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Full Length Research Paper

Effect of EDTA on biofilm formation and antibiotic susceptibility of multidrug resistant uropathogenic *Escherichia coli* clinical isolates in Egypt

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Urinary tract infections are mainly caused by uropathogenic *Escherichia coli* (UPEC). Biofilm-producer UPEC tends to have a high level of resistance to antibiotics and this leads to recurrent episodes of urinary tract infections. The study tested the effect of a non-antibiotic adjuvant, ethylenediaminetetraacetic acid (EDTA) on the bacterial susceptibility to antibiotics and biofilm formation by multidrug resistant (MDR) strong biofilm producer UPEC from Egypt. The ability for *in vitro* biofilm formation was detected in 88 MDR UPEC isolates in the absence and presence of two concentrations of EDTA (10 and 20 mM). The minimum inhibitory concentrations (MIC) of the tested antibiotics were detected in the presence and absence of sub-inhibitory concentration of EDTA (2 mM) by the two-fold broth microdilution method. The effect of polyvinylchloride gelatin-EDTA coat on biofilm formation by strong and moderate biofilm producers was tested. The addition of 2 mM EDTA to antibiotics resulted in a decrease in the antimicrobials MIC values with the highest effect recorded with Meropenem (81.6%) and Nitrofurantoin (61.4%). EDTA with concentrations (10 and 20 mM) and Gelatin-EDTA coat inhibited biofilm formation by strong and moderate biofilm producing UPEC by 45.8, 78.8, and 81.1%, respectively. The combination of Carbapenems with EDTA in parenteral preparations to treat life threatening infections could greatly improve the clinical outcome. There is a continuous need for the development of new strategies for treatment of MDR biofilm-producer UPEC. Novel approaches to control microbial biofilm are needed.

Key words: Ethylenediaminetetraacetic acid (EDTA), *Escherichia coli*, biofilm, antibiotic resistance.

INTRODUCTION

Urinary tract infection (UTI) is one of the most common infectious diseases affecting all ages (Neupane et al., 2016). Catheter associated UTI (CAUTI) is common in patients with indwelling bladder catheter leading to an increase in the length of hospitalization and prolonging the antibiotic therapy period than non-catheterized

patients (Jacobsen et al., 2008). Uropathogenic *Escherichia coli* (UPEC) is responsible for more than 80% of UTI in healthy people and are the most common isolates in catheterized patients with UTI (Kumar et al., 2017). The multidrug resistant (MDR) UPEC strains are major public threat worldwide (Lee et al., 2016) and are highly prevalent in Egypt (El-Sokkary and Abdelmegeed, 2015; Abdel-Moaty et al., 2016).

Biofilms are the microbial communities of the surface attached to cells embedded in a self-produced extracellular polymeric matrix (Niveditha et al., 2012) and biofilm-producers show higher resistance to antimicrobial agent and this leads to recurrent episodes and persistence of UTI (Tayal et al., 2015). Biofilm-producer UPEC are also the most common cause of UTI (Bang et al., 2016), which are difficult to treat with a single antibiotic (Wu et al., 2015). Several strategies have been tested to inhibit biofilm formation on the indwelling urinary catheter (Cai et al., 2016), including coating catheters with natural products as green tea and Dandasa, fresh garlic extract, honey and Oregano essential oil (Sadekuzzaman et al., 2015); ethylenediaminetetraacetic acid (EDTA)-gallium gelatin coating (Zhu et al., 2013); and using levofloxacin and vitamin C (El-Gebaly et al., 2012).

EDTA is a polyamine carboxylic acid used as a metal chelator with established anticoagulant activity (Raad et al., 2003) and in low concentrations act as a food preservative and in combination with antibiotics (Lerma et al., 2014); ZOSYN® (Wyeth Pharmaceuticals Inc.) is a commercially available antibiotic combination (Piperacillin/Tazobactam) for intravenous use that contains EDTA in the formulation. It is used intravenously, in combinations with vitamins and minerals in treatment of various diseases including atherosclerotic vascular disease and renal ischemia. EDTA is shown to be safe up to 40 mg/kg/body weight when administrated intravenously to swiss albino mice (Chaudhary et al., 2012) and can be administered with a daily dosage of 50 mg/kg of body weight in humans (ENDRATE®, Hospira inc).

EDTA prevents curli production and inhibits bacterial adhesion which is required for biofilm development. EDTA chelates divalent ions present in lipopolysaccharide layer of biofilm (Chaudhary et al., 2013); potentiating the antibiotic effect by enhancing the drug penetration and disrupting the lipopolysaccharide present in the outer membrane, hence increasing the porosity of membrane and increasing the drug permeability (Abd et al., 2000; Chaudhary et al., 2013).

This study was conducted to evaluate the effect of a non-antibiotic adjuvant EDTA on *in vitro* biofilm formation and the antibiotic susceptibility of clinical MDR strong biofilm-producer UPEC from Egypt.

MATERIALS AND METHODS

Bacterial strains and identification

The study was performed on a total number of 88 MDR UPEC from inpatients and outpatients. Seventy seven (77) isolates were collected from Mansoura University Hospital (Dakhalia Governorate), and 11 isolates were collected from Misr University for Science and Technology (MUST) Hospital (Giza Governorate) in the period between January 2014 and December 2015. All experiments in this study were conducted in accordance with and approval of the ethical committee at Cairo University, Cairo, Egypt with approval number MI (1045).

Identification of the isolates was done by Gram staining and isolation on MacConkey agar (Oxoid, UK) and eosin methylene blue (Oxoid, UK) (Brenner, 1984). The molecular identification of *E. coli* was done by the PCR amplification of *uspA* gene (Chen and Griffiths, 1998). Multiplex PCR for detecting *gadA, chuA, yjaA* and *TspE4.C2* genes was used to determine the phylogenetic groups for each UPEC isolate (Doumith et al., 2012).

The antibiotic susceptibility testing of 88 MDR UPEC isolates was performed by using double disk diffusion using the following antibiotics (Cockerill et al., 2012): amikacin (30 µg), amoxicillin/clavulanic acid (30 µg), ampicillin (10 µg), ampicillin/sulbactam (10/10 µg), cefixime (5 µg), cefotaxime (30 µg), ceftazidime (30 µg), cefuroxime (30 µg), ciprofloxacin (5 µg), CO-trimoxazole (25 µg), gentamicin (10 µg), levofloxacin (5 µg), nalidixic acid (30 µg), nitrofurantion (300 µg), and norfloxacin (10 µg) all were supplied from Himedia, India; aztreonam (30 µg), imipenem (10 µg), meropenem (10 µg), piperacillin/tazobactam (100/10 µg), and tetracycline (30 µg) were supplied from (Oxoid, UK). *E. coli* strain ATCC 25922 was used as a reference strain and the result was interpreted according to CLSI guidelines (CLSI, 2012). The isolates were classified as MDR according to Magiorakos et al. (2012).

Effect of EDTA on the bacterial susceptibility to antibiotics

The MIC of EDTA (E. MERCK. Darmstadt, GERMANY) and the following antibiotics: ciprofloxacin, levofloxacin, amoxicillin/ clavulanic acid (Sedico Pharmaceutical Co., 6th of October city, Giza, Egypt), nalidixic acid, gentamicin (Memphis Pharmaceutical Co, Cairo, Egypt), nitrofurantoin (El-Kahera Pharmaceutical Co, Cairo, Egypt), cefotaxime and ceftazidime (EPICO, 10th of Ramadan), and meropenem (AstraZeneca Co, Cairo, Egypt) were performed using the microdilution broth method (Andrews, 2001). The antimicrobials MICs were determined in the absence of EDTA and in the presence of sub MIC of EDTA (2 mM EDTA).

Effect of EDTA on curli production

The presence of curli fibers were determined using Luria-Bertani agar (L.B.) (Difco Laboratories, U.S.A) without salts containing 40 mg/L congo red dye (Aldrich Chemical Co. Ltd. England) (Baugh et al., 2013). The effect of EDTA on curli production by curli positive strains was tested using two different concentrations of EDTA (5 and 10 mM) (Chaudhary et al., 2013).

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Effect of EDTA on *in vitro* biofilm formation

The ability of *in vitro* biofilm formation was determined using the microtiter plate assay (SarojGolia et al., 2012) in a 96-well microtiter plate (Greiner Bio-one, Stuttgart, Germany), in the absence and presence of EDTA (10 and 20 mM), in triplicates. The optical density was measured at 570 nm with ELISA reader (BioTek®, MQX 200, USA) and the degree of biofilm formation was estimated (SarojGolia et al., 2012).

Effect of coating polyvinyl chloride microtiter plate with 50 mM EDTA on *in vitro* biofilm formation

Coating of polyvinyl chloride (PVC) microtiter plates was performed using an EDTA-gelatin coating according to Zhu et al. (2013) with some modifications. The surface coat was developed by adding 150 µl of a mixture of 0.5% gelatin and 50 mM EDTA in triplicates to each well of a 96-well microtiter plate (Greiner Bio-one, Stuttgart, Germany) and drying overnight at 40°C. After drying, 125 µl of fresh Brain Heart Infusion broth (Difco Laboratories, U.S.A) supplemented with 2% sucrose (EL Naser Chemical Co. Egypt) (BHIS) was transferred to each well. Finally, these wells were inoculated with 25 µl bacterial suspension (10^8 CFU/ml) and incubated at 37°C for 24 h and then washed three times with sterile phosphate buffer saline (PBS) and air dried for 45 min (Chazotte, 2012). The wells were stained with 0.1% (w/v) crystal violet (Winlab, UK) for 15 min. The excess dye was removed by washing three times with bi-distilled water and then 200 µl of 95% ethanol was added for 1 h to release the attached dye and the optical density was measured at 595 nm using ELISA reader (BioTek®, MQX 200, USA). A negative control was performed (Rukayadi and Hwang, 2006). The extent of *in vitro* biofilm formation was also measured in PVC microtiter plates coated with gelatin only and in the absence of an EDTA-gelatin coat for comparison (SarojGolia et al., 2012).

Scanning electron microscope (SEM)

The biofilms produced by strong biofilm-producers MDR UPEC in the absence and presence of two concentrations of EDTA (10 and 20 mM) were scanned using SEM (JSM-840 SEM, JEOLE ltd., Tokyo, Japan). The biofilm was prepared in 6-well cell culture plate (Greiner Bio-one, Stuttgart, Germany) using BHI broth containing 5% sucrose. The biofilm produced was fixed with glutaldehyde 2.5% (v/v) in Dulebecco PBS (PH 7.2) for 1.5 h, rinsed with PBS and then dehydrated through ethanol series. The sample was dried and coated with gold-platinum coat (Soboh et al., 1995).

RESULTS

Bacterial strains, identification and antibiotic susceptibility

A total of 88 MDR UPEC isolates presumptively identified using the conventional culture methods and molecularly identified, were included in the study. The phylogenetic analysis of the 88 UPEC isolates revealed that a percentage of 62.5% (55/88), 18.2% (16/88), 13.6% (12/88) and 5.7% (5/88) belonged to the following phylogenetic groups B2, D, A and B1, respectively. High resistance levels were recorded with ampicillin (97%, 86/88) and cefuroxime (85.2%, 75/88), while high susceptibility was recorded with amikacin (12.5%, 11/88). Several patterns of antibiotic resistance were recorded as shown in Supplementary Table S1; patterns C, D and F were recorded each in 2 isolates from Mansoura hospital and all belonged to phylogenetic group B2, while pattern G was recorded in 3 isolates; all of them were isolated from Mansoura hospital and they all belonged to phylogenetic group A.

Effect of EDTA on bacterial susceptibility to antibiotics

The addition of a sub-MIC (2 mM EDTA) with antibiotics resulted in a decrease in the antimicrobials MIC values. The decrease in the fold of antimicrobials MIC in the presence of sub-MIC of EDTA is shown in Supplementary Table S2. The highest inhibitory effect of EDTA was observed with meropenem and nitrofurantoin rendering 81.6 and 61.4%, respectively of resistant UPEC to sensitive as shown in Figure 1.

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

The degree of biofilm formation in the tested MDR UPEC clinical isolates revealed that 85.2 (75/88), 11.3 (10/88) and 3.4% (3/88) of the isolates were strong, moderate and weak biofilm producers, respectively.

The degree of *in vitro* biofilm formation was determined for strong and moderate biofilm producers (85 isolates) in the presence of two different concentrations of EDTA (10 and 20 mM). The ability of *in vitro* biofilm formation decreased with the increase in EDTA concentration as shown in Supplementary Table S3 and Figure 2; where 45.8 (39/85), 43.5 (37/85), 3.5 (3/85) and 7% (6/85) were rendered negative, weak, moderate and still strong (no effect) biofilm-producers, respectively after the addition of 10 mM EDTA. Also, 78.8 (67/85), 17.6 (15/85) and 3.5% (3/85) were rendered negative, weak and still strong (no effect) biofilm producers, respectively after the addition of 20 mM EDTA.

The curli production was detected in 67% (59/88) of tested isolates; they showed bright red colonies on congo red agar plate (CRA) and were confirmed to be curli producers. The ability for curli production was tested in the presence of two different concentrations of EDTA (5 and 10 mM), where the ability of curli production decreased by increasing the concentration of EDTA; 69.4% (41/59) and 89.8% (53/59) of curli producing isolates were negative producers after the addition of 5 and 10 mM EDTA, respectively.

SEM analysis showed reduction in biofilm formation following treatment with EDTA at both tested concentrations, with the highest reduction following the addition of 20 mM EDTA, as shown in Figure 3.

Effect of coating polyvinyl chloride microtiter plate with 50 mM EDTA on *in vitro* biofilm formation

Gelatin coating alone had no effect on biofilm formation.

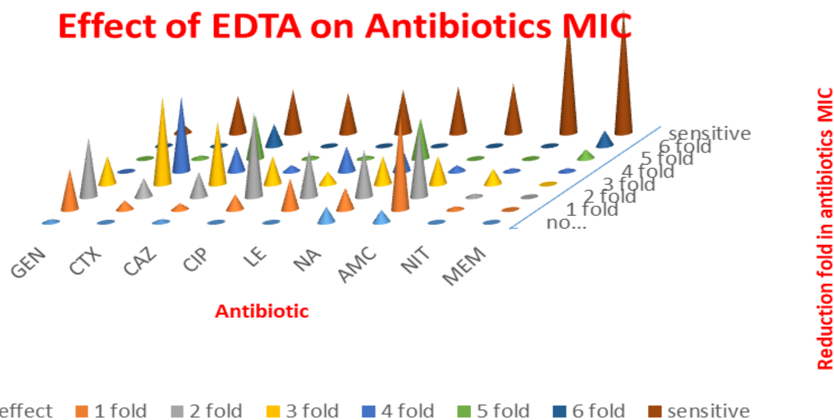

Figure 1. The effect of EDTA on bacterial susceptibility to antibiotics. GEN, Gentamicin; MEM, meropenem; CFM, cefotaxime; CAZ, ceftazidime; CIP, ciprofloxacin; LE, levofloxacin; NA, nalidixic acid; AMC, amoxycillin/clavulanic acid; NIT, nitrofurantoin.

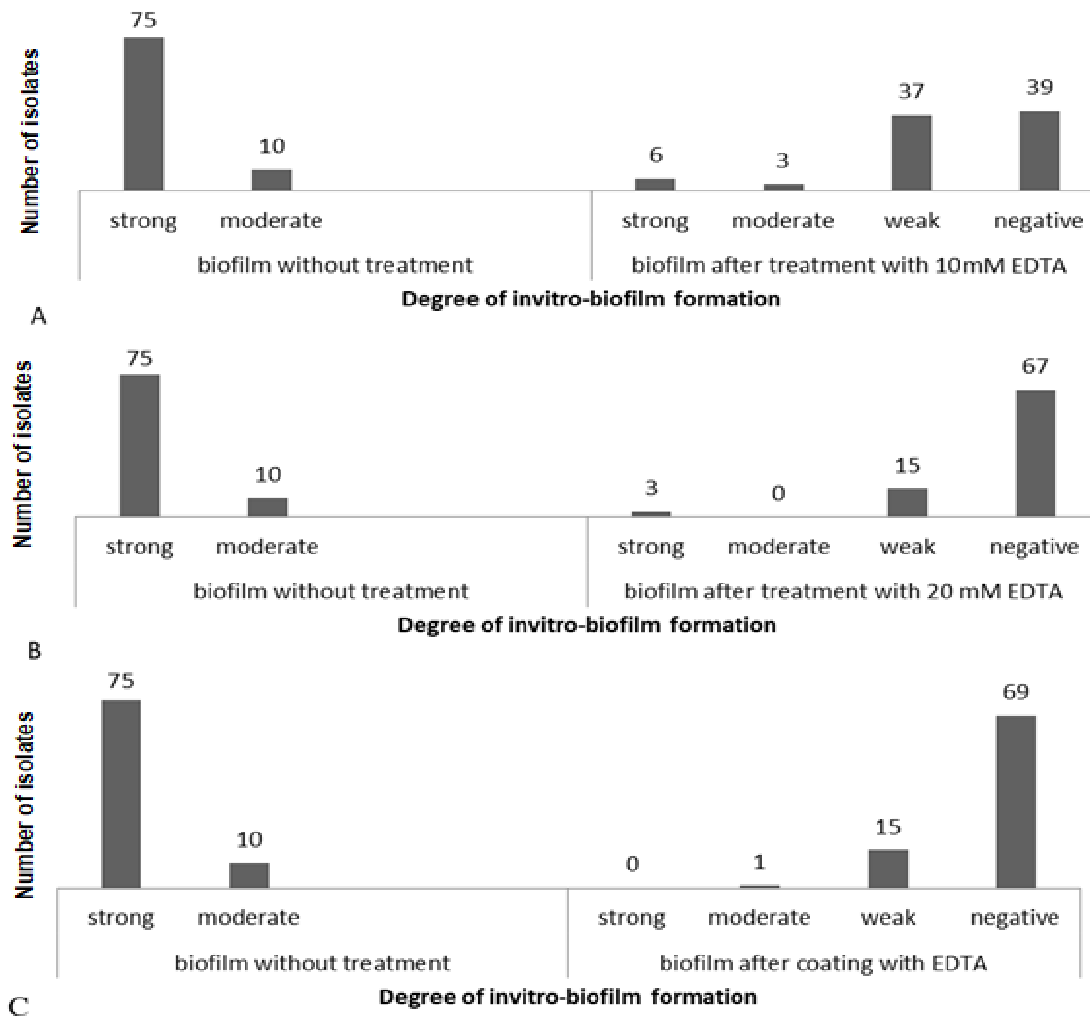

Figure 2. (A) The effect of addition of 10 mM EDTA on biofilm formation by MDR strong and moderate biofilm producing UPEC. **(B)** The effect of addition of 20 mM EDTA on biofilm formation by MDR strong and moderate biofilm producing UPEC. **(C)** The effect of coating of microtiter plates with Gelatin-EDTA coat on biofilm formation by MDR strong and moderate biofilm producing UPEC.

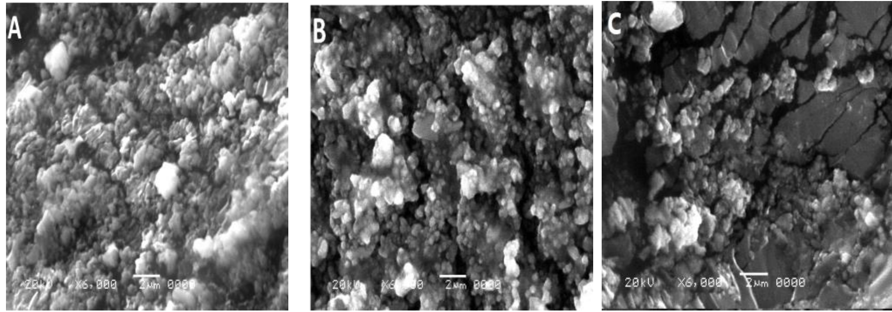

Figure 3. Scanning electron micrographs of a strong biofilm producer MDR UPEC isolate; where (A) is biofilm without treatment with EDTA, (B) biofilm in the presence of 10 mM EDTA and (C) Biofilm in presence of 20 mM EDTA.

EDTA at concentration 50 mM in gelatin coat effectively inhibited biofilm formation, where 81.1 (69/85), 17.6 (15/85) and 1.17% (1/85) were negative, weak and moderate biofilm-producers, respectively as shown in Figure 2.

DISCUSSION

UTI is a major cause of morbidity and may sometimes lead to mortality (Tajbakhsh et al., 2016) and represents a major health threat due to antibiotic resistance and high recurrence rate (Ponnusamy and Nagappan, 2013). Microbial biofilms in CAUTIs play an important role in antibiotic resistance and limits the therapeutic options (Deotale et al., 2015), so the effect of a non-antibiotic adjuvant EDTA on *in vitro* biofilm formation and antibiotic susceptibility of MDR strong biofilm producing UPEC clinical isolates from Egypt was studied.

The results revealed that using EDTA with concentrations 5 and 10 mM inhibited curli production, the first step in biofilm production. A similar study in India showed that EDTA at concentrations 4 and 5 mM can inhibit curli production (Chaudhary et al., 2013). It was also shown that EDTA with concentrations of 10 and 20 mM inhibited biofilm formation in UPEC biofilm producers by 45.8 and 78.8%, respectively and Chaudhary and colaboraters (2013) showed a decrease in biofilm formation by increasing EDTA concentrations.

In the present study, a novel approach was used to eradicate *in vitro* biofilm production and further evaluated the effect of EDTA coating of PVC microtiter plates, the material is often used for medical implants such as urinary catheter, on biofilm production. The results indicated that EDTA-gelatin coat was effective in inhibiting biofilm formation in 81.1% of tested isolates. Another study in China used EDTA and gallium coat in gelatin to inhibit the bacterial biofilms (Zhu et al., 2013). Trials to sustain the release of EDTA in wound dressings and contact lenses were done, using the therapeutic polymer of chitosan-EDTA (Netsomboon et al., 2017) and polylactic-glycolic acid disc containing 10% EDTA (Nishi et al., 1996). From the results, the coating of urinary catheters using combinations of EDTA with other anti-biofilm agents could greatly improve the clinical outcome.

Very low concentrations of EDTA (2 mM) was found to reduce the antimicrobials' MIC of MDR UPEC in the findings; the reduction of antimicrobials' MIC in the presence of EDTA was highly observed with Meropenem 81.6%, Nitrofurantoin 61.4%, Levofloxacin 26.4%, Ciprofloxacin 23.2% and Nalidixic acid 20.3%, Amoxicillin Clavulanic acid 18.8%, Ceftazidime 18.7%, Cefotaxime 15.8% and Gentamycin 6.5%.

Carbapenems are broad spectrum antimicrobial agent used as last resort treatment for Gram-negative bacteria. Emergence of resistance to carbapenems is a major threat and started to increase in the Middle East, and in this study, 55.6% (49/88) of isolates were resistant to Meropenem. In similar studies from Egypt, 44% of tested Gram negative bacteria were Carbapenem resistant (Khalifa et al., 2017). High prevalence of Carbapenem resistance among Gram negative bacteria was recorded worldwide, where similar studies in North Lebanon recorded Carbapenem resistance among 24.4% of tested Enterobacteriacea (Christophy et al., 2017) and in Germany, 16% of Carbapenem resistance organisms were detected among MDR Gram negative organisms (Maechler et al., 2015). EDTA is an inhibitor of metallo β-lactamases (MBLs) activity (Franklin et al., 2006), and in the present study, the addition of 2 mM EDTA to meropenem rendered 82.7% of meropenem resistant isolates to completely sensitive ones. Yet, no pharmaceutical preparations are available in the market that combines carbapenems with EDTA in parenteral preparations to treat life threatening infections.

Conclusions

The high prevalence of MDR phenotype among strong biofilm producers UPEC from Egypt is recorded and the combination of carbapenems with EDTA in parenteral

preparations to treat life threatening infections could greatly improve the clinical outcome. There is a continuous need for the development of new strategies for treatment of biofilm-producing UPEC with MDR profile and novel approaches to control microbial biofilm are needed.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

REFERENCES

Abd AKH, Abu-Raghif AR, Al-Azzawi AN (2000). The Effect of EDTA with Single or Combination of Antibiotics on *Pseudomonas aeruginosa* Isolates in Vitro. Iraq. J. Med. Sci. P 285.

Abdel-Moaty MM, Mohamed WS, Abdel-All SM, & El-Hendawy HH (2016). Prevalence and molecular epidemiology of extended spectrum β-lactamase producing *Escherichia coli* from hospital and community settings in Egypt. Appl. Pharm. Sci. 6(1):42-47.

Andrews JM (2001). Determination of minimum inhibitory concentrations. J. Antimicrob. Chemother. 48(suppl 1):5-16.

Bang CS, Kruse R, Johansson K, Persson K (2016). Carbon monoxide releasing molecule-2 (CORM-2) inhibits growth of multidrug-resistant uropathogenic *Escherichia coli* in biofilm and following host cell colonization. BMC Microbiol. 16(1):64.

Baugh S, Phillips CR, Ekanayaka AS, Piddock LJ, Webber MA (2013). Inhibition of multidrug efflux as a strategy to prevent biofilm formation. J. Antimicrob. Chemother. 69(3):673-681.

Brenner DJ (1984). Family I. Enterobacteriaceae. Bergey's Manual of Systematic Bacteriology 1:408-420.

Cai T, Gallelli L, Meacci F, Brugnolli A, Prosperi L, Roberta S, Eccher C, Mazzoli S, Lanzafame P, Caciagli P, Malossini G (2016). The efficacy of umbelliferone, arbutin, and N-acetylcysteine to prevent microbial colonization and biofilm development on urinary catheter surface: Results from a preliminary study. J. Pathog. 2016.

Chaudhary M, Kumar S, Payasi A (2012). A novel approach to combat acquired multiple resistance in *Escherichia coli* by using EDTA as efflux pump inhibitor. J. Microb. Biochem. Technol. 4:126-130.

Chaudhary M, Kumar S, Payasi A (2013). Role of CSE1034 in *Escherichia coli* biofilm destruction. J. Microb. Biochem. Technol. 5:54-58.

Chazotte B (2012). Labeling Golgi with fluorescent ceramides. Cold Spring Harb Protoc, 2012(8), pdb-prot070599.

Chen J, Griffiths MW (1998). PCR differentiation of *Escherichia coli* from other Gram-negative bacteria using primers derived from the nucleotide sequences flanking the gene encoding the universal stress protein. Lett. Appl. Microbiol. 27(6):369-371.

Christophy R, Osman M, Mallat H, Achkar M, Ziedeh A, Moukaddem W, Hamze M (2017). Prevalence, antibiotic susceptibility and characterization of antibiotic resistant genes among carbapenem-resistant Gram-negative bacilli and yeast in intestinal flora of cancer patients in North Lebanon. J. Infect Public Health (In Press).

CLSI (2012). Performance Standards for Antimicrobial Disk Susceptibility Tests, Approved Standard, 7th ed., CLSI document M02-A11. Clinical and Laboratory Standards Institute, 950 West Valley Road, Suite 2500, Wayne, Pennsylvania 19087, USA.

Cockerill FR, AWikler M, Alder J, Dudley M, Eliopoulos G, Ferraro M, Patel J (2012). Performance standards for antimicrobial disk susceptibility tests. M100-S22, CLSI, 32(3):84-86.

Deotale VS, Attal R, Joshi S, Bankar N (2015). Correlation between biofilm formation and highly drug resistant uropathogens (HDRU). Int. J. Curr. Res. Rev. 7(2):61.

Doumith M, Day MJ, Hope R, Wain J, Woodford N (2012). Improved multiplex PCR strategy for rapid assignment of the four major *Escherichia coli* phylogenetic groups. J. Clin. Microbiol. JCM-01468.

El-Gebaly E, Essam T, Hashem S, El-Baky RA (2012). Effect of levofloxacin and vitamin c on bacterial adherence and preformed biofilm on urethral catheter surfaces. J. Microb. Biochem. Technol. 4:131-136.

El-Sokkary MMA, Abdelmegeed ES (2015). Characterisation of class 1 integron among Escherichia coli isolated from Mansoura University Hospitals in Egypt. Adv. Microbiol. 5(4):269.

Franklin C, Liolios L, Peleg AY (2006). Phenotypic detection of carbapenem-susceptible metallo-β-lactamase-producing gram-negative bacilli in the clinical laboratory. J. Clin. Microbiol. 44(9):3139-3144.

Jacobsen SM, Stickler DJ, Mobley HLT, Shirtliff ME (2008). Complicated catheter-associated urinary tract infections due to *Escherichia coli* and *Proteus mirabilis*. Clin. Microbiol. Rev. 21(1):26-59.

Khalifa HO, Soliman AM, Ahmed AM, Shimamoto T, Hara T, Ikeda M, Kuroo Y, Kayama S, Sugai M, Shimamoto T (2017). High Carbapenem Resistance in Clinical Gram-Negative Pathogens Isolated in Egypt. Microbial Drug Resistance 2017.

Kumar N, Nahid F, Zahra R (2017). Association of virulence factors, phylogenetic groups and antimicrobial resistance markers in *Escherichia coli* from Badin city, Pakistan. J. Chemother. 29(1):8-13.

Lee JH, Subhadra B, Son YJ, Kim DH, Park HS, Kim JM, Choi CH (2016). Phylogenetic group distributions, virulence factors and antimicrobial resistance properties of uropathogenic *Escherichia coli* strains isolated from patients with urinary tract infections in South Korea. Lett. Appl. Microbiol. 62(1):84-90.

Lerma LL, Benomar N, Valenzuela, AS, Muñoz MDCC, Gálvez A, Abriouel H (2014). Role of EfrAB efflux pump in biocide tolerance and antibiotic resistance of *Enterococcus faecalis* and *Enterococcus faecium* isolated from traditional fermented foods and the effect of EDTA as EfrAB inhibitor. Food Microbiol. 44:249-257.

Maechler F, Diaz LP, Schröder C, Geffers C, Behnke M, Gastmeier P (2015). Prevalence of carbapenem-resistant organisms and other Gram-negative MDRO in German ICUs: first results from the national nosocomial infection surveillance system (KISS). Infection 43(2):163-168.

Magiorakos AP, Srinivasan A, Carey RB, Carmeli Y, Falagas M E, Giske CG, Paterson DL (2012). Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. Clin. Microbiol. Infect. 18(3):268-281.

Netsomboon K, Suchaoin W, Laffleur F, Prüfert F, Bernkop-Schnürch A (2017). Multifunctional adhesive polymers: Preactivated thiolated chitosan-EDTA conjugates. Eur. J. Pharm. Biopharm. 111:26-32.

Neupane S, Pant ND, Khatiwada S, Chaudhary R, Banjara MR (2016). Correlation between biofilm formation and resistance toward different commonly used antibiotics along with extended spectrum beta lactamase production in uropathogenic *Escherichia coli* isolated from the patients suspected of urinary tract infections visiting Shree Birendra Hospital, Chhauni, Kathmandu, Nepal. Antimicrob. Resist. Infect Control 5(1):5.

Nishi O, Nishi K, Saitoh I, Sakanishi K (1996). Inhibition of migrating lens epithelial cells by sustained release of ethylenediamine tetraacetic acid. J. Cataract Refract. Surg. 22:863-868.

Niveditha S, Pramodhini S, Umadevi S, Kumar S, Stephen S (2012). The isolation and the biofilm formation of uropathogens in the patients with catheter associated urinary tract infections (UTIs). J. Clin. Diagn. Res. 6(9):1478.

Ponnusamy P, Nagappan R (2013). Extended spectrum beta-lactamase, biofilm-producing uropathogenic pathogens and their antibiotic susceptibility patterns from urinary tract infection-an overview. Int. J. Microbiol. Res. 4(2):101-118.

Raad I, Chatzinikolaou I, Chaiban G, Hanna H, Hachem R, Dvorak T, Costerton W (2003). In vitro and ex vivo activities of minocycline and EDTA against microorganisms embedded in biofilm on catheter surfaces. Antimicrob. Agents Chemother. 47(11):3580-3585.

Rukayadi Y, Hwang J K (2006). Effect of coating the wells of a polystyrene microtiter plate with xanthorrhizol on the biofilm formation of Streptococcus mutans. J. Basic Microbiol. 46(5):410-415.

Sadekuzzaman M, Yang S, Mizan MFR, Ha SD (2015). Current and recent advanced strategies for combating biofilms. Compr. Rev. Food Sci. Food Saf. 14(4):491-509.

SarojGolia D, Hittinahalli V, Karjigi S K, Reddy KM (2012). Correlation between biofilm formation of uropathogenic *Escherichia coli* and its antibiotic resistance pattern. J. Evol. Med. Den Sci. 1(3):166.

Soboh F, Khoury A E, Zamboni AC, Davidson D, Mittelman MW (1995). Effects of ciprofloxacin and protamine sulfate combinations against catheter-associated Pseudomonas aeruginosa biofilms. Antimicrob. Agents Chemother. 39(6):1281-1286.

Tajbakhsh E, Ahmadi P, Abedpour-Dehkordi E, Arbab-Soleimani N, Khamesipour F (2016). Biofilm formation, antimicrobial susceptibility, serogroups and virulence genes of uropathogenic *E. coli* isolated from clinical samples in Iran. Antimicrob. Resist. Infect. Control 5(1):11.

Tayal RA, Baveja SM, De Anuradha S (2015). Analysis of biofilm formation and antibiotic susceptibility pattern of uropathogens in patients admitted in a tertiary care hospital in India. Int. J. Health Allied Sci. 4(4):247.

Wu H, Moser C, Wang HZ, Høiby N, Song ZJ (2015). Strategies for combating bacterial biofilm infections. Int. J. Oral Sci. 7(1):1-7.

Zhu Y, Jin F, Yang S, Li J, Hu D, Liao L (2013). Pre-treatment with EDTA-gallium prevents the formation of biofilms on surfaces. Exp. Ther. Med. 5(4):1001-1004.

Supplementary Table S1. Antibiotic susceptibility patterns of tested isolates.

Pattern number	Sample number	AK	GEN	IPM	MEM	CXM	CFM	CTX	CAZ	COT	CIP	NX	LE	NA	NIT	AMP	AMC	A/S	TZP	ATM	TE
A	2					■	■	■	■	■	■	■	■	■		■	■	■	■	■	■
A	6					■	■	■	■	■	■	■	■	■		■	■	■	■	■	■
A	10					■	■	■	■	■	■	■	■	■		■	■	■	■	■	■
A	65					■	■	■	■	■	■	■	■	■		■	■	■	■	■	■
B	72	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
B	73	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
B	78	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
B	84	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
B	97	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
B	113	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
B	160	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
B	171	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
C	7		■			■	■	■	■	■	■	■	■	■		■	■	■		■	■
C	144		■			■	■	■	■	■	■	■	■	■		■	■	■		■	■
D	8		■			■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
D	83		■			■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
E	69					■	■	■	■	■	■	■	■	■		■	■	■		■	■
E	85					■	■	■	■	■	■	■	■	■		■	■	■		■	■
E	102					■	■	■	■	■	■	■	■	■		■	■	■		■	■
F	16		■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
F	119		■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
G	87		■	■	■	■	■	■	■			■	■	■	■	■	■	■	■	■	■
G	88		■	■	■	■	■	■	■	■			■	■	■	■	■	■	■	■	■
G	121		■	■	■	■	■	■	■	■		■	■	■	■	■	■	■	■	■	■
H	1					■				■				■		■	■				■
I	3					■	■	■	■	■	■	■	■	■		■	■	■	■	■	
J	4			■		■	■	■	■	■				■	■	■	■	■		■	■
K	5					■	■	■	■			■		■	■	■			■	■	■
L	9			■		■	■	■		■	■			■	■	■	■	■			■
M	12					■										■	■				■
N	14			■	■	■	■	■	■	■	■	■		■		■	■	■	■	■	■
O	15			■	■	■	■	■	■	■				■	■	■	■	■	■	■	
P	17				■	■	■	■	■					■		■	■			■	
Q	18				■	■	■	■	■	■		■		■	■	■	■	■	■	■	
R	19				■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	
S	20	■	■		■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
T	22				■	■				■	■	■		■		■	■	■	■		
U	23				■	■	■	■	■	■	■	■		■	■	■	■	■	■		■
V	27	■		■	■	■	■	■	■	■	■	■	■	■	■	■	■			■	
W	28					■				■	■	■		■		■	■	■			■
X	29					■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
Y	30					■	■	■	■	■	■	■		■	■	■	■	■		■	■
Z	32	■	■	■	■	■	■	■	■	■	■	■		■	■	■	■	■	■	■	■
AA	33			■	■	■	■	■	■	■						■	■	■	■	■	■
AB	34				■	■	■	■	■					■	■	■	■			■	
AC	35		■			■	■	■	■	■	■	■	■	■		■	■	■	■	■	■
AD	36				■	■		■		■	■	■		■		■	■	■	■		

Supplementary Table S1. Contd.

AE	37
AF	39
AG	41
AH	43
AI	45
AJ	51
AK	52
AL	55
AM	56
AN	57
AO	60
AP	61
AQ	62
AR	67
AS	68
AT	70
AU	71
AV	75
AW	79
AX	80
AY	81
AZ	86
BA	89
BB	91
BC	93
BD	96
BE	98
BF	100
BG	106
BH	107
BI	117
BJ	120
BK	122
BL	129
BM	137
BN	138
BO	141
BP	143
BQ	152
BR	156
BS	157

AK, Amikacin ; AMC, amoxicillin/clavulanic acid ; AMP, Ampicillin ; A/S, ampicillin/sulbactam; CFM, cefixime; CTX, cefotaxime; CAZ, ceftazidime; CXM, cefuroxime; CIP, ciprofloxacin (), CO-Trimoxazole (COT), Gentamicin (GEN), Levofloxacin (LE), Nalidixic Acid (NA), Nitrofurantion (NIT), Norfloxacin (NX), Aztreonam (ATM), Imipenem (IMP), Meropenem (MEM), Piperacillin/Tazobactam (TZP), and Tetracycline (TE). Black filled cells represent resistant results while white cells represent susceptible results.

Supplementary Table S2. Antibiotics MIC in the absence and presence of subMIC of EDTA.

Isolates number	NIT MIC		NA MIC		GEN MIC		MEM MIC		CIP MIC		LE MIC		AMC MIC		CAZ MIC		CTX MIC	
	- EDTA	+ EDTA	- EDTA	+ EDTA	- EDTA	+ EDTA	- EDTA	+ EDTA	- EDTA	+ EDTA	- EDTA	+ EDTA	- EDTA	+ EDTA	- EDTA	+ EDTA	- EDTA	+ EDTA
1	<2	<2	>512	32	<2	<2	<2	<2	<2	<2	<2	<2	512	128	<2	<2	<2	<2
2	<2	<2	>512	32	<2	<2	<2	<2	128	<2	64	16	512	<2	>512	32	>512	<2
3	<2	<2	512	32	<2	<2	<2	<2	128	16	64	16	256	32	>512	16	>512	64
4	64	4	>512	32	<2	<2	<2	<2	<2	<2	<2	<2	128	64	>512	32	256	64
5	256	4	>512	8	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2	512	32	512	64
6	<2	<2	256	<2	<2	<2	<2	<2	128	<2	64	16	512	<2	512	4	>512	<2
7	<2	<2	>512	32	>512	256	<2	<2	128	4	64	16	256	128	>512	128	>512	64
8	256	4	512	32	>512	256	<2	<2	128	32	64	16	256	128	>512	16	512	64
9	128	8	>512	512	<2	<2	<2	<2	128	32	<2	<2	512	256	<2	<2	>512	64
10	<2	<2	>512	128	<2	<2	<2	<2	64	16	32	16	256	128	>512	32	512	32
12	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2	128	128	<2	<2	<2	<2
14	<2	<2	>512	8	<2	<2	512	<2	128	32	<2	<2	256	128	>512	32	512	128
15	512	64	512	32	<2	<2	512	8	<2	<2	<2	<2	256	128	512	16	512	256
16	128	4	>512	128	>512	128	512	<2	128	16	128	16	128	64	>512	16	512	64
17	<2	<2	512	128	<2	<2	8	<2	<2	<2	<2	<2	64	32	>512	4	>512	128
18	256	32	>512	512	<2	<2	512	<2	<2	<2	<2	<2	128	64	>512	16	>512	128
19	256	64	512	256	<2	<2	64	<2	8	<2	16	<2	128	64	128	8	128	<2
20	128	32	>512	>512	>512	256	512	<2	128	32	64	16	256	16	128	8	256	32
22	8	4	>512	256	<2	<2	16	<2	128	8	<2	<2	32	16	<2	<2	<2	<2
23	16	<2	>512	32	<2	<2	512	<2	64	<2	<2	<2	64	8	32	4	512	64
27	128	32	>512	256	<2	<2	8	<2	128	8	64	16	128	8	512	128	512	64
28	8	4	>512	256	<2	<2	<2	<2	64	8	<2	<2	16	8	<2	<2	<2	<2
29	128	4	512	32	<2	<2	<2	<2	128	16	256	16	64	8	>512	128	>512	64
30	128	4	>512	256	<2	<2	<2	<2	128	<2	<2	<2	64	64	>512	32	>512	64
32	64	8	>512	32	>512	512	32	<2	64	<2	<2	<2	256	128	>512	32	512	64
33	<2	<2	<2	<2	<2	<2	64	<2	<2	<2	<2	<2	128	64	>512	4	512	128
34	128	8	512	16	<2	<2	16	<2	<2	<2	<2	<2	16	4	>512	16	>512	64
35	<2	<2	>512	<2	>512	512	<2	<2	128	32	256	32	128	128	16	<2	>512	64
36	<2	<2	>512	<2	<2	<2	32	<2	128	64	<2	<2	128	64	<2	<2	512	64
37	128	4	>512	256	>512	128	64	<2	<2	<2	128	8	256	32	256	8	512	64
39	<2	<2	512	32	>512	128	64	<2	<2	<2	64	4	128	64	<2	<2	512	64
41	64	8	>512	256	512	128	<2	<2	128	16	256	16	512	128	>512	64	>512	128
43	256	4	512	64	256	64	<2	<2	128	16	64	8	64	8	>512	4	512	<2
45	128	4	>512	32	16	<2	512	<2	64	16	128	16	512	128	>512	32	512	64
51	<2	<2	<2	<2	<2	<2	<2	<2	64	<2	<2	<2	128	4	<2	<2	<2	<2
52	<2	<2	>512	256	64	4	<2	<2	<2	<2	<2	<2	64	<2	>512	4	256	4
55	16	4	>512	64	<2	<2	<2	<2	128	32	32	4	128	64	128	16	>512	128
56	256	4	>512	32	>512	256	512	8	<2	<2	128	4	256	32	128	16	<2	<2

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57	8	4	>512	32	512	128	<2	<2	<2	<2	<2	<2	128	16	>512	32	512	<2
60	<2	<2	>512	128	>512	128	<2	<2	128	32	64	16	256	64	512	<2	512	128
61	512	64	256	128	<2	<2	<2	<2	<2	<2	<2	<2	256	128	256	16	128	64
62	<2	<2	256	32	<2	<2	>512	<2	64	32	<2	<2	128	8	<2	<2	<2	<2
65	<2	<2	>512	128	<2	<2	<2	<2	128	<2	32	4	128	8	128	64	>512	64
67	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2	64	16	32	4	>512	64
68	<2	<2	<2	<2	64	16	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2
69	<2	<2	>512	32	<2	<2	<2	<2	128	32	128	16	64	16	512	128	>512	64
70	128	16	>512	32	<2	<2	64	4	64	16	<2	<2	128	64	512	64	256	64
71	128	64	>512	>512	>512	512	<2	<2	128	32	<2	<2	512	128	>512	128	512	<2
72	32	2	512	32	512	128	16	<2	128	32	256	8	512	128	>512	64	>512	64
73	512	4	512	4	>512	128	16	<2	64	16	256	4	>512	128	>512	128	512	<2
75	32	4	256	<2	<2	<2	<2	<2	<2	<2	<2	<2	256	128	16	<2	512	<2
78	512	64	>512	256	128	64	16	<2	128	32	256	16	>512	128	>512	128	>512	64
79	<2	<2	>512	16	256	64	16	<2	64	32	32	4	>512	64	512	32	256	<2
80	64	8	32	32	128	64	<2	<2	32	8	<2	<2	256	64	>512	128	>512	64
81	256	4	>512	>512	<2	<2	4	<2	64	32	<2	<2	64	16	>512	16	256	64
83	32	4	256	16	32	8	<2	<2	64	32	8	<2	128	32	>512	128	>512	64
84	128	4	>512	>512	128	64	32	<2	64	16	64	16	512	128	>512	32	>512	64
85	<2	<2	>512	256	<2	<2	<2	<2	128	<2	16	4	512	128	512	128	512	<2
86	<2	<2	128	16	<2	<2	<2	<2	128	<2	<2	<2	128	64	<2	<2	64	<2
87	64	<2	>512	256	>512	512	16	<2	<2	<2	256	16	512	128	>512	32	512	64
88	256	4	>512	256	128	32	32	<2	<2	<2	256	16	>512	128	>512	16	>512	64
89	<2	<2	<2	<2	256	128	64	<2	<2	<2	256	16	256	32	<2	<2	>512	64
91	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2	16	<2	64	16	<2	<2	<2	<2
93	64	4	<2	<2	256	64	16	<2	<2	<2	64	16	128	64	128	16	>512	64
96	<2	<2	>512	128	<2	<2	32	<2	128	32	64	16	128	64	64	<2	<2	<2
97	64	<2	>512	128	>512	512	256	8	128	32	256	64	128	64	>512	128	>512	64
98	<2	<2	>512	16	>512	512	16	<2	128	32	64	16	128	8	>512	128	512	64
100	16	4	>512	512	<2	<2	8	<2	128	32	32	16	128	64	128	128	128	64
102	<2	<2	>512	512	<2	<2	<2	<2	256	32	32	16	64	64	128	32	512	64
106	<2	<2	<2	<2	>512	256	512	8	<2	<2	32	<2	64	16	256	32	512	64
107	<2	<2	>512	<2	>512	512	64	<2	128	32	256	16	256	128	>512	128	>512	64
113	512	4	>512	32	>512	512	64	<2	128	16	64	16	256	128	>512	128	>512	64
117	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2	128	8	<2	<2	<2	<2
119	256	64	>512	512	>512	512	512	8	128	<2	64	4	128	64	>512	32	512	64
120	128	4	>512	32	<2	<2	16	<2	<2	<2	32	16	512	128	512	64	512	64
121	128	4	32	8	256	64	16	<2	32	8	64	4	512	128	>512	32	>512	64
122	<2	<2	<2	<2	<2	<2	64	<2	<2	<2	<2	<2	64	16	256	64	512	64
129	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2	256	128	128	32	256	<2
137	128	4	>512	256	>512	256	16	<2	128	32	32	16	<2	<2	256	128	>512	64

Supplementary Table S2. Contd.

138	512	64	4	<2	512	128	64	<2	<2	<2	<2	<2	<2	<2	128	32	256	32
141	<2	<2	>512	256	<2	<2	<2	<2	128	<2	32	16	256	32	128	16	512	64
143	<2	<2	>512	512	<2	<2	16	<2	128	<2	32	16	128	8	>512	64	512	64
144	<2	<2	512	<2	128	128	<2	<2	128	16	32	16	32	8	>512	64	512	64
152	256	4	512	32	512	128	512	8	128	16	64	16	512	128	256	64	<2	<2
156	<2	<2	<2	<2	>512	128	128	4	128	32	32	16	64	16	>512	64	256	32
157	128	4	256	32	>512	256	16	<2	<2	<2	32	4	128	64	>512	32	<2	<2
160	256	8	512	128	>512	128	32	<2	128	32	128	64	512	128	512	64	512	64
171	128	4	>512	128	>512	128	64	<2	128	32	16	4	256	64	16	4	>512	64

GEN, Gentamicin; MEM, meropenem; CFM, cefotaxime; CAZ, ceftazidime; CIP, ciprofloxacin; LE, levofloxacin; NA, nalidixic acid; AMC, amoxycillin/clavulanic acid; NIT, nitrofurantoin.

Supplementary Table S3. The ability of *in vitro* biofilm formation by strong and moderate biofilm-producers MDR UPEC in the presence of increased concentrations of EDTA.

Isolates number	Biofilm without treatment	Biofilm after 10 mM EDTA	Biofilm after 20 mM EDTA	Biofilm after coating with 50 mM EDTA
1	Strong	Negative	Negative	Negative
2	Strong	Negative	Negative	Negative
3	Strong	Weak	Negative	Negative
4	Moderate	Negative	Negative	Negative
5	Strong	Weak	Negative	Weak
6	Moderate	Negative	Negative	Negative
7	Strong	Negative	Negative	Negative
8	Strong	Negative	Negative	Negative
9	Strong	Negative	Negative	Weak
10	Weak	Negative	Negative	Negative
12	Moderate	Negative	Negative	Negative
14	Strong	Negative	Negative	Negative
15	Strong	Weak	Negative	Negative
16	Strong	Weak	Negative	Negative
17	Strong	Negative	Negative	Negative
18	Strong	Weak	Negative	Negative
19	Strong	Weak	Negative	Negative
20	Strong	Weak	Negative	Negative
22	Moderate	Negative	Negative	Negative
23	Weak	Negative	Negative	Negative
27	Strong	Weak	Negative	Negative
28	Strong	Weak	Negative	Weak

Supplementary Table S3. Contd.

29	Strong	Weak	Negative	Weak
30	Strong	Negative	Negative	Moderate
32	Strong	Weak	Negative	Weak
33	Strong	Weak	Negative	Weak
34	Strong	Weak	Weak	Negative
35	Strong	Weak	Negative	Negative
36	Strong	Negative	Negative	Negative
37	Moderate	Negative	Negative	Negative
39	Strong	Weak	Negative	Negative
41	Moderate	Negative	Negative	Negative
43	Strong	Negative	Negative	Negative
45	Strong	Negative	Negative	Negative
51	Strong	Weak	Negative	Negative
52	Strong	Negative	Negative	Negative
55	Strong	Weak	Negative	Negative
56	Strong	Weak	Negative	Negative
57	Strong	Strong	Strong	Negative
60	Strong	Weak	Negative	Negative
61	Strong	Negative	Negative	Negative
62	Moderate	Moderate	Weak	Negative
65	Strong	Negative	Negative	Negative
67	Strong	Moderate	Weak	Negative
68	Strong	Weak	Negative	Negative
69	Strong	Weak	Negative	Negative
70	Strong	Negative	Negative	Negative
71	Strong	Negative	Negative	Negative
72	Strong	Weak	Negative	Negative
73	Strong	Negative	Negative	Negative
75	Strong	Weak	Weak	Weak
78	Strong	Negative	Negative	Negative
79	Strong	Weak	Weak	Negative
80	Strong	Weak	Weak	Weak
81	Strong	Weak	Negative	Negative
83	Strong	Negative	Negative	Negative
84	Strong	Strong	Strong	Weak
85	Strong	Strong	Strong	Weak
86	Strong	Weak	Weak	Weak

Supplementary Table S3. Contd.

87	Strong	Weak	Weak	Negative
88	Strong	Strong	Weak	Weak
89	Strong	Moderate	Weak	Weak
91	Strong	Strong	Weak	Weak
93	Strong	Weak	Weak	Weak
96	Strong	Negative	Negative	Negative
97	Strong	Negative	Negative	Negative
98	Strong	Weak	Weak	Negative
100	Strong	Weak	Negative	Negative
102	Moderate	Negative	Negative	Negative
106	Strong	Weak	Negative	Negative
107	Strong	Negative	Negative	Negative
113	Strong	Strong	Weak	Negative
117	Strong	Weak	Weak	Negative
119	Strong	Weak	Negative	Negative
120	Strong	Negative	Negative	Negative
121	Strong	Negative	Negative	Negative
122	Moderate	Negative	Negative	Negative
129	Strong	Negative	Negative	Negative
137	Strong	Negative	Negative	Negative
138	Strong	Negative	Negative	Negative
141	Weak	Negative	Negative	Negative
143	Strong	Weak	Negative	Negative
144	Moderate	Negative	Negative	Negative
152	Strong	Negative	Negative	Negative
156	Strong	Negative	Negative	Negative
157	Strong	Negative	Negative	Negative
160	Strong	Weak	Negative	Weak
171	Strong	Weak	Negative	Negative