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Anti-biofilm activity of ibuprofen and diclofenac against some biofilm producing *Escherichia coli* and *Klebsiella pneumoniae* uropathogens

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Ibuprofen (IBU) and Diclofenac (DIC) are non-steroidal anti-inflammatory drug which have shown to have broad antimicrobial activity and limit biofilm formation. Aim of this study was to evaluate the effects of IBU and DIC against biofilm formation strong strains *Escherichia coli* and *Klebsiella pneumoniae* associated with urinary tract infections (UTIs). Additionally some virulence factors, antibiotic resistance, ESBL production were estimated to find correlation with the biofilm production and acil homoserine lactone (AHL). Our results suggest that IBU and DIC, could be useful in the treatment of urinary tract infections caused by *E. coli* and *K. pneumoniae.* IBU with CMIB 8, 30 and 125 mg/L, caused dramatic reductions in some cases and inhibited virulence factors which changes in the morphotypes expressed on congo red agar. DIC with CMIB 30 and 50 mg/L caused reductions in biofilm formation 50.1%. In this study, were evidenced the relationship between virulence factors, production of AHL and multi drugs resistance with biofilm production.

Key words: Diclofenac, ibuprofen, biofilm, acil homoserine lactone (AHL), *Escherichia coli*, *Klebsiella pneumoniae*.

INTRODUCTION

Escherichia coli and *Klebsiella pneumoniae* are the two predominant pathogens commonly isolated in urine.

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These uropathogens have also developed resistance to commonly prescribed antimicrobial agents, exerts their antimicrobial resistance against beta-lactams by producing extended spectrum beta-lactamases (ESBLs) enzymes that confers bacterial resistance to all beta-lactams except carbapenems, cephamycins and clavulanic acid (Ullah et al., 2009), severely limits the treatment options of an effective therapy and pose treatment problem resulting in high morbidity, high mortality, and increased health care costs (Schwaber and Carmeli, 2007). Uropathogenic bacteria may be responsible for many recurrent UTIs by biofilm producing. A biofilm is a complex aggregate of microorganisms in which cells adhere to each other and to a surface in a self-produced matrix of extracellular polymeric substance/slime. Biofilm production is a mechanism exhibited by several microbes to survive in unfavorable conditions. Bacteria communicate with one another using chemical signal molecules, this process of chemical communication called quorum sensing (QS), exerts a great influence on the production of virulence factors through control of transcriptional regulators as a result of changes in population density (Waters and Bassler, 2005). QS operate through a wide range of signals such as: (1) Oligopeptides, (2) Acylhomoserine lactones (AHLs), (3) Furanosyl borate (autoinducer-2), and (4) Fatty acids (McDougald et al., 2007). Most QS systems described to date in gram-negative bacteria depend on N-acyl-homoserine lactones (AHL) as signal molecules, and these QS systems found in more than 100 bacterial species (Viswanath et al., 2015).

Bacteria within biofilms are intrinsically more resistant to antimicrobial agents than planktonic cells. Antimicrobial concentrations sufficient to inactivate planktonic organisms are generally inadequate to inactivate biofilm organisms. Antibiotic resistance can increase 1000 fold (Stewart and Costerton, 2001). According to a research, more than 60% of all infections are caused by bacteria growing in biofilms (Lewis, 2001). These infections tend to be chronic as they resist innate and adaptive immune defense mechanisms, and the treatment presents a considerable unmet clinical need. To date, there are no drugs that specifically target bacteria in biofilms; however, several approaches are in early-stage (Marvig et al., 2012).

Several reports sugared when designing and testing new drug candidates, it is important that the biofilm phenotype is taken into consideration. Recently non-steroidal anti-inflammatory drug (NSAID) that specifically blocks the biosynthesis of mammalian prostaglandins by inhibiting one or both of the COX isoenzymes has been shown that dramatically decrease biofilm production in fungal and bacteria (Alem et al., 2004). In this study, we evaluated the effects of ibuprofen and diclofenac drugs against isolates from of urinary tract infection with ability to strong biofilm formation. Additionally, some virulence factors, antibiotic resistance, ESBL production, detection and identification of signaling molecules involved in the quorum sensing were estimated. This is the first this type of study conducted in Colombia.

MATERIALS AND METHODS

Bacterial strains

A total of 100 isolates obtained from patients with UTI's who were referred to two hospitals in Cartagena, Colombia, were included in this study. Specimens collected were clean catch midstream urine (54%) and urine from vesicular sounds (46%) using standard sterile procedures. The isolates were identified based on observing colonial morphology on EMB medium. Lactose-fermenting colonies were confirmed using standard biochemical tests (Voges-Proskauer, methyl red, and nitrate reduction, motility at 37°C, indole production and gas production from glucose). All the strains were stored in thioglycolate broth with 15% glycerol at -80°C. *E. coli* ATCC 25922, *K. pneumoniae* ATCC 700603 and *Staphylococcus aureus* ATCC 25923 were used as control in all tests.

Antibiotic susceptibility testing

Antibiotic susceptibilities were determined according to the manufacturer's recommendations by overnight microdilution method with commercial dehydrated panels (NUC 60) provided by Dade Behring MicroScan (Sacramento, Calif.) that were read by the autoSCAN-4 and interpreted according to Clinical Laboratory Standards Institute (CLSI) guidelines (CLSI, 2010). Multidrug resistance (MDR) was defined as non-susceptibility to at least one agent in three or more antimicrobial categories (Magiorakos et al., 2012).

Detection of ESBL

Phenotypic screening of ESBL

Isolates were screened for resistance to three oxyimino-cephalosporins: Ceftazidime (30 µg), cefotaxime (30 µg), ceftriaxone (30 µg) and the monobactam: Aztreonam (30 µg) by disk diffusion test. Zone diameters were read using criteria (CLSI, 2012). An inhibition zone of ≤17 mm ceftazidime, ≤ 22 mm cefotaxime, ≤ 19 mm ceftriazone and ≤17 mm aztreonam indicated a probable ESBL producing strain requiring phenotypic confirmatory testing.

Phenotypic confirmatory method of ESBL

ESBL production was detected by the double disc synergy test (DDST) using clavulanic acid-amoxicillin (20/10 µg) and ceftazidime (30 µg), cefotaxime (30 µg), aztreonam (30 µg) and cefepime (30 µg) on Mueller Hinton agar as recommended by French Society for Microbiology (FSM, 2009) (http://www.sfm-microbiologie.org). The presence of ESBL was manifested by the synergistic effect of the inhibitor and discs (effect of egg, fish tail or American soccer ball) (Lezameta et al., 2010).

Analysis of biofilm formation capacity

Congo red agar method by

Suspension of tested strains was inoculated onto a specially

prepared solid medium brain heart infusión broth (BHI) supplemented with 5% sucrose and Congo red The medium was compose of BHI (37 g/L), sucrose (50 g/L), agar no.1 (10 g/L) and Congo red stain (0.8 g/L). Congo red was prepared as concentrated aqueous solution and autoclaved at 121°C for 15 min, separately from other medium constituents and was then added when the agar had cooled to 55°C. Plates were inoculated and incubated aerobically for 24 to 48 h at 37°C (Freeman et al., 1989).

Screening of morphotypes (Congo red agar assay)

The morphotypes of each strain was determined by the morphology of the colonies after incubation for 24 h at 37°C. All plates were visually examined and the morphotypes were categorized 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 and smooth and white (saw), indicating expression of neither cellulose nor fimbriae (Bokranz et al., 2005).

Microtiter plate assay (quantitative assays for biofilm formation)

A crystal violet staining method was employed to examine biofilm-forming abilities of the isolates (O'Toole and Kolter, 1998) with modifications. The isolates were inoculated into 1 mL LB broth and grown overnight at 37ºC with constant shaking. Overnight cultures were transferred to new culture medium (diluted by 1:100) and grown to OD 600 between 0.45 and 0.65 and for each strain assay, it was done in triplicate. Thirty microliters of bacteria in log phase growth were inoculated into 96-well polystyrene plates containing 100 mL fresh LB broth and incubated at 37°C for 24 h. The plates were rinsed 3 times with deionized water and the adherent bacteria cells were stained with 0.5% crystal violet for 30 min. After being rinsed 3 times with deionized water, the crystal violet was liberated by 80% ethanol and 20% acetone following a 15 min incubation. The OD values of each well were measured at 492 nm. The tested strains were classified according to the criteria of Stepanovic et al. (2007) into non-biofilm producer (OD ≤ ODc), weak biofilm producer (OD > ODc, but ≤ 2x ODc), moderate biofilm producer (OD>2x ODc, but ≤ 4x ODc), and strong biofilm producer (OD> 4x ODc).

Detection of AHL by colorimetric method

Extraction and quantification of the acyl homoserine lactone activity was performed as described by Dietrich et al. (2010). The isolates were incubated in 5 mL Mueller Hinton broth overnight at 37°C. 1.5 mL of the suspension was centrifuged at 10,000 rpm for 15 min, the supernatant was transferred and this step was repeated twice. Subsequently liquid-liquid extraction using ethyl acetate for 10 min and the organic phase (top) was removed. Next, the samples were dried at 40°C, they transferred 40 µL to a microplate and added 50 µL of 1:1 solution of hydroxylamine 2 M: NaOH 3.5 M and 50 µL of 1:1 solution of $FeCl_3$ 10% in HCl 4 M: Ethanol 95%. Finally, the optical density at 520 nm was measured in a plate reader.

Determination of AHL functional groups

N-Acyl homoserine lactone functional groups were identified by FT-IR as described by Shikh-Bardsiri and Shakibaie (2013), in five well-characterized and strong biofilm-producers *E. coli* (3) and *K. pneumoniae* (2) strains isolated. *E. coli* ATCC 25922 and *K. pneumoniae* ATCC 700603 were used as controls. Briefly, the AHL extract of each sample was mixed with 100 mg potassium bromide (KBr) and subjected to Shimadzu 8400S FT-IR Spectrometer; pellets were scanned at 6 cm^{-1} resolution in the spectral range of 4000 to 500 cm^{-1}. Peaks at 1764.33, 1377.99, and 1242.90 cm^{-1} correspond to the C=O bond of the lactone ring, the N=H bond, and the C-O bond, respectively. These results support the AHL data and confirm the presence of a lactone ring in the AHL (Taghadosi et al., 2015).

Anti-biofilm activity of diclofenac (DIC) and ibuprofen (IBU) on biofilm formation

The patterns IBU and DIC were obtained of Drug Analysis Laboratory (LAM), Faculty of Pharmaceutical Sciences at the University of Cartagena. The effect of IBU and DIC in solution was assessed on the ability to form biofilms by *E. coli* strains and *K. pneumoniae* previously identified as strong biofilm forming using concentrations, 2, 8, 30 and 125 mg/L IBU and 5, 15, 30 and 50 mg/L of DIC; sub-MIC concentrations as reported (Naves et al., 2010; Reślíński et al., 2013). The biofilm producer *K. pneumoniae* ATCC 700603 strain and the non-biofilm producer *E. coli* ATCC 25922 were used as controls.

The assays were done as previously mentioned for biofilm formation, after carrying out incubation in LB broth at 37°C for 24 h, 10 µL of the suspension was transferred to polystyrene plates of 96 wells, 180 µL of LB with then he added 10 µL of solution treatment and incubated for 24 h at 37°C. Washing, staining the biofilm and subsequent reading it was performed as described previously. Untreated strains were used as negative control (O´Toole and Kolter, 1998).

Minimum inhibitory concentration (MIC) was defined as the minimum concentration of a compound that produces reductions of at least half of OD 630 nm values compared with an untreated control and minimum inhibitory biofilm concentration (MBIC) of each compound was defined as the minimum concentration that reduces biofilm formation significantly compared with an untreated control. To exclude diminutions of biofilm formation due to inhibitory effects on bacterial growth, sub-inhibitory concentrations of each compound were selected for testing (Naves et al., 2010).

Statistical analysis

All assays were done in triplicate in independent assays. The effect of compounds in biofilm production was evaluated with paired two-tailed Student's t-test. $P < 0.01$ was considered significant. Statistical Package GraphPad Prism 7 was used for data analysis. P-value < 0.05 was considered as statistically significant.

RESULTS AND DISCUSSION

Characterization of *E. coli* and *K. pneumoniae*

100, non-repeat, clinical isolates of *E. coli* (83 isolates) and *K. pneumoniae* (17 isolates) collected over a period of 12 months, were studied. Phenotypic identification of each isolate was performed based on conventional biochemical test (Table 1).

In the past years have been reported an increased incidence of UTI due to ESBL-producing *E. coli* and *K. pneumoniae*. In this study we found that ESBL production was seen in 44 (23.81%) isolates of Enterobacteriaceae. ESBL production was higher in *E. coli* followed by *K.*

Table 1. Biochemical tests used to identify *E. coli* and *K. pneumoniae*.

Biochemical test	*E. coli*	*K. pneumoniae*
Catalase	+	+
Triple sugar agar TSI	+	+
Indole production	+	-
Methyl red	+	-
Urease	-	+
Voges proskauer	-	+
Simmon´s citrate	-	+
H_2S	-	-

*Results based on the types of substrate utilization: Positive (+) and negative (-).

Figure 1. ESBL production double disc synergy test (DDST) using clavulanic acid-amoxicillin (20/10 µg) and ceftazidime (30 µg), cefotaxime (30 µg), aztreonam (30 µg) and cefepime (30 µg) on Mueller Hinton agar.

pneumoniae. 83% of the strains identified as *E. coli*, found that 12.05% were ESBL producers and for *K. pneumoniae* total (17%) strains were 11.76% ESBL-producing strains (Figure 1).

These findings are in agreement with previous studies such as was reported by Akram et al. (2007) and Kalsoom et al. (2012) on urinary infection also showed that *E. coli* and *Klebsiella* spp. are the most common urinary pathogens in UTI. A study by de Paz et al. (2015) reported a higher prevalence of ESBL-producing strains of *E. coli* that found in this research with 21.5%. As it has been reported that the overall prevalence of ESBL producing *K. pneumoniae* is about of 17.7% (Iabadene et al., 2008).

In this contribution we report an antimicrobial activity and outcomes in 100 isolates subject to biofilm detention. The antimicrobial resistance profiles for the isolates were determined using the MicroScan Gram Negative Urine Combo Panel Type 60 of 20 antimicrobials and breakpoints for classification as resistant used were determined according to the guidelines of the (CLSI, 2010) (Table 2).

The outcomes of susceptibility testing performed on the panel MicroScan show that only 6 of the 22 antibiotics tested (amikacin, doripenem, ertapenem, meropenem and combinations cefotaxime/acid clavulanate, ceftazidime/clavulanic acid) were effective against more than 95% of the strains tested.

Prakash et al. (2013), reported similar data to those obtained where the greatest resistance of *E. coli* and *K. pneumoniae* to antibiotics such as ciprofloxacin, ceftazidime, cefotaxime and ceftriaxone. And they are also very sensitive to antibiotics such as carbapenems meropenem and imipenem, amikacin, and aminoglycoside.

In our study, was observed that half of antibiotics evaluated showed a degree of resistance between 40 and 50% of the strains, and even antibiotics often used as ciprofloxacin and ampicillin, it was found that 55 and 75% respectively of the strains were resistant. In addition, they identified about 20% of strains with ESBLs were so resistant to antibiotics such as aztreonam, cefotaxime, ceftazidime, and ceftriaxone. 60% of the strains were sensitive to antibiotics such as ceftriaxone, aztreonam and ceftazidime. For cefotaxime and amoxicillin/ clavulanate a smaller percentage, 42 and 45% respectively was found. And more than 20% of strains were resistant to these antibiotics, highlighting cefotaxime, in which 40% of the strains were resistant and 20% intermediate. These data are confirmatory of susceptibility testing by the MicroScan panel, obtaining again, resistance in 60% of strains with the group of antibiotics evaluated compared with 55% obtained in antibiogram. These outcomes are similar to those reported previously by Arce et al. (2012) where it was found that the resistance of *E. coli* and *K. pneumoniae* isolated from patients with UTI against ceftazidime, cefotaxime, ceftriaxone, and aztreonam are about 40, 45, 32 and 20%, respectively (Stepanović et al., 2004).

The rate of multiple antibiotic resistance (MAR) was determined for each of the strains. 65% of isolates had an index ≥ 0.2, indicating that the bacterium has multiple resistance (Vanegas et al., 2009). These outcomes have a huge impact when selecting empiric treatment of these infections. Since, in general, *E. coli*, as in other studies (Alós et al., 2005; Andreu et al., 2005), shows a high rate of resistance to most of the antibiotics greater clinical use. The use of antibiotics with limited antimicrobial activity in the ITU is associated with lower clinical efficacy in pyelonephritis, with more failures and less removal, and serious infections were associated with increased mortality, and therefore must be avoided as initial empiric treatment these infections (Gomez, 2007). The choice of antibiotic treatment for UTI must be based on the results of urine culture and sensitivity testing. However, in

Table 2. Antibiotic susceptibility pattern of *E. coli* and *Klebsiella* species isolates.

Antimicrobial Agent	Interpretive break points		S (%)	R (%)	I (%)
	Susceptible	Resistant			
Amikacin	≤16	≥64	96	2	2
Amp/Sulbactam	≤8/4	≥32/16	34	46	20
Ampicilin	≤8	≥32	25	75	0
Aztreonam	≤8	≥32	72	27	1
Cefazolin	≤8	≥32	61	31	8
Cefepime	≤8	≥32	73	27	0
Cefotaxime	≤8	≥64	73	27	0
Cefoxitin	≤8	≥32	88	7	5
Ceftazidime	≤8	≥32	68	28	4
Ceftriaxone	≤8	≥64	73	24	3
Ciprofloxacin	≤1	≥4	46	50	4
Doripenem	≤0,5	≥	99	1	0
Ertapenem	≤2	≥8	99	1	0
Gentamicin	≤4	≥16	74	25	1
Meropenem	≤4	≥16	98	2	0
Nitrofurantoin	≤32	≥128	89	6	5
Pip/tazo	≤64/4	≥128/4	83	11	6
Piperacilin	≤16	≥128	52	45	3
Tobramycin	≤4	≥16	66	28	6
Trimet/sulfa	≤2/38	≥4/76	51	49	0

situations in which the symptoms or the patient's condition does not allow wait to get this information. It is important to know which antibiotic to use empirically in this initial period, it relied on the knowledge of local resistance treatment (Hawser et al., 2011).

Based on these results, it is possible to suggest that the treatment regimen of patients with UTI should be geared to the use of carbapenem drugs, as well as the combination of cefotaxime/clavulanate or ceftazidime/ clavulanate. Also, the use of amikacin aminoglycoside would have enough sense. However, should take into account the risk of ototoxicity and nephrotoxicity with the latter, as well as the location of the UTI, whether adult or child, whether pregnant or not and this way to individualize the patient. But these results mark a reference point for further *in vivo* investigations, which provide greater knowledge to make recommendations more evidence on the treatment schedule of the patients with UTI.

Detection and quantification biofilm formation

For this study, 99% of the strains analyzed presented biofilm forming activity in some degree. 1% of clinical isolates not form biofilm, 45% of the strains showed weak and biofilm formation 49% showed moderate activity. 5% of the isolates were strong ability to form biofilms; *E. coli* was 3 and K. *pneumoniae* was 2 (Figure 2).

In other studies on bacterial resistance and biofilm formation in *E. coli* and *Klebsiella* spp. you can see the same trend in the distribution of the groups. Shikh-Bardsiri and Shakibaie (2013), reported in its study for a total of 88 isolates the percentages of non-forming strains and weak, moderate and strong production capacity biofilms were 6.8, 36, 39.7 and 17.4%, respectively. Furthermore, a relationship between biofilm formation and antibiotic resistance was observed, since most strains with multiple resistance, are the same as had higher optical densities for biofilm formation, strains with biofilm-forming ability of moderate and strong. This relationship, multidrug resistance-biofilm formation has been reported by Lopez et al. (2008) and Hirales-Casillas et al. (2009), so their importance in the clinical setting for proper patient management. In this communication, we also report the ability of primary uropathogens isolates of *E. coli* and *K. pneumoniae* to express curli fimbriae and cellulose on LB agar without salt, which is coupled to the biofilm-formation capability of the organism. Three different morphotypes were found, 50% of the isolates was rdar, 27.3% bdar and 22.7% rdar.

Detection of AHL by colorimetric method

For quantification of AHL optical density it was measured in a microplate reader at a wavelength of 520 nm (Figure 3). The AHL was no or only weak activity for those

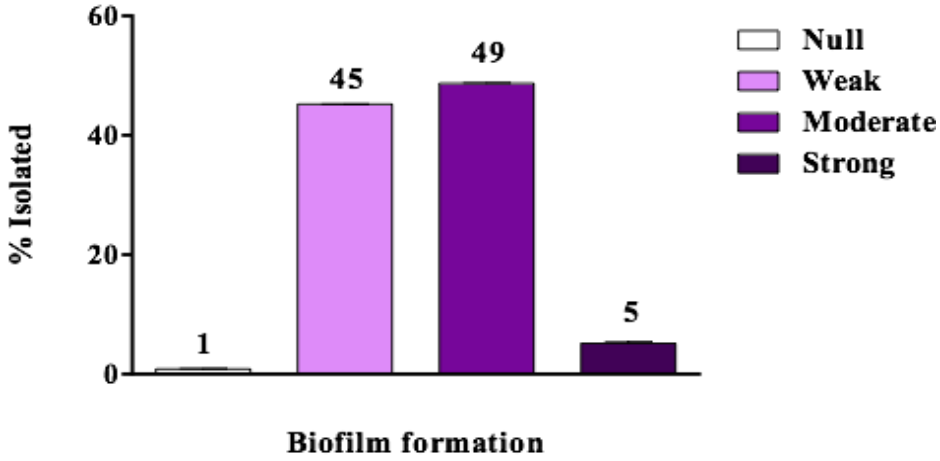

Figure 2. Classification of isolates according to their ability to form biofilms. Error bars represent the standard deviation from the mean of three observations.

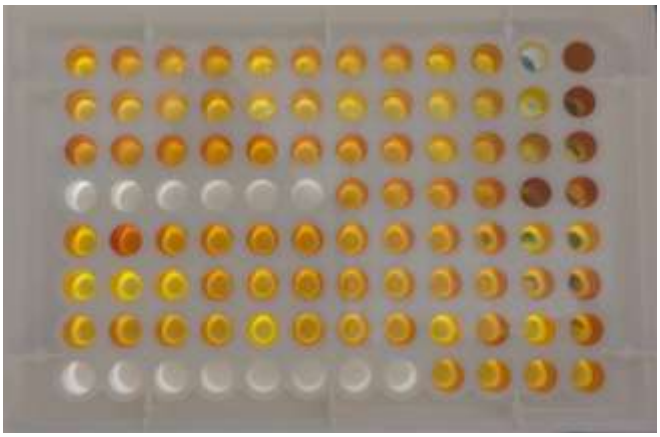

Figure 3. Quantification of AHL (optical density) measured in a microplate reader at a wavelength of 520 nm.

isolates that showed an optical density ≤0.98. Strains that showed activity AHL are those that were strong forming biofilms (5 strains), one of the strains that is within this group did not exceed the threshold of activity AHL, however, it was close to the limit with a value of 0.962 (Figure 4). Control strains *E. coli* ATCC 25922 and *K. pneumoniae* ATCC 700603. AHL activity showed values below the threshold, 0.720 and 0.414, respectively. Only four isolates showed activity AHL above the threshold, the highest activity was 1.329 (*E. coli)*, followed by 1.190 (*E. coli*), 0.993 (*K. pneumoniae* and *E. coli*).

Several studies have reported that *E. coli* is not able to produce AHL, yet has the ability to alter their pattern of gene expression and phenotypic properties in response to AHL by the AHL responsive transcriptional regulator SdiA, according to the presence of other locations of Gram-negative bacteria (Ahmer, 2004). Although the association between biofilm formation and other virulence factors has been variable, in the present study biofilm formation in *E. coli* and *K. pneumoniae* strains capable of producing relatively high AHL was detected. Already has been reported in recent studies by Taghadosi et al. (2015), uropathogenic *E. coli* strains producing biofilms with capacity to produce and communicate through AHL molecules type. More research is needed to understand the molecular genetics of this compound in *E. coli*.

Determination of AHL functional groups

Strains with the highest activities were selected for AHL analysis of functional groups by FT-IR spectroscopy (Figure 5). The results show amide carbonyl bond absorptions I and II was found at 1547 and 1516 cm^{-1} and characteristic absorptions near 1678 and 1647 cm^{-1} these signals are attributable to the presence of γ-lactone and ketone carbonyls. The band bending vibration of the O=CN bond was found to 680 cm^{-1} (Wang et al., 2011; Yang et al., 2006). These outcomes support the activity data AHL and confirm the presence of a lactone ring in producing strains biofilm with high activity AHL; however the spectra obtained for the extracts have bands too broad and in some cases overlap with other bands, due to the presence of other compounds in the extracts. So this technique is not allowed to obtain clear results apparently by low concentrations to those found in the AHL extract (Cuadrado Silva, 2009).

Effect of soluble compounds on bacterial growth and biofilm formation

IBU reduced biofilm formation significantly in three of the five strains, of which two are *K. pneumoniae* and is *E.*

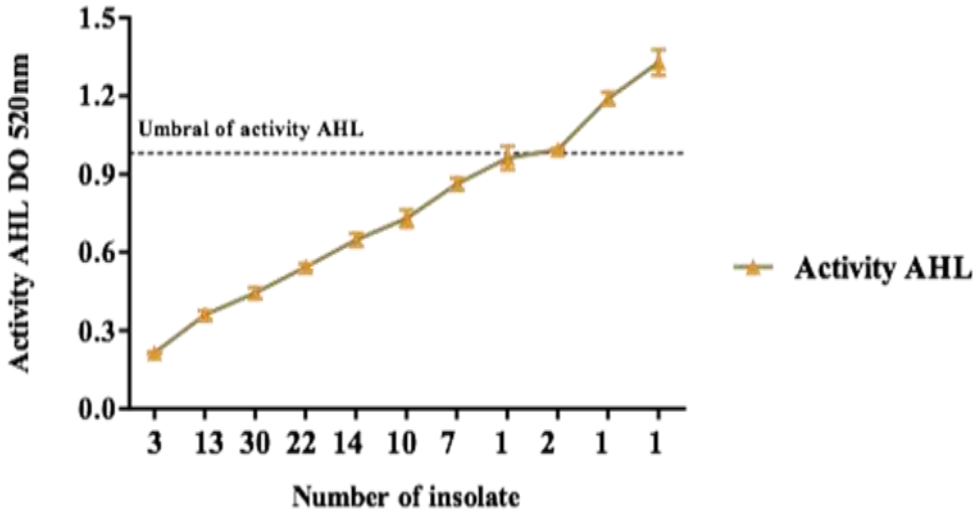

Figure 4. AHL- activity by uropathogenic *E. coli* and *K. pneumoniae* exhibiting strong biofilm. The results below threshold indicate weak or no AHL activity.

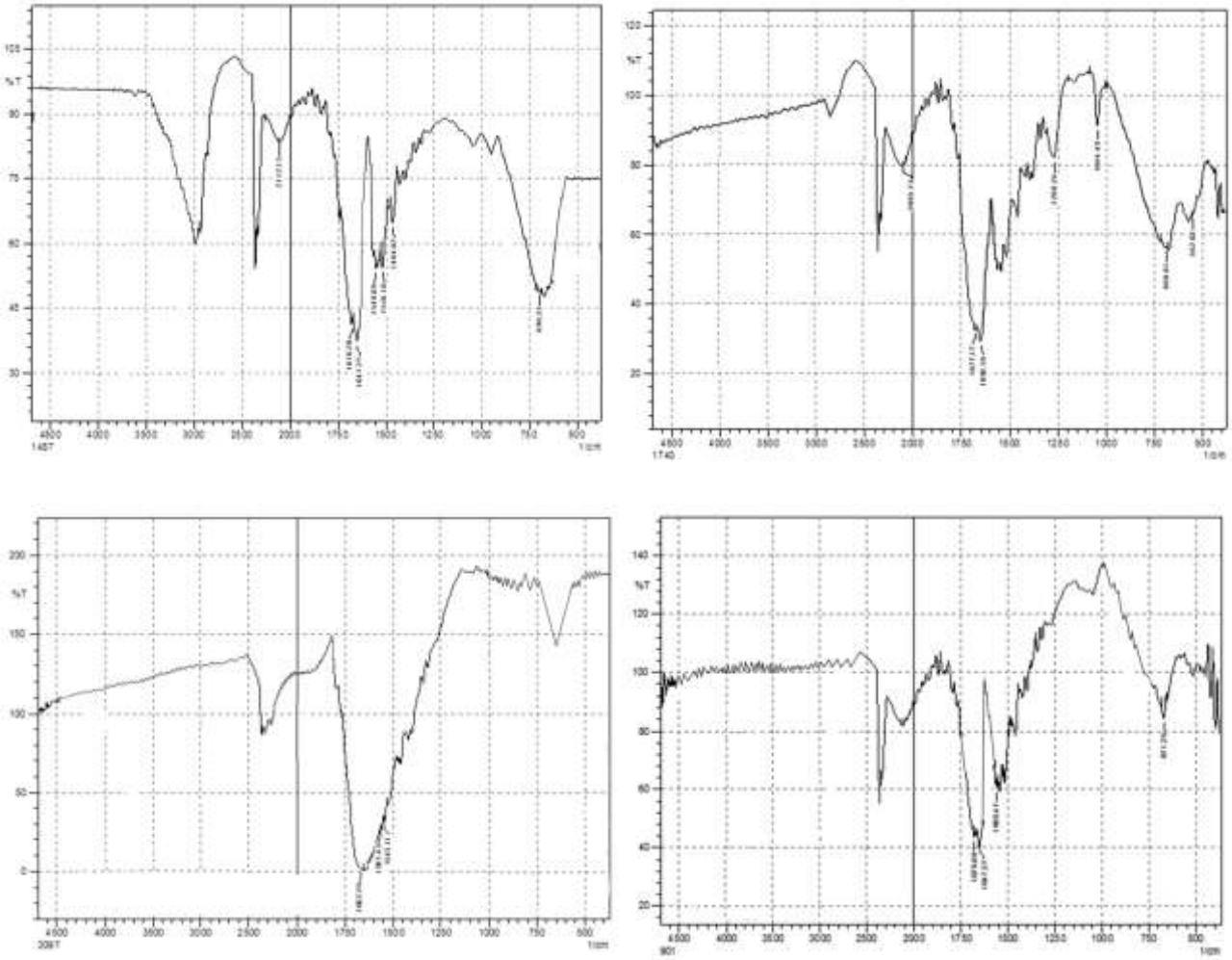

Figure 5. FT-IR spectra of AHL functional groups produced by uropathogenic *E. coli.* The AHL was extract from organism by LLE- methods as described in the text. The pure compound was then subjected to FT-IR spectroscopy. The lactone ring was shown at 1764.69 cm^{-1} wave number.

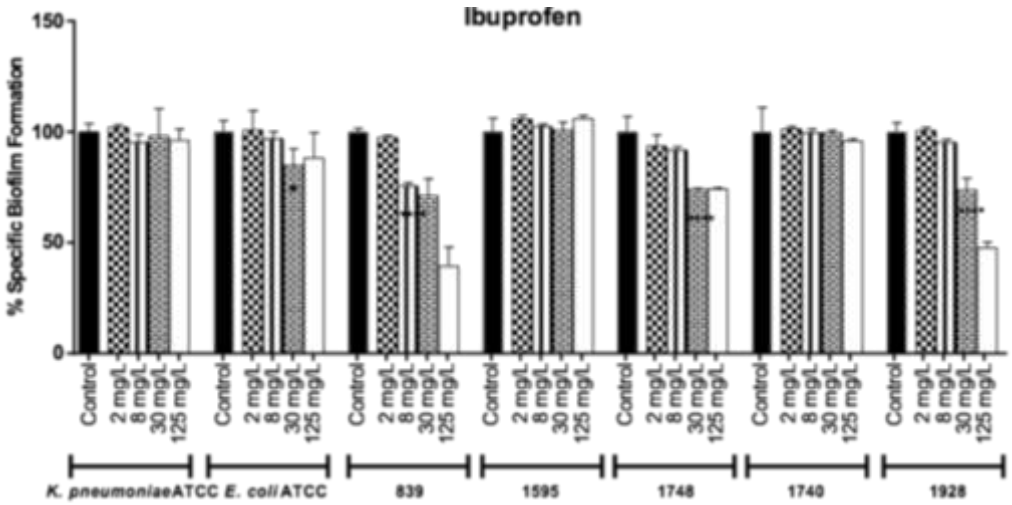

Figure 6. Ibuprofen effect on biofilm formation in *E. coli* strains *K. pneumoniae* strong forming and biofilm.

(A) **(B)**

Figure 7. Morphotypes on red Congo agar. A. rdar that displayed cellulose and curli fimbriae. B. bdar that displayed only curli fimbriae, but not cellulose.

coli, with CMIB 8 and 30 mg/L, decreases observed 20 to 25%. While in some cases the presence of the highest concentration (125 mg/L) caused dramatic reductions in biofilm formation, ranging from 52.4 to 60.6% (Figure 6). Naves et al. (2010), studied the effects of ibuprofen on biofilm formation in *E. coli* strains, identified as forming strong biofilm. In this study it was found that the concentration of IBU in urine obtained under a treatment regimen is much greater than the concentration of IBU to reduce biofilm formation by five of the seven strains of *E. coli* tested. Therefore, IBU administered at standard doses may prevent biofilm formation by *E. coli* in indwelling urinary catheters. These outcomes can be as was explained by Naves (2010) and coworkers when they detected not biofilm reduction were observed, suggesting that IBU could be washed out from the wells during the treatment. With the consequence that the concentration of IBU is down more than usual.

A study performed by Drago et al. (2002), reports that sub-MIC concentrations IBU inhibit the production of fimbriae by *E. coli* strains, to reduce bacterial adhesion uro-epithelial cells. However, in our study we can demonstrate that the sub-MIC concentrations at which biofilm formation is inhibited, the morphotype expressed by these strains incubated with sub-MIC concentrations (8 and 30 mg/L) on Congo red agar changes and evidence inhibiting production of fimbriae (Figure 7).

Moreover, bacterial hydrophobicity and hemolysin production by *E. coli* strains are also affected by incubation with drug (Drago et al., 2002). Also, it has

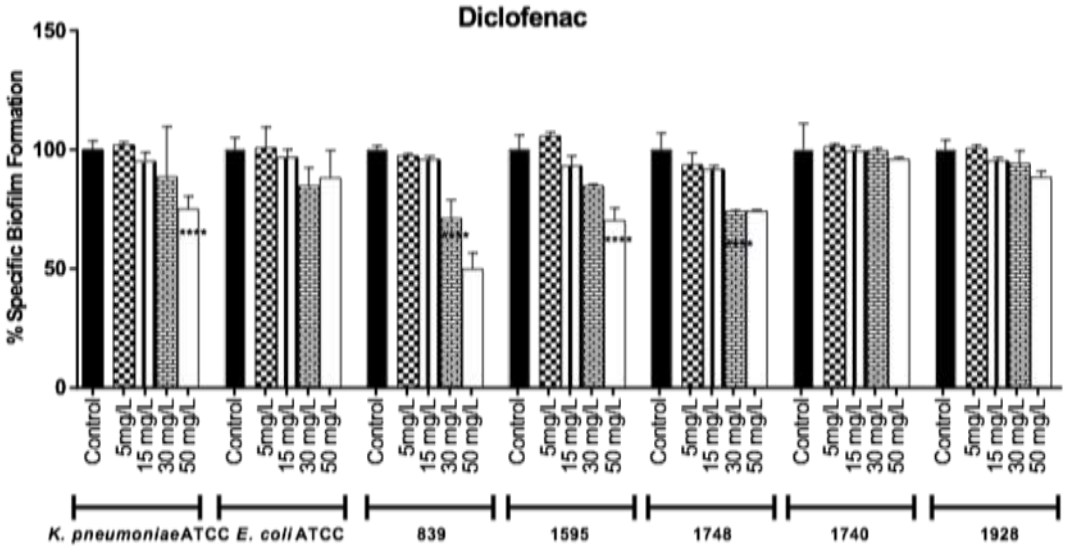

Figure 8. Diclofenac effect on biofilm formation in *E. coli* strains *K. pneumoniae* strong forming and biofilm.

demonstrated anti-adhesive effect of IBU in *Candida albicans* using a disc model system *in vitro* catheter (Alem and Douglas, 2004). Additionally, some *in vivo* studies have reported beneficial effects when combined IBU and antibiotics for the treatment of otitis media and experimental pneumococcal in a rat model of chronic pulmonary infection with *P. aeruginosa* (Diven et al., 1995).

DIC reduced biofilm formation significantly in three of the five isolates, of which two are *K. pneumoniae* and the other is *E. coli*, with CMIB 30 and 50 mg/L respectively, decreases observed 26 to 30%. Even for a sample found that the presence of the highest concentration (50 mg/L) caused reductions in biofilm formation 50.1% (Figure 8). Has been reported that diclofenac and ibuprofen at concentrations equal to those obtained from human blood drugs (serum) inhibits biofilm formation of *S. aureus* and *E. coli* in a polypropylene mesh surface (Reśliński et al., 2013). A strain of *E. coli* (1740), showed not significant changes in biofilm formation with exposure to IBU and DIC. And all strains evaluated when treated with these compounds showed not significant increase in biofilm forming capacity relative to the control. So far there has evaluated the effect of IBU and DIC in biofilm formation in *K. pneumoniae* strains. However other studies have reported compounds such as alkaloids, chitosan, linoleic acid, curcumin and eugenol (Magesh et al., 2013) and N-acetyl cysteine, which has been used at a concentration of 2000 mg/L inhibits growth of a variety of Gram-positive and negative organisms, including *K. pneumoniae*, *E. cloacae* and *E. coli* (Olofsson et al., 2003).

In tis contribution was found that isolates with the highest AHL activities also exhibited strong adherence to microplate wells (P≤0.05). A significant relationship between strong biofilm production and production of AHL, suggesting the importance of this mechanism in bacterial pathogenicity and its role in sensitivity to antibiotics. IBU and DIC reduced biofilm formation significantly in three of the five strains, *K. pneumoniae* and *E. coli*, IBU with CMIB. While in some cases the presence of the highest concentration (125 mg/L) caused dramatic reductions in biofilm formation, ranging from 52.4 to 60.6%. Our results suggest that IBU and DIC could be useful in the treatment of urinary tract infections caused by *E. coli* and *K. pneumoniae* due to their inhibitory effect on both bacterial growth and biofilm formation.

Conflict of Interests

The authors have not declared any conflict of interests.

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