REVIEW ARTICLE

The Inhibitory Effect of Garlic and Onion Root Exudates on *Escherichia coli* from Urinary Tract Infection and Molecular Detection of *hlyA* Virulence Gene

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ABSTRACT

162 urine samples were collected from UTIs during October to December 2017 at Saladin General Hospital. *E. coli* was 38.9% of the collected samples. Women are more vulnerable to infection. The resistance percentage to Chloramphenicol, Ofloxacin, Ceftriaxone and Imipenem were 17% to 100%. The root exudates were prepared by using hydroponics with different periods: (7, 10 and 14 days). Onion (*Allium cepa* L.) root exudates have not any effect on the *E. coli* through the periods while root exudates of garlic (*Allium sativum* L.) gave the highest inhibitory effect at the period of 10 and 14 days. The virulence factors of *E. coli* were screened for the presence of *hlyA* through PCR and showed that all the resistant isolates could produce beta-hemolysis.

Keywords: *Allium cepa*, *Allium sativum*, *hlyA,* UTIs, Root exudates.

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INTRODUCTION

Urinary tract infection (UTI) is a response to infection of the urinary system as a result of infection by pathogenic bacteria, and it is one of the common infections, and comes second after the respiratory tract infection was causing a high death rate, 95% of UTIs was bacterial, while 5% are fungal and viral infections.[1] UTIs are the main causes of septicemia, and it is usually correlated with a high number of bacteria associated with its clinical symptoms and can occur anywhere in the urinary tract, and the incidence of infection varies depending on the severity of the bacterial infection, exposure rate and individuals exposed.[2]

Antibiotics have an active role in the treatment of UTI in the past and at present.[3] However, the antimicrobial activity of antibiotics has been affected by the increased ability of bacteria to resist antibiotics in different methods, which leads to treatment is no benefit.[4] This resistance increases significantly when a random increase in the use of antibiotics due to the presence of plasmids responsible for this resistance, which can between the bacteria.[5] The discovery of antibiotics and their use as a treatment leads to disease control and distributed. Antibiotics show a significant role in improving public health by helping to reduce deaths caused by bacterial infections.[6]

Roots have many major functions and are a site for manufacturing and releasing root exudates, which is a complex mixture of organic and inorganic chemical compounds that are released into the soil by roots that determine interference under the soil surface.[7] The chemical composition of root exudate is determined by several factors, including plant species, growth stage, organisms, and wide variation in environmental factors.[8] Root exudates include inorganic ions as well as oxygen and water but consist essentially of carbon compounds estimated at 5-60 % of the total carbon fixed by photosynthesis that is transferred to the rhizosphere by releasing it from the root system.[9]

Garlic (*Allium sativum* Linn.) and Onion (*Allium cepa* L.) are one of the most important medicinal plants and is considered a major research target.[10] A number of medical studies have led to the production of commercial garlic products such as Dried Garlic Powder (DGP) and Aged Garlic Extract (AGE) and it is rich in amino acid (S-Allylcysteine) and other organic sulfur compounds.[11] The great efficacy of garlic plants and their extracts against microorganisms is due to the diversity of their content in the composition compounds, which can be obtained by different separation methods.[12] Studies have shown that Onions contain many

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active ingredients including Scordinin, Phytonicidin, Alliin, mucous substances, sulfuric materials, androgen hormone and contains vitamins, A, B1, B2, C and carbohydrate, cellulose, protein, in addition to mineral salts.[13] The volatile oils of Onion juice have the effect of killing most of *Staphylococcus* as well as *Streptococcus*, with the inhibiting against *Brucella abortus* and examine the antimicrobial activity of Onions on the gram-negative bacteria such as *E. coli* and *Pseudomonas aeruginosa*, where the effect is less than on the gram-positive bacteria.[14] Several studies have indicated that Garlic and Onion acts as an antimicrobial agent and is usually given as a treatment for infections caused by *Helicobacter pylori* and *Candida albicans* and temporarily in yeast infections, *Salmonella typhimurium*, *Shigella dysenteriae*, *E. coli*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*, has been inhibited by S-Allylcysteine, the active ingredient in garlic.[15]

The virulence factor gene (*hlyA*) is important hemolysin toxin gene produced by *E. coli* that encodes and production of α-hemolysin protein help the bacteria to enter the tissues and the incidence of infection, causing intestinal diseases, damage of tissue and release of bacterial nutrients.[16] *hlyA* gene is one of the virulence factors of these bacteria and is one of the most important diagnostic and differential characteristics of these bacteria from other.[17]

No information on the antibacterial activity of Onion and Garlic root exudates are available. Thus, this study aims to determine the antibacterial activity of garlic and onion root exudates on UTIs under *In vitro* conditions in Saladin General Hospital in Tikrit city of Iraq and to identify the virulence factor of *the hlyA* gene in bacteria isolated from UTIs in case of the use of natural sources.

MATERIALS AND METHODS

Sampling

162 samples isolates were obtained from UTIs of the patients (male and female) in the Saladin General Hospital who suffered from symptoms of UTIs and is difficult to urinate for the period from October to December 2017.

Bacterial Isolation and diagnosis

Bacterial samples were isolated from UTI by inoculation on Blood agar and Macconkey agar at 37°C overnight and then the sample growth was inoculated on deferential media. The Microscopic and Biochemical diagnosis test was done according to Whitman *et al*., 2012,[18] and the diagnosis was confirmed by the VITEK 2 compact system (Biomerieux, France).

Susceptibility test

The disc diffusion test method was used to determine the antimicrobial susceptibility of bacterial isolated against 10 antibiotics (Amoxyclave (AMC) 30µg, Amoxicillin (AMX) 25µg, Chloramphenicol (C)10µg, Erythromycin (E) 15µg, Streptomycin (S) 10µg, Imipenem (IMP)10µg, Ceftriaxone (CRO)10µg, Ciprofloxacin (CIP)10µg, Cefepime (CPM) 30µg, Ofloxacin (OFX)10µg) were from (Bioanalyse Co. Turkey). Susceptible isolates were done to intermediate or resistant according to the (CLSI).[19]

Root exudates preparation

Hydroponic Culture System (HCS) was used to collect root exudate from locally grown Garlic and Onion which sterilized by 1% Sodium hypochlorite for 2 minutes, then rinse with sterile distilled water and are grown in sterile glass containers containing sterile distilled water covered with two layers of gauze and after seeds germination, the root exudates were collected in dark glass containers after three-time (7, 10 and 14 days). The exudates were filtered using the Millipore filter (0.22 µm), and the exudates were kept sterile in test tubes at 4ºC.[20]

Root exudates susceptibility test

The freshly isolates a prepared inoculum was spread on Muller Hinton Agar, three wells of 6 mm diameter were prepared using sterile cork – borer and 50µL of root exudates were filled in the wells and incubated at 37°C for 18-24 hours.[18]

Plasmid DNA extraction

The Plasmid bacterial DNA was extracted from *E. coli* using Miniprep plasmid DNA Kit (Promega, USA).

Virulence gene detection

The hemolysin toxin genes (*hlyA*) were amplified using Polymerase chain reaction (PCR) for the detection of *E. coli* virulence gene. Specific primer was supplied by the (Bioneer Co. Korea), this primer was designed from the National Center for Biotechnology Information (NCBI) published sequence *E. coli hlyA* gene (code: AF037577.1). PCR master mix kit (AccuPower® PCR PreMix kit.Bioneer. Korea) was achieved in a 20 µL mixture included: 2.5 µL 1x PCR buffer, 0.75 µL MgCl2, 1 µL dNTP mixture, 1 µL of each forward and reverse primer, 0.5 µlTaq DNA polymerase, 5 µL genomic DNA and complete the mixture by 8.85 µL double distilled water. A 150 bp fragment of *hlyA* was amplified by PCR primer sequences forward and reverse were: 5'AGAATGGCACGGCGATTACT-3' 5'CAGCACTGCCGCCTAATTTA-3'.The following thermocycler (Prime. UK) conditions; initial denaturation temperature of 95 °C for 5 min; followed by 30 cycles at denaturation 95 °C for 30 s, annealing 58 °C for 30 s, and extension 72 °C for 1min and then final extension at 72 °C for 5 min. The PCR products were tested by electrophoresis in a 2% agarose gel.

RESULTS

Samples included both sexes and all age groups, 59.3% of females and 40.7% of males. 38.9% showed positive bacterial growth; this is due to the use of antibiotics for patients that led to the disappearance of bacteria causing the UTIs, or maybe the cause of anaerobic bacteria and cannot be isolated by the same methods used in this study. Eosin Methylene Blue (EMB) medium was used to isolated and identified *E.coli*, which appeared in Green metallic color and using conventional bio-chemical method and VITEK 2 compact system.[21]

The Susceptibility test of 10 antibiotics was performed for *E. coli* isolated using the disk diffusion method. Figure 1 shows the susceptibility, and the figure indicates 100% resistance to Amoxicillin, Erythromycin and Imipenem. Streptomycin and Amoxyclave inhibitors gave a high resistance rate of 96%, and the bacterial isolates gave a 75% resistance to Cefepime. 42% to Ofloxacin and 38% to Ciprofloxacin, whereas Chloramphenicol was the least resistant 17%.

Hydroponic Culture System (HCS) was used to collect the root exudates. The Onion and Garlic imported and available in the market were used for eating and did not show roots when growing it in the HCS, to treat preservatives for conservation as long as possible and avoid contamination, these substances prevent the growth of roots, and the diversity differences in plant species. Then the different sizes of Onion and Garlic mother bulb (7g, 13g, and 18g) were used, and their growth in HCS under sterile conditions to have a good root group, the exudates were collected at different time intervals from the moment of root group growth.

The exudates were used at 7, 10, and 14 days. The effect of these exudates was tested on all isolates by inoculating on the Muller Hinton Agar, after filling these wells with exudates and recording the result of diameters after 18-24 hours. Onion exudates did not give any antimicrobial effect on the bacterial isolate, although its root group has grown well. The garlic exudates gave the most antimicrobial effect at age 14 days, with different inhibitors, and less effective in 10 days, while no antimicrobial effect was given at age 7 days (Figure 2).

E.coli showed varying sensitivity to 10-day garlic root exudates 37.5% with a 10 mm inhibitor diameter, 54.1% with an inhibitory diameter of 11 mm and 8.3% with no sensitivity. A 14-day inhibition area with a 10 mm inhibitor diameter of 8.3% was observed. A diameter of 14 mm inhibition was observed for 33.3 isolates, 13 mm inhibition for 41.6% of isolates, and a diameter of 12 mm for 16.6% of isolates.

Plasmid DNA was extracted for antibiotic resistance isolates to detect the presence of *hlyA* virulence genes. The extracted DNA was used in PCR technique. The result of the *hlyA* gene in *E.coli* was shown clear bands at 150 bp when tested by electrophoresis in a 2% agarose gel (Figure 3), indicating that these isolates have antibiotic resistance. This gene is responsible for the production of hemolysin, which is an important virulence factor.

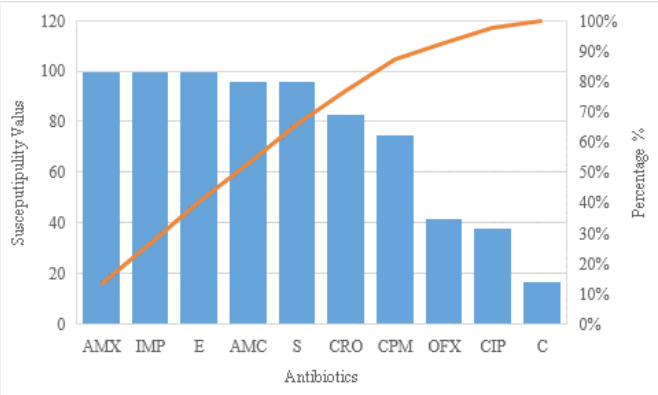

Figure 1: Antibiotics susceptibility profile.

DISCUSSION

The increase of UTIs in females due to the presence of these bacteria as normal flora and the nature of the urinary tract, their short urethra, and it is near the anus and analgesia, and probably because of the sexual activity in this age group.[22]

The high incidence of *E. coli* were explained bacterial leaving of the intestine as a normal flora and when it arrives the urinary tract causing infection, and it has many dangerous factors, including Fimbriae, that adhere to specific receptor molecules in urinary epithelial cells, as well as their ability

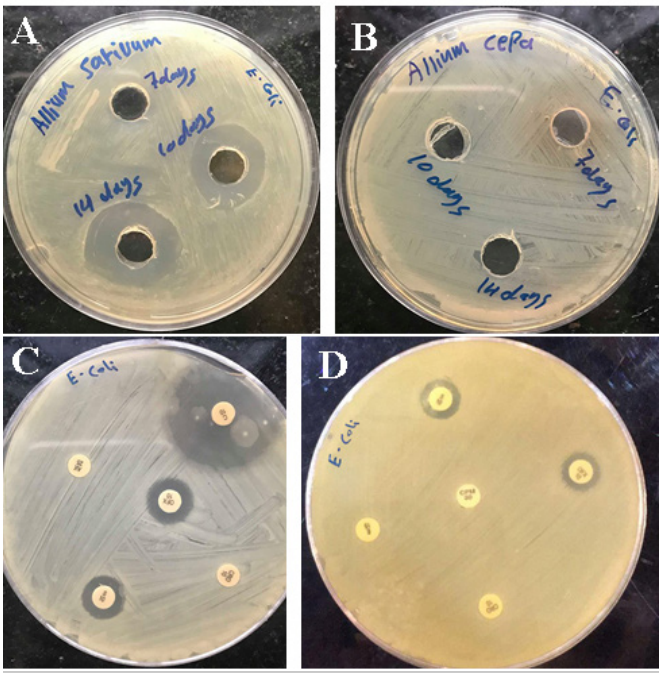

Figure 2: Kirby-Bauer diffusion of (A) *Allium sativum*; (B) *Allium cepa*; (C and D) Disk diffusion of Antibiotics

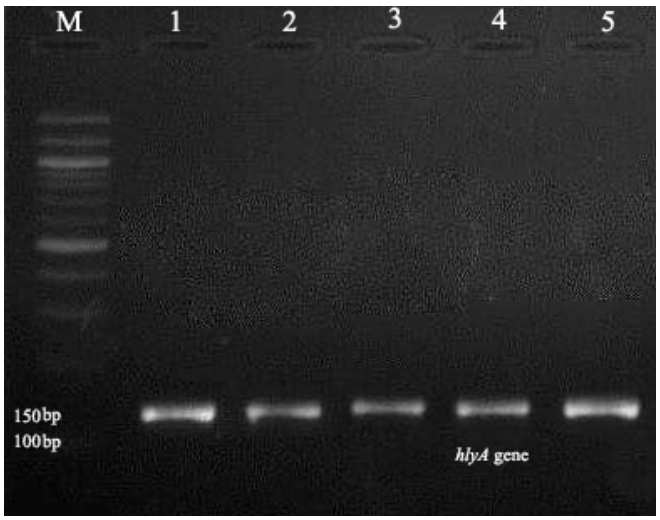

Figure 3: Electrogram of PCR of the *hlyA* gene in *E. coli*. Agarose gel (2%), DNA ladder (100-1000) lane M., lane (1-6,) samples indicate to hemolysin toxin *hlyA* at 150bp PCR product

to produce hemolysin, antibiotic resistance, and it has short generation time, in addition to the presence of urinary pathogens, including the necrosis of the cells, and the adhesion factors that play a large role in UTIs.[23]

Most studies indicate that these bacteria are resistant to different types of antibiotics.[24] These bacteria are common in hospitals and in patients who are immunologically inhibited, so treatment is difficult because they have different mechanisms to resist these antibiotics.[25] The increase of the resistance of bacterial isolates to antibiotics is due to the random use of antibiotics or their acquisition of genetic traits as multiple resistance by conjugation,[26] or chromosomal mutations that encode resistance from other bacterial sources, and exposure to various antibiotics for a long time increases the ability of bacteria to resistance development against antibiotics.[27]

The ability of uropathogenic *E. coli* to cause UTI is related to general virulence factors such as α-hemolysin together with pili-mediated adherence to uroepithelial cells (P pili).[28] A change in the pathogenesis of the sensitive cellular wall of the antibiotics led to an increase in thickness or a lack of correlations causing a change in the target sites of the antibiotics or resistance maybe by reducing the permeability of the outer membrane of the gram-negative bacteria and may affect the rate of absorption of these antibodies.[29] These mechanisms are found exclusively in the Gram-negative bacteria because they contain the outer membrane, while the Gram-positive bacteria are lacking. One of the factors that cause bacterial resistance against the aminoglycoside group of Streptomycin is the change in the target site (the 30S), or resistance may result from the production of an enzyme by antimicrobial resistance or the lack of outer membrane permeability.[30]

The garlic exudates showed a significant antibacterial effect at 14 days because time is longer, and its concentration increases and the amount of water evaporated from the hydroponic culture during this period is higher; it increases the concentration of the exudates substances from the root in the hydroponic culture.[31] The effect of *E.coli* on the root exudates is due to the presence of compounds in the exudates having bactericidal activity; this interference is known as the allelopathy, which is known as biochemical interactions between plants and microorganisms. No comparisons have been made for the absence of previous studies on the effect of exudates on pathogenic bacteria.

The ability to detect the hemolysin toxin gene (*hlyA*) in *E. coli* was increased several times in the application of PCR. In this study, 6 out of 24 isolates were found to contain the *hlyA* gene. Several studies have shown that hemolysin plays an important role in the occurrence of UTIs.[32,33] It is considered an important factor in association with other genes not mentioned in this study. There is a strong relationship to the infection in UTIs and severity due to several factors, including the age of the patient, the incidence of chronic diseases, pregnancy, education, and excessive use of antibiotics without consulting the doctor.[34]

CONCLUSIONS

Our findings suggest that isolated bacteria can cause UTI and that virulent gene may help these isolates survive even with the use of appropriate antibiotics and are responsible for recurrent infections. The study included some limitations, including the treatment of plant root exudates used for the first time with pathogenic bacteria; Limited information has been collected about the patient.

REFERENCES

1. Gambogou B, Ouattara AK, Taale E, Karou SD, Ameyapoh YA, Simpore J (2018) Garlic as Alternative Therapy to Treat Uropathogene Bacteria in Women with Urinary Tract Infection in Lomé, *Togo,* no. September, pp. 1–11, doi:10.20944/preprints201809.0077.v1
2. Stein R, Dogan HS, Hoebeke P, Kočvara R, Nijman RJ, Radmayr C, Tekgül S (2015) "Urinary Tract Infections in Children : EAU/ESPU Guidelines, Eur Urol., 67(3): 546-58. doi: 10.1016/j.eururo.2014.11.007.
3. Wong CKM, Kung K, Au-Doung PLW, Ip M, Lee N, Fung A, Wong SYS (2018) Antibiotic resistance rates and physician antibiotic prescription patterns of uncomplicated urinary tract infections in southern Chinese primary care, PLoS One., 12(5):1-13, e0177266., doi: 10.1371/journal.pone.0177266.
4. Tong SY, Davis JS, Eichenberger, E, Holland TL, FowlerVG Jr (2015) *Staphylococcus aureus* infections: epidemiology, pathophysiology, clinical manifestations, and management. Clin. Microbiol. Rev., 28(3):603-61doi: 10.1128/CMR.00134-14.
5. Grabe M, Bartoletti R, Johansen TEB, Cai T, Köves MÇB, Naber KG, Pickard RS, Tenke P, Wagenlehner F, Wullt B (2015) Guidelines on Urological Infections, European Association of Urology Available: https://uroweb.org/wp-content/uploads/19-Urological-infections_LR2.pdf
6. Rossignol L, Vaux S, Maugat S, Blake A, Barlier R, Heym B, Le Strat Y, Blanchon T, Hanslik T, Coignard B (2017) Incidence of urinary tract infections and antibiotic resistance in the outpatient setting a cross-sectional study, infection, 45(1):33-40 doi: 10.1007/s15010-016-0910-2.
7. Bais HP, Park SW, Weir TL, Callaway RM, Vivanco JM (2004) How plants communicate using the underground information superhighway, Trends Plant Sci., 9(1):26-32. doi:10.1016/j.tplants.2003.11.008.
8. Badri DV, Loyola-Vargas VM, Broeckling CD, De-la-Pen˜a C, Jasinski M, Santelia D, Martinoia E, Sumner LW, Banta LM, Stermitz F, Vivanco JM (2008) Altered Profile of Secondary Metabolites in the Root Exudates of Arabidopsis ATP-Binding Cassette Transporter Mutants, Plant Physiology, 146,762–771. https://doi.org/10.1104/pp.107.109587
9. Walker TS, Bais HP, Grotewold E, Vivanco JM (2003) Root Exudation and Rhizosphere Biology, Plant Physiol, 132,44-51, DOI: https://doi.org/10.1104/pp.102.019661
10. Madineh H, Yadollahi F, Yadollahi F, Mofrad EP, Kabiri M (2017) Impact of garlic tablets on nosocomial infections in hospitalized patients in intensive care units, Electron Physician, 25;9(4):4064-4071. doi: 10.19082/4064.
11. Pulipati S, Babu PS, Narasu ML, Anusha N (2017) An overview on urinary tract infections and effective natural remedies, Journal of Medicinal Plants Studies, 5(6): 50-56.

12. Mansour A, Hariri E, Shelh S, Irani R, Mroueh M (2014) Case Report Efficient and Cost-Effective Alternative Treatment for Recurrent Urinary Tract Infections and Interstitial Cystitis in Women : A Two-Case Report, Case Reports in Medicine, Volume 2014, Article ID 698758, 4 pages, https://doi.org/10.1155/2014/698758.
13. Nemeth K, Piskula MK (2007) Food Content, Processing, Absorption and Metabolism of Onion Flavonoids, Critical Reviews in Food Science and Nutrition, 47(4):397–409. https://doi.org/10.1080/10408390600846291.
14. Kabrah AM, Faidah HS, Ashshi AM, and Turkistani SA (2016) Antibacterial Effect of Onion, Sch. J. App. Med. Sci., 4(11D):4128-4133 10.21276/sjams.2016.4.11.53.
15. Millet COM, Lloyd D, Williams C, Williams D, Evans G, Saunders RA, Cable J (2011) Effect of garlic and allium-derived products on the growth and metabolism of *Spironucleus vortens*. Experimental Parasitology, 127(2), 490–499. https://doi.org/10.1016/j.exppara.2010.10.001.
16. Wiles TJ, Kulesus R. R., and Mulvey M. A. 2008. Origins and virulence mechanisms of uropathogenic *Escherichia coli*, Exp Mol Pathol., 85, 11–19. 10.1016/j.yexmp.2008.03.007.
17. Afrough P, Pourmand MR, Sarajian AA, Saki M Saremy S (2013) Molecular Investigation of *Staphylococcus aureus*, *coa* and *spa* Genes in Ahvaz Hospitals, Staff Nose Compared With Patients Clinical Samples., Jundishapur Journal of Microbiology, 6 (4): 5377. https://doi.org/10.5812/jjm.5377.
18. Goodfellow M, Kämpfer P, Busse HJ, Trujillo M, Ludwig W, Whitman W (2012) Part A. and B; Bergey's Manual of Systematic Bacteriology, (5th ed.). Springer-Verlag New York. https://doi.org/10.1007/978-0-387-68233-4
19. Patel JB, Weinstein MP, Eliopoulous GM, Jenkins SG, Lewis JS, Limbago B, Mathers AJ, Mazzulli T, Patel R, Richter SS, Satlin M, Swenson JM, Traczewski MM, Turnidge JD, Zimmer BL (2017) M100-S26 Performance Standards for Antimicrobial susceptibility testing. Clinical and laboratory standards institute. M100 27th ed. 37(1).
20. Hameed KM, Saghir AR, Foyj CL (1973) Influence of Root Exudates on *Orobanche* Seed Germination, Weed. Res., 13(242), 114–117.
21. Balat A, Hill LL (1999) Genitourinary Abnormalities in Children with Urinary Tract Infections, Tr. J. of Medical Sciences, 29, 59–63.
22. Moore EE, Hawes SE, Scholes D, Boyko EJ, Hughes JP, Fihn SD (2008) Sexual Intercourse and Risk of Symptomatic Urinary Tract Infection in Post-Menopausal Women, Journal of General Internal Medicine, 23(5):595–599. https://doi.org/10.1007/s11606-008-0535-y.
23. Flores-mireles AL, Walker JN, Caparon M, Hultgren SJ (2015) Urinary tract infections : epidemiology, mechanisms of infection and treatment options. Nature Reviews Microbiology, 13(5), 269–284. https://doi.org/10.1038/nrmicro3432.
24. Belongia EA, Knobloch MJ, Kieke BA, Davis JP, Janette C, Besser RE (2005) Impact of Statewide Program To Promote Appropriate Antimicrobial Drug Use, Emerg Infect Dis. 11(6):912-20. https://doi.org/10.3201/eid1106.050118.
25. Orrett FA (2004) Antimicrobial Susceptibility Survey of *Pseudomonas Aeruginosa* Strains Isolated from Clinical Sources, J Natl Med Assoc., 96(8):1065–1069. PMID: 15303411.
26. Pathak SD, Day JM, Nair A, Sawaya WJ, Kristal MM (2007) Complexity and Adaptively in Supply Networks : Building Supply Network Theory Using a Complex Adaptive Systems Perspective., Decision Sciences, 38(4):547 – 580.
27. Chen C, Huang Y (2014) New epidemiology of *Staphylococcus aureus* infection in Asia. Clin. Microbiol. Infect., 20(7):605-23. https://doi.org/10.1111/1469-0691.12705.
28. Wilson ML, Gaido L (2004) Laboratory Diagnosis of Urinary Tract Infections in Adult Patients, Clin Infect. Dis., 38(8):1150-8. doi/10.1086/383029.
29. Morosini MI, Garcı´a-Castillo M, Coque TM, Valverde A, Novais A, Loza E, Baquero F, Canto´n R (2006) Antibiotic Coresistance in Extended-Spectrum-Lactamase-Producing *Enterobacteriaceae* and In Vitro Activity of Tigecycline, Antimicrobial Agents and Chemotherapy, 50(8):2695–2699. https://doi.org/doi:10.1128/AAC.00155-06
30. Furtula V, Farrell EG, Diarrassouba F, Rempel H, Pritchard J, Diarra MS (2010) Veterinary pharmaceuticals and antibiotic resistance of *Escherichia coli* isolates in poultry litter from commercial farms and controlled feeding trials, Poultry Science, 89(1): 180–188. https://doi.org/10.3382/ps.2009-00198.
31. Khan, MA, Zhihui C (2010) Influence of garlic root exudates on cyto-morphological alteration of the hyphae of *phytophthora capsici*, the cause of *phytophthora* blight in pepper, Pak. J. Bot., 42(6), 4353–4361.
32. Clermont O, Bonacorsi P, Bingen E (2000) Rapid and Simple Determination of the *Escherichia coli* Phylogenetic Group, Appl Environ Microbiol, 66(10): 4555–4558.
33. Ghenghesh KS, Elkateb E, Berbash N, Nada RA, Ahmed SF, Rahouma A, Seif-Enasser N, Elkhabroun MA, Klena JD (20090 Uropathogens from diabetic patients in Libya : virulence factors and phylogenetic groups of *Escherichia coli* isolates, J Med Microbiol., 58(Pt 8):1006–1014. https://doi.org/10.1099/jmm.0.007146-0.
34. Rijavec M, Mu¨ller-Premru M, Zakotnik B, Z˘gur-Bertok D (2008) Virulence factors and biofilm production among *Escherichia coli* strains causing bacteraemia of urinary tract origin, Journal of Medical Microbiology, 57, 1329–1334. https://doi.org/10.1099/jmm.0.2008/002543-0.