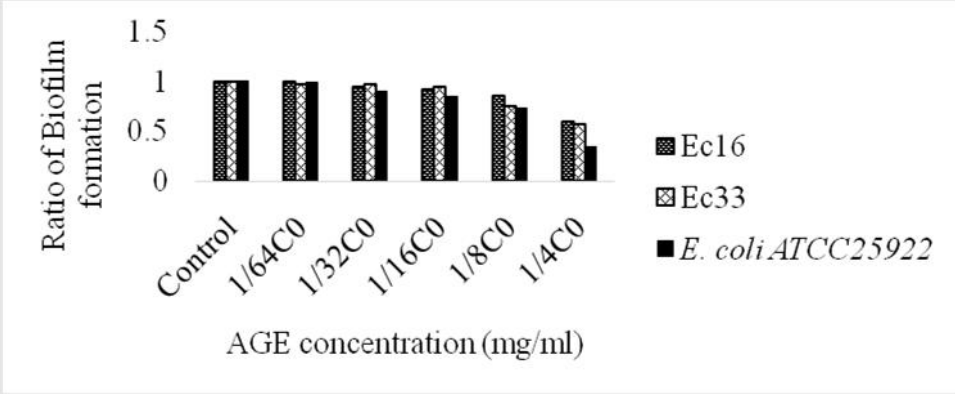

Figure 4: The growth of *E. coli* ATCC 25922, Uropathogenic *Escherichia coli* (UPEC) Ec16 and Ec33 are evaluate by measuring optical density (OD) value at 600 nm after 24 h exposure to AGE and represent as growth ratio relative to control.

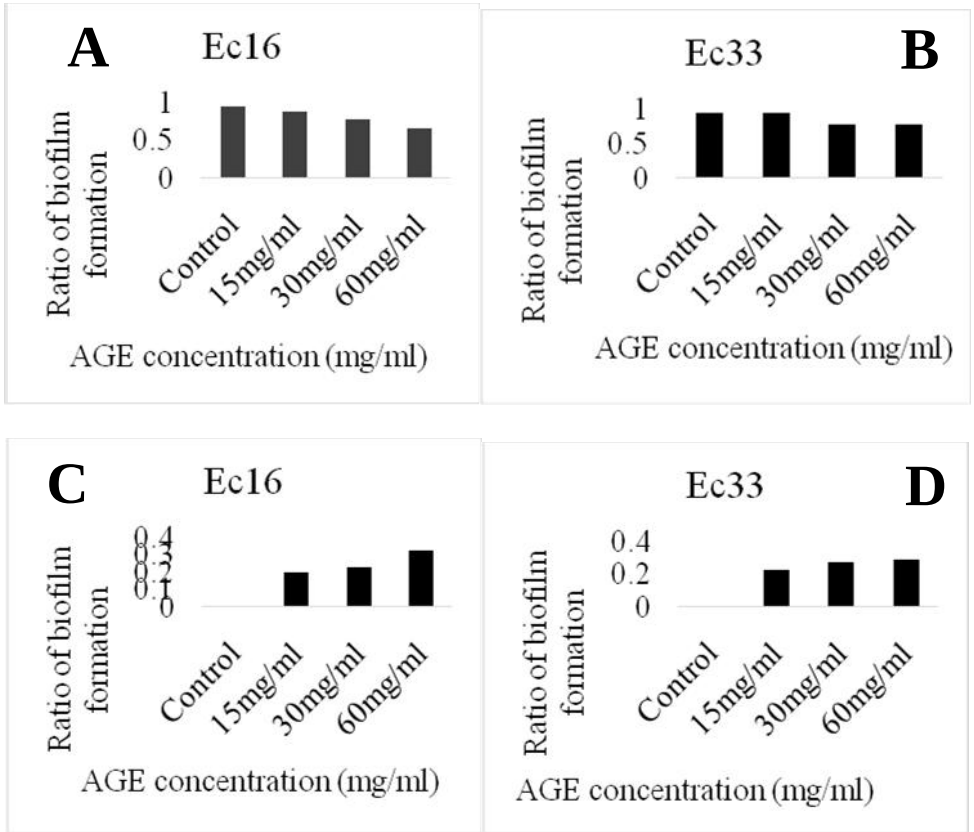

Figure 5: The effect of sub-inhibitory concentrations (sub-MICs) of AGE on UPEC biofilm formation (**A**, **B**) and dispersal (**C**, **D**).
Legend: The biofilm formation is evaluate by measuring OD value at 595 nm and represente as ratio of biofilm formation relative to control.
Ratio of biofilm dispersal = (OD value of control – OD value of AGE treatment)/OD value of control.

UPEC Ec16 and Ec33 are grow in LB broth in the presence and absence of AGE. Concentrations above 62.5 mg/mL of AGE slightly decrease the growth of Ec16 and Ec33 (Figure 4). A similar trend is observed for *E. coli* ATCC 25922 in Figure 4. Sub-MICs of AGE (15, 30 and 60 mg/mL) were chosen for subsequent experiments since their effect on the growth of UPEC was negligible. The UPEC biofilm levels are present in Figure 5 for both biofilms pre-treated or post-treated with AGE. In the case of AGE pre-treatment, Ec16 and Ec33 formed less biofilm when incubate with AGE. 30 and 60 mg/mL AGE decreased Ec16 biofilm by 17 and 28% compared to the control respectively (Figure 5A) while Ec33 biofilm formation was approximately 17% for the two sub-MICs (Figure 5B). Furthermore, all concentrations of AGE tested dispersed the biofilm significantly. Ec16 biofilm dispersed approximately 23 and 32% compared to the control (untreated biofilm) (Figure 5C) and 28 and 29% for Ec33 biofilm (Figure 5D) when post-treated with 30 and 60 mg/mL of AGE for 1 h respectively. Thus, AGE can inhibit UPEC biofilm formation and promote their dispersal with 30 and 60 µg/mL of AGE for 1 h. Thus, AGE can inhibit UPEC biofilm formation and promotetheir dispersal.

Discussion

UTI is a major cause of morbidity and may sometimes lead to mortality (Rasamiravaka *et al.,* 2015) and represents a major health threat due to antibiotic resistance and high recurrence rate (Nadembega *et al.,* 2017). Microbial biofilms in UTI play an important role in antibiotic resistance and limits the therapeutic options (Sara and Soto, 2014), so the effect of AGE on *in vitro* biofilm formation and antibiotic susceptibility of MDR strong and moderate biofilm producing UPEC clinical isolates from Togo was studied.

The dissemination of antibiotic-resistant bacteria contributes to morbidity and mortality from infectious diseases (Rasamiravaka *et al.,* 2015: Neupane *et al.,* 2016). Emergence of resistance is a major threat and started to increase in Togo (Toudji *et al.,* 2017), and in this study, UPEC isolates (57.14% – 100%) showed high resistance to penecilline group, cephalosporin fluoroquinolone, and tetracycline groups, and while resistance to cefoxitime and amikacin was found to be 28.57% and 17.15%, respectively. Amikacin has to be administered parenterally and it is nephrotoxic. However, isolates were found more sensitive to gentamicin (5.3%) and imipenem (11.42%). These findings were in accordance with other studies (Toudji*et al*, 2017; Mohammad *et al.,* 2015). Increased resistance might be due to widespread, inappropriate use of antibiotics and transfer of resistance genes in these isolates (Mohammad *et al.,* 2015). It is remarkable that antibiotics were not able to kill all the UPEC, and thus, it did not eradicate the UTI. A novel antimicrobial will be found to treat the UTI caused by MDR UPEC.

Plant based medicine gain much popularity in developing countries due to fewer side effects and ease in availability (Khan *et al.,* 2016). Researchers are focusing on medicinal aspects of plant and explore their contents that are beneficial in cure of various health disorders (Khan *et al.,* 2016). Chemical composition analysis plays a vital role to assess the nutritional quality and quantity of plant materials constituting the human diet. In this study, garlic moisture, carbohydrate, oil content, total ash and protein were different from the findings of other authors (Chen *et al.,* 2018; Fratianni *et al.,* 2016). In this study, aqueous garlic extract at stock solution showed maximum inhibition against MDR UPEC (32±4.5mm) and 29±2 mm as minimum inhibition (Table 3). The MIC observed for *A. sativum* in the present study (62.5 - 100 mg/mL) against UPEC was lower than MIC observed by Chen and al. (2018) which observed 11.25 mg/mL. Probably, the difference is due to environmental conditions. Garlic is well-recognized anti-*Escherichia coli* agent against both multidrug resistance Uropathogenic *E. coli* in an *in vitro* model (Gupta *et al.,* 2015; Tshibind *et al.,* 2014) as observed in the present study.

Biofilms are the basis of many persistent diseases (Sara and Soto, 2014). Most of the important infections results from bacterial adherence on some tissue surface (Sarkar, 2016, Wiedemann *et al.,* 2014). Adhesion ability are necessary for biofilm establishment (Sarkar *et al.,* 2016) and they are also important for maintaining virulence and pathogenicity of UPEC. Therefore, it is important to carry out studies on novel strategies against adhesion and biofilm formation. Natural products or food-derived (garlic) agents are attracting great attention for their antimicrobial activity (Chen *et al.,* 2018).

In the present study, garlic was used to eradicate *in vitro* biofilm production. The sub-MICs AGE inhibited curli production, at 54.28% (19/35), 74.28% (26/35) and 91.43% (32/35) respectively, and reported to be effective in inhibiting adhesion in 81.1% of tested isolates. A similar study with allicine, one of garlic compound, in China showed that concentrations

of 10 and 20 mMcan inhibit curli production (Yang *et al*., 2016). The garlic ability to affect the biofilm formation and dispersal was investigate. Sub-MICs exhibited no effect on UPEC growth but decreased biofilm formation (Figure 4). Ahmad *et al.*, (2015) have seen the similar result on *Proteus mirabilis*. Moreover, a major established biofilm dispersal was observed under the treatment of AGE. AGE quickly broke up the biofilms of UPEC within 1 h. Therefore, AGE may be used to protect the immune system so that these antibiotics are able to kill these dormant cells, which are likely to be responsible for UTIs (Wiedemann *et al.*, 2015). As we know, initial attachment of UPEC not only aids to establish biofilm (Ahmad *et al.*, 2015), but also results in colonization and invasion to host cells (Floyd *et al.*, 2015). It is clear that one of the most efficient way to prevent UTI is to reduce the attachment of UPEC.

Conclusion

The antimicrobial properties of aqueous garlic extract (AGE) as an antimicrobial agent on multidrug resistance, on adherence, on biofilm formation and dispersal was evaluated.

The high prevalence of MDR phenotype among strong biofilm producers UPEC from Togo is recorded and the multidrug resistance, biofilm formation, adhesion ability, and disperse developed UPEC biofilm are decrease by aqueous garlic extract at MIC and sub-MICs. These effects may be due to the influence of garlic on virulence factor. There is a continuous need for the development of new strategies for treatment of UTIs and recurrent episodes a further investigations are necessary to appreciate the mechanism action of garlic on virulence factors and the effect in *in vivo.*

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