**Biofilm formation and overcoming strategies of *E.coli* isolated from human and chickens**

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**Abstract**

*E. coli* is one of the commonest causes of bacteremia in humans around the world, accounting for 15% - 40% of all significant bacteremia isolates. The present study aimed toassess the role of aspirin as an overcoming strategy for biofilm formation in E. coli isolated from humans urinary tract infection samples and chickens. A total number of 435 samples collected (200 from human urine samples and 235 chickens) from Alexandria governorate. The obtained results revealed that *E.coli* was isolated from 158 out of 200 (79%) human samples and 110 out of 235 (46.8 %) samples from chickens. A total number of 81 isolates were evaluated for biofilm formation (50 isolates from human and 31 from chickens). Forty-five isolates out of 50 isolates (90%) from human urine and 19 out of 31 isolates (61.3%) from chickens revealed the presence of biofilm in 19 samples with percentage of 61.29%. Twenty-six isolates were tested by real time PCR (RT-PCR) for evaluation of ndvb gene expression. Twenty isolates positive for biofilm formation were treated with Aspirin from human urine samples and 6 isolates from chicken samples. Six isolates negative for biofilm formation were used as a negative control. It could be concluded that there is a relation between biofilm formation and antibiotic resistance. Treatment of the isolated *E.coli* with aspirin reduced biofilm formation and increase sensitivity of E.coli to antibiotics. On conclusion aspirin may be used as an adjuvant to antibiotics in the management of drug resistant E. coli infections. However, more studies conducting the same perspective should be conducted in the near future.

**Kay words:** E. coli , Biofilm, , ndvb gene, Human, Chickens

**I-Introduction**

*Escherichia coli* is one of the commonest causes of bacteremia in humans around the world, accounting for 15% - 40% of all significant bacteremia isolates **(**[**Beauchamp and Sofos, 2010**](file:///C:\Users\Mohamed\Downloads\5-%20introduction.docx#_ENREF_7)**).** Despite being a common and serious infection, there have been few recent studies describing the clinical features of *E. coli* bacteremia, and few data are available on its population incidence ***(Kennedy et al., 2008).***

Poultry food products are important sources of *E. coli*, because at the time of slaughter, fecal contamination from the intestines contaminated the carcass. As a result, poultry meat can be contaminated with fecal material or ingesta and with bacteria associated with these contaminants **(*Seidavi et al., 2010)*.**

*E. coli* biofilms are found to be the major causative agent of many intestinal infection ***(***[***Prakash et al., 2003***](file:///C:\Users\Mohamed\Downloads\5-%20introduction.docx#_ENREF_67)***)*.** The most frequent causative agent for UTI has been recognized as E. coli and most of these isolates were recognized as resistant to antibiotics ampicillin, amoxicillin- clavulanic acid, norfloxacin, cefuroxime, ceftriaxone and co-trimoxazole. Diabetes, renal disease and use of intra uterine device are some of the risk factors associated with UTI which complicate the infection and increases the cost of treatment, morbidity and mortality ***(***[***Niranjan and Malini, 2014***](file:///C:\Users\Mohamed\Downloads\5-%20introduction.docx#_ENREF_64)***)*.**

There is an urgent need for the development of new therapeutic strategies to eradicate biofilm infections by E. coli ***(***[***Chibeu et al., 2012***](file:///C:\Users\Mohamed\Downloads\5-%20introduction.docx#_ENREF_16)***).*** Only few studies have reported on the SA anti-biofilm effects on a mature and well-developed biofilm typical of microbial systems. Moreover, the SA anti-biofilm mechanism of action, the nature of its binding targets and cellular receptors remain unknown ***(Damman 2013; Kumar 2014).*** Thus the present study aimed toassess the role of aspirin as an overcoming strategy for biofilm formation in E. coli isolated from humans UTI samples and chicken.

**II- Material and methods**

**1- Samples**:

The total number of 435 samples collected (200 abnormal urine samples from human and 235 cloacal swab , kidney and spleen samples from apparently diseased chicken) from Alexandria governorate. The collected samples were transferred in an ice box to the lab of Dept. Microbiology, Fac. of Vet. Med. Alex. University.

**2- Bacteriological examination of *E.coli*:**

**A. Cultivation and purification of samples:**

They collected samples were inoculated into nutrient broth and incubated at 37°C for 24h. A loopful for each incubated broth was streaked onto MacConkey’s agar medium and incubated at 37°C for 24h to 48h according to **Cruickshank et al., (1975)**.

**B-Identification of the bacterial isolates:**

The suspected *E.coli* isolates were identified biochemically according to **Cruickshank et al., (1975)**. The isolated *E.coli* isolates were tested for their sensitivity to antibiotic sensitivity test **(Table 1)** was performed using the Kirby-Bauer method on Mueller-Hinton agar and interpreted according to **NCCLS (1990).**

**Table (1):** antibiotic discs used in the antibiotic sensitivity test of E.coli isolated from human and chickens:

|  |  |
| --- | --- |
| Antibiotic | Concentration |
| Ampicillin (AMP) | 10 µg |
| Amoxicillin (AML) | 10 µg |
| Cephalexin (CL) | 30 µg |
| Neomycin (N) | 30 µg |
| Erythromycin (E) | 10 µg |
| Nitrofurantoin(F) | 300 µg |
| Gentamycin (CN) | 10 µg |
| Tetracycline (TE) | 10 µg |
| Ciprofloxacin (CIP) | 5 µg |
| Kanamycin (K) | 30 µg |

**3- DNA extraction:** DNA was extracted from E.coli isolated using a commercial kit (PREP- NA Plus kit) **(Table 2)**.

Table (2): showed reagent for DNA extraction

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Kit Reagents | Quantity | | | |
| **PREP –NA Plus** | | **PREP –NA** | |
| Lysis buffer | 15 ml | 1 Vial | 30ml | 1 Vial |
| Precipitation Buffer | 20 ml | 1 Vial | 40ml | 1 Vial |
| Washout solution N-1 | 25 ml | 1 Vial | 50ml | 1 Vial |
| Washout solution N-2 | 15 ml | 1 Vial | 30ml | 1 Vial |
| Dilution Buffer | 15 ml | 1 Vial | 1.25ml | 4 tubes |
| Negative control ( C- ) | - | - | 1.5ml | 2 tubes |
| Internal control ( RNA-IC ) | - | - | 1ml | 1 tubes |
| Internal control ( DNA-IC ) | - | - | 1ml | 1 tubes |

**4- Biofilm formation:**

A total number of 81 isolates were evaluated for biofilm formation (50 isolates from human and 31 from chickens). Biofilm formation was tested according to **O'Toole G.A. (2011).**

**5- Real time-Polymerase Chain Reaction (rPCR) using** (PREP- NA Plus kit) **product number** : (P-002/2EU)

The ndvB gene was amplified by PCR using purified genomic DNA as a template.

Oligonucleotide primers were synthesized to amplify the intact region of ndvB gene as (in table 4). The forward primer for ndvB, 5′ GAGGTGGCAAAATGGGCAAG 3′ and the reverse primer, 5′- CATGCAGGCAAGAATCGACG 3′, these primers correspond to the gene ndvB and the final expected PCR product was 781bp **(Silva *et al.*, 2013)**

Table 4 :Real time-Polymerase Chain Reaction (Rt-PCR)

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Step | Temperature | Min | Sec | Number of cycles | Optical Measurement | Type of the step |
| 1 | 80.0 | 0 | 30 | 1 |  | **Cycle** |
| 94.0 | 1 | 30 |  |
| 2 | 94.0 | 0 | 30 | 5 |  | **Cycle** |
| 64.0 | 0 | 15 | V |
| 3 | 94.0 | 0 | 10 | 45 |  | **Cycle** |
| 64.0 | 0 | 15 | V |
| 4 | 94.0 | 0 | 5 | 1 |  | **Cycle** |
| 5 | 10.0 | - | - | - |  | **Holding** |

**6- Treatment of *E.coli* isolates by salicylic acid :**

Twenty isolates positive from human urine samples and 6 isolates from chicken samples for biofilm formation were treated with aspirin (acetylsalicylic acid)tested at a concentration of 1 mM inhibited biofilm formation according to **Alem and Douglas (2004) .**

**7- Antibiotic sensitivity test after treatment**

It was done by agar plate method (agar diffusion method or disk diffusion method) according to **Gillies RR, Dodds TC (1976).**The result was interpreted according to **NCCLS (1990).**

**III- Results:**

E.coli was isolated from158 out of 200 (79%) human samples and 110 out of 235 (46.8%) from chicken samples as shown in table (4)

Table (4) Incidence of E. coli isolated from human and chickens:

|  |  |  |  |
| --- | --- | --- | --- |
|  | Total samples | Positive samples | |
| Human urine samples | 200 | 158 | 79% |
| Chickens | 235 | 110 | 46.8% |
| Total | 435 | 268 | 61.6% |
| Data is expressed as percentage and frequency. | | | |

As shown in table (5) out of 66 E. coli isolates from human tested for antibacterial sensitivity, 30 *E. coli* isolates were resistant Ampicillin and Amoxicillin with percentage of 63.33%and 60% respectively, moderately sensitive to Nitrofurantoin, Neomycin, Tetracycline, Gentamycin, Cephalexin, Kanamycin and Erythromycin with percentage of 63.33%, 43.33%, 53.33%, 64.15%, 62.72%, 49.09% and 47.57% respectively and sensitive to Ciprofloxacin with percentage 55.09%.

**Table (5):** Result of antibiotic sensitivity of *E. coli* isolated from human urine

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Antibiotic | Sensitive | | Intermediate sensitivity | | Resistant | |
| No. | % | No. | % | No. | % |
| Ampicillin | 0 | 0 | 11 | 36.66% | 19 | 63.33% |
| Amoxicillin | 0 | 0 | 12 | 40% | 18 | 60% |
| Cephalexin | 5 | 16.66% | 16 | 53.33% | 9 | 30% |
| Neomycin | 10 | 33.33% | 13 | 43.33% | 7 | 23.33% |
| Erythromycin | 12 | 40% | 9 | 30% | 9 | 30% |
| Nitrofurantoin | 11 | 36.66% | 19 | 63.33% | 0 | 0 |
| Gentamycin | 14 | 46.66% | 13 | 43.33% | 3 | 10% |
| Tetracycline | 0 | 0 | 16 | 53.33% | 14 | 46.66% |
| Ciprofloxacin | 16 | 53.33% | 8 | 26.66% | 6 | 20% |
| Kanamycin | 12 | 40% | 10 | 33.33% | 8 | 26.66% |

**No, of tested isolates 30.**

The result presented in **table ( 6 ) revealed that 36** *E. coli from chickens were*  resistant to Ampicillin and Amoxicillin with percentage of 57.09% and 53.06%, respectively, moderately sensitive to Nitrofurantoin, Neomycin, Tetracycline, Gentamycin, Cephalexin, Kanamycin and Erythromycin with percentage of 87.87%, 83.33%, 83.33%, 74.25%, 72.72%, 59.09% and 57.57%, respectively and sensitive to Ciprofloxacin with percentage of 53.03%.

**Table ( 6 ):** Results of antibiotic sensitivity of *E. coli*. Isolated from chickens

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Antibiotic | Sensitive | | Intermediate sensitivity | | Resistant | |
| No. | % | No. | % | No. | % |
| Ampicillin | 0 | 0 | 13 | 36.11% | 23 | 63.88% |
| Amoxicillin | 0 | 0 | 14 | 38.88% | 22 | 61.11% |
| Cephalexin | 9 | 25% | 13 | 36.11% | 14 | 38.88% |
| Neomycin | 9 | 25% | 11 | 30.55% | 16 | 44.44% |
| Erythromycin | 5 | 13.88% | 16 | 44.44% | 15 | 41.66% |
| Nitrofurantoin | 17 | 47.22% | 19 | 52.77% | 0 | 0 |
| Gentamycin | 12 | 33.33% | 18 | 50% | 6 | 16.66% |
| Tetracycline | 11 | 30.55% | 17 | 47.22% | 8 | 22.22% |
| Ciprofloxacin | 21 | 58.33% | 8 | 22.22% | 7 | 19.44% |
| Kanamycin | 17 | 47.22% | 10 | 27.77 | 9 | 25% |

No. of tested isolates 36.

As shown in table (7), a total number of 81 isolates tested for biofilm formation, 45 out of 50 (90%) of it from Human urine samples and 19 out of 31 (61.3%) from chicken samples.

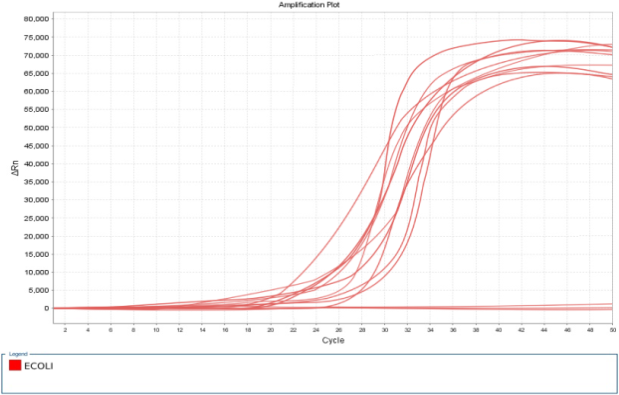
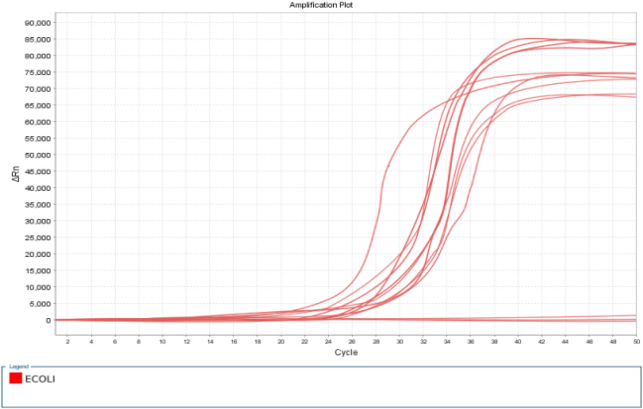
Table (7): Results of biofilm formation of the resistant E.coli isolated from human and chickens.

|  |  |  |
| --- | --- | --- |
| Biofilm formation | | |
| Human urine samples (n= 50) | 45 | 90% |
| chicken (n= 31) | 19 | 61.3% |
| Total (n= 81) | 64 | 79.0% |
| Data is expressed as percentage and frequency. | | |

A total number of 26 samples were tested for biofilm formation gene expression by rPCR (table, 8).

Table (8) PCR results of gene expression of biofilm in the Multi-drug resistant E.coli isolates:

|  |  |  |
| --- | --- | --- |
| Isolates | Ct Urine samples | Ct chickens |
| 1 | 25.42 | 28.25 |
| 2 | 23.76 | 30.25 |
| 3 | 26.85 | 22.95 |
| 4 | 27.31 | 26.84 |
| 5 | 25.58 | 27.66 |
| 6 | 26.95 | 24.93 |
| 7 | 29.42 | 28.42 |
| 8 | 23.51 | 25.61 |
| 9 | 24.27 | 24.36 |
| 10 | 26.99 | 23.85 |
| 11 | 0 | 0 |
| 12 | 0 | 0 |
| 13 | 0 | 0 |

  Figure 1 the amplification plot of 13 human Urine samples  Figure 2 the amplification plot for 13 Chicken samples

Twenty of them were positive biofilm formation from human urine samples and chicken samples and 6 isolate negative for biofilm formation were used as negative control. The results presented in table (9) revealed that 20 out of 26 isolates were positive for gene of biofilm formation (ndvb gene).

Table (9) PCR results of gene expression of biofilm in the Multi-drug resistant isolated samples after aspirin treatment:

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | Mean & SD | Median | Minimum | Maximum | IQR |
| Ct UTI | 24.80 ± 1.896 | 24.69 | 21.16 | 29.42 | 23.59, 26.11 |
| Ct | 27.04 ± 2.425 | 27.66 | 22.95 | 30.25 | 24.93, 28.42 |
| Ct | 25.28 ± 2.618 | 24.99 | 21.34 | 29.34 | 23.25, 27.52 |
| Ct total | 25.13 ± 2.181 | 24.88 | 21.16 | 30.25 | 23.67, 26.93 |
| Data is expressed as mean and standard deviation, median, Minimum, Maximum and Inter-quartile range. | | | | | |

The results of antibacterial sensitivity (Table,10) show that 20 out of 40 E. coli isolates were tested for antibacterial sensitivity, from urine samples were resistant to Ampicillin and Amoxicillin with percentage of 30%and 25% respectively, moderately sensitive to Nitrofurantoin, Neomycin, Tetracycline, Gentamycin, Cephalexin, Kanamycin and Erythromycin with percentage of 60%, 50%, 50%, 50%, 50%, 45% and 55% respectively and sensitive to Ciprofloxacin with percentage 50%.

Table (10): Results of antibacterial sensitivity of E.coli isolated from human and treated with Aspirin.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Antibiotic | Sensitive | | Intermediate sensitivity | | Resistant | |
| No. | % | No. | % | No. | % |
| Ampicillin | 6 | 30% | 8 | 40% | 6 | 30% |
| Amoxicillin | 5 | 25% | 9 | 45% | 6 | 30% |
| Cephalexin | 7 | 35% | 10 | 50% | 3 | 15% |
| Neomycin | 6 | 30% | 10 | 50% | 4 | 20% |
| Erythromycin | 5 | 25% | 11 | 55% | 4 | 20% |
| Nitrofurantoin | 8 | 40% | 12 | 60% | 0 | 0 |
| Gentamycin | 9 | 45% | 10 | 50% | 1 | 5% |
| Tetracycline | 5 | 25% | 10 | 50% | 5 | 25% |
| Ciprofloxacin | 10 | 50% | 8 | 40% | 2 | 10% |
| Kanamycin | 8 | 40% | 9 | 45% | 3 | 15% |

**No. of tested E.coli isolates was 40 .**

Sensitivity of E.coli isolates from chickens (table,11 ), revealed that 20 E. coli were resistant to Ampicillin and Amoxicillin with percentage of 35% and 40%, respectively, moderately sensitive to Nitrofurantoin, Neomycin, Tetracycline, Gentamycin, Cephalexin, Kanamycin and Erythromycin with percentage of 35%, 25%, 30%, 30%, 20%, 25% and 30% respectively and sensitive to Ciprofloxacin with percentage of 50%.

**Table (11):** Result of antibiotic sensitivity of *E. coli* isolated from chickens and aspirin treatment

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Antibiotic | Sensitive | | Intermediate sensitivity | | Resistant | |
| No. | % | No. | % | No. | % |
| Ampicillin | 7 | 35% | 7 | 35% | 6 | 30% |
| Amoxicillin | 8 | 40% | 6 | 30% | 6 | 30% |
| Cephalexin | 12 | 60% | 4 | 20% | 4 | 20% |
| Neomycin | 11 | 55% | 5 | 25% | 4 | 20% |
| Erythromycin | 9 | 45% | 6 | 30% | 5 | 25% |
| Nitrofurantoin | 13 | 65% | 7 | 35% | 0 | 0 |
| Gentamycin | 13 | 65% | 6 | 30% | 1 | 5% |
| Tetracycline | 11 | 55% | 6 | 30% | 3 | 15% |
| Ciprofloxacin | 14 | 70% | 4 | 20% | 2 | 10% |
| Kanamycin | 13 | 65% | 5 | 25% | 2 | 10% |

No. of tested isolates was 20.

***IV- Discussion***

The biofilms have evolved significantly increased antibiotic resistance relative to their free-floating counterparts, severely hampering the successful treatment of biofilm-associated infections. The increased antimicrobial resistance results from the simultaneous operation of multiple biofilm-specific mechanisms that are still not fully understood. Deeper understanding of these mechanisms will be useful for the development of new antibiofilm agents, from which innovative therapeutic measures may be developed to eradicate persistent infections ***(Mohammed et al., 2014)***.

Biofilm formation occurs by at least three different mechanisms. In one mechanism, type IV pili-mediated twitching motility encourages surface aggregation. Alternatively, attached cells spread outward and upward by binary division to form cell clusters, or cells are recruited from the bulk fluid to form of biofilms. The relative contribution of these mechanisms depends on the organisms, the nature of the surface, and the physical – chemical condition of the environment ***(Beloin et al., 2008)***.

The twitching motility, growth rate, cell signaling, exopolysaccharide production, and the physical growth environment all play a significant role in the biofilm structure. Bacteria growing in biofilms are responsible for a large number of persistent infections and are often more resistant to antibiotics than are free floating bacteria ***(Méndez-Ortiz et al., 2006)***.

Biofilms offer a mode of growth for bacteria that allow them to survive and indeed thrive in a host. The survival of bacteria exposed to toxic compounds is a multifactorial phenomenon, involving well known molecular mechanisms of resistance but also less-well-understood mechanisms of tolerance that need to be clarified. In particular, the contribution of biofilm formation to survival in the presence of toxic compounds, such as nickel, was investigated in this study ***(Domka et al., 2006)***.

ndvB is a gene that encodes a glucosyl transferase involved in the formation of cyclic glucans. The glucans are cyclic polymers of 12 to 15 β-(1→3) linked glucose molecules with phosphoglycerol substitutions. Inactivation of ndvB blocked glucan production but did not affect growth, the kinetics of biofilm formation, or the architecture of the biofilms ***(Mohammed et al., 2014)***.

Expression of erbR in ∆ndvB biofilms was restored after the treatment of the biofilm with periplasmic extracts derived from wild-type biofilm cells. Inactivation of ethanol oxidation genes increased the sensitivity of biofilms to tobramycin. Together, these results reveal that ndvB affects the expression of multiple genes in biofilms and that ethanol oxidation genes are linked to biofilm-specific antibiotic resistance ***(Rangel et al., 2005)***.The aim of the present study is to assess the role of aspirin as an overcoming strategy for biofilm formation in E. coli isolated from humans UTI samples and chicken.

Unluckily, there is a paucity of studies studying the effect of aspirin of ndvB gene and biofilm formation in E. coli organisms. However, studies investigating the influence of salicylate on biofilm formation by staphylococci also showed its effectiveness only at a concentration as high as 5 mM ***(Teichberg et al., 1993; Muller et al., 1998)***.

Salicylic acid (SAL) is a small molecule derived from plants with pleiotropic effects on eukaryote and prokaryote cells. In addition, SAL is the main biometabolite of aspirin, the popular nonsteroidal antiinflammatory agent regularly utilized by millions of individuals worldwide due to its known analgesic and cardiovascular protective activities. Furthermore, vegetarian individuals contain similar plasma concentrations of SAL when compared with patients consuming low daily doses of aspirin ***(Dotto et al., 2017)***. The expression of virulence factors and regulatory genes is modified by SAL in several bacterial species ***(Pomposiello et al., 2001; Denkin et al., 2005)***.

Previous findings from demonstrated that exposure of encapsulated S. aureus strains to low concentrations of SAL reduced capsular polysaccharide (CP) production and increased the Eap adhesin expression under planktonic conditions ***(Alvarez et al., 2010; Alvarez et al., 2011)***. On the other hand, Johnson et al. (2008) observed that an increase of Eap expression under depleted iron conditions contributed to biofilm formation, a finding that becomes relevant due to the fact that SAL can form complexes with iron cations ***(Cheng et al., 1996; Pozdnyakov et al., 2015)***.

The inhibitory effect on biofilms appeared to be dose related. Over 70% inhibition was observed at aspirin concentrations between 100 µM and 1 mM. Lower concentrations (50 to 75 µM) produced only about 20% inhibition, while 10 µM aspirin had no effect on biofilm formation. Aspirin concentrations of 50 to 200 µM can be achieved in humans by the use of therapeutic doses of the drug (**Wu, K. K. 2000 and Xu , X.-M., L.et al .,1999** ), suggesting that the antibiofilm effect observed in vitro might also be relevant in vivo.

Multi-drug resistance (to at least three antimicrobials) was found in *E. coli* from both avian and human sources (Table 5 &6 ), but was higher in frequency and proportion in avian isolates. However, when these multi-drug resistant organisms were compared, although it is clear that they do not have common sources of resistant bacteria , but Avian and human isolates are common in resistant to ampicillin and amoxicillin . Most avian multi-drug resistant isolates exhibited resistance to a combination of antimicrobials that included neomycin and erythromycin resistance, while most human isolates were resistant to kanamycin and tetracycline and cephalexin and erythromycin.

Our results revealed that most human E. coli were resistant to ampicillin and amoxicillin (63.33 and 60% respectively). In addition, 46.66 and 26.66% of human samples showed resistance to tetracycline and kanamycin respectively. Besides, resistance to cephalexin and erythromycin was encountered in 30% of cases. Regarding E.coli isolated from chicken samples, ampicillin and amoxicillin resistance was detected in 63.88 and 61.11% of specimens respectively. Furthermore, neomycin and erythromycin resistance were detected in 44.44 and 41.66% of specimens respectively. The obtained results were similar to those described by ***Tricia. et al., 2006*** who showed that Avian *E. coli* expressed resistance to the β-lactams, ampicillin and amoxicillin/clavulanic acid at frequencies of 20.6% and 2.9% respectively and Most of the human *E. coli* isolates were 43.8% and 33.3% showed resistance to the aminoglycosides, kanamycin and gentamicin, respectively, and 43.8% were resistant to tetracycline.

The PCR test for amplification of ndvB gene has demonstrated that the gene can be downregulated after aspirin treatment. In human samples, a significant increase in gene cut was observed (24.8 vs. 36.06 after treatment – p < 0.001). Regarding chicken, it showed the same response (27.04 vs. 35.35 after aspirin – p < 0.001).

On assessment of drug resistance after treatment, it was significantly decreased from 64% down to 40% after aspirin treatment (p < 0.001). In E.coli isolated from human samples, ampicillin and amoxicillin resistance decreased down to 30%, whereas tetracycline decreased down to 25%. In E.coli isolated from chicken samples, ampicillin and amoxicillin resistance decreased to the same percentage as humans, while neomycin and kanamycin resistance dropped to 20 and 25% respectively.

The biofilm formation by E. coli contributes to the occurrence of various infections and makes their eradication difficult. Factors like different extracellular appendages which contribute in E. coli surface colonization and their finely regulated expression and activity lead to formation of mature biofilms ***(Sharma et al., 2016)***.

It is still not fully understood how biofilm cells become more resistant to antibiotics. Since the genetic makeup of the cells in the biofilm has not been altered, the increased resistance likely involves the altered expression of specific genes in the biofilm. It is well documented that the gene expression profile of biofilm cells is markedly different from that of planktonic cells. Thus, a subset of these genes likely functions to protect biofilm cells from antibiotics ***(Beaudoin et al., 2012)***.

In parallel with our findings, aspirin showed significant inhibition of both quorum sensing signals. The activity of aspirin against the las and rhl systems led to investigate its effects on Pseudomonas virulence factor production. P. aeruginosa produces hydrolytic enzymes elastase, protease and hemolysin that degrade tissue components and interfere with host defense mechanisms ***(Bandara et al., 2006)***.

Aspirin at sub-MICs caused significant reduction (p < 0.01) in elastase, hemolysin, protease and pyocyanin activities, to the level of the mutant strain PAOJP2. Furthermore, the percentage reduction in virulence factors was consistent with the level of reduction in QS signaling molecules ***(El-Mowafy et al., 2014)***. Biofilm development is associated with antibacterial resistance and can contribute to severe infections ***(Høiby et al., 2010)*** and the QS system participates in control of biofilm formation. PAO1 biofilm production was significantly reduced with 6 mg/ml aspirin (p < 0.01), likely via its effect on QS ***(El-Mowafy et al., 2014)***.

Nevertheless, in the same study, the inhibitory effect of aspirin on virulence factors was specific for P. aeruginosa as sub-MICs of aspirin did not affect motility or biofilm production in E. coli K-12, likely because the E. coli QS circuit differs from that of P. aeruginosa ***(El-Mowafy et al., 2014)***.

In similar studies, salicylic acid, the primary metabolite of aspirin, is a potent inhibitor of pqs-dependent signaling which may account for its QSI effect ***(Yang et al., 2009)***. Salicylic acid down regulates rhlR and lasA in P. aeruginosa PA14 ***(Prithiviraj et al., 2005)*** with subsequent inhibition of pyocyanin, protease, and elastase activities ***(Schuster and Greenberg, 2006; Guo et al., 2013)***.

Within the same context, another study the possible effect of aspirin on Candida spp. Biofilm-producing capacity. The significant effects of aspirin on growth and biofilm formation of Candida spp. were achieved only with suprapharmacological concentrations of the drug. The influence of the inoculum size on the effect of aspirin on biofilm formation was determined for C. albicans only and a significant decrease was observed also at suprapharmacological concentrations of aspirin, irrespective of the inoculum size. The results obtained in that study showed that aspirin may have the potential to affect and suppress biofilm formation by Candida spp. ***(Stepanović et al., 2004)***.

On the contrary, treatment of mice with aspirin induced a significant increase of S aureus colonization. It is suggested that the elevated PIA expression induced by aspirin might be responsible for the high nasal colonization observed in mice. Aspirin-induced biofilms may contribute to S. aureus infection persistence in vegetarian individuals as well as in patients that frequently consume aspirin ***(Dotto et al., 2017)***.

Our study suggest that aspirin may be used as an adjuvant to antibiotics in the management of drug resistant E. coli infections. However, more studies conducting the same perspective should be conducted in the near future.

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