International Journal of Studies in Natural and Medical Sciences
Volume 02 Issue 01, January, 2023
ISSN (E): 2949-8848
Scholarsdigest.org

Detection the Ability of Escherichia Coli to Produce Biofilm and their Resistance to Some Antibiotics

Esraa Ahmed Abbas * , Ali Jaffar Saleem**
*esra.ahmed.abbas@uodiyala.edu.iq
** College of Education for Pure Science - Diyala University,
alijaffar3@yahoo.com

Abstract
In current study, 150 samples were collected from patients with urinary tract infection of both genders and all ages from October 2021 to January 2022 in Diyala and Baghdad governorates , Iraq . They were tested by phenotypic methods and the results showed that 73 isolates belonged to E.coli.
The ability of the isolates to produce biofilm was detected by two different methods: the first method is Congo red agar method (CRA), showed that E. coli 72.60% . As for the second method is Microtiter Plate Method (MTP), showed that E.coli 83.56%.
The sensitivity of all isolates to antibiotics was tested according to the Kerby Bauer method, The results showed that (79.45%) of them were resistant to Ampicillin, Amoxicillin-clavulanate (73,97%) , Sulfamethoxazole - Trimethoprim (57,53%) , Tetracycline (52,05%), Cefotaxime (41,09%) , Norfloxacin (38,35%), Azithromycin (28,76%), Gentamicin (16,43%) , Chloramphenicol (9,58%), Piperacillin_Tazobactam (2.73%) , no isolate showed resistance to both meropeneme and nitrofurantoin .
Most of the isolates showed resistance to multiple antibiotics76.71% .

Keywords: E.coli, Biofilm , Antibiotics, MDR,UTI

Introduction

Urinary tract infections (UTI) are of interest to researchers and medical professionals , It is one of the most common and widespread diseases and the second most common type of injury after respiratory infections [1] . Among the infections related to society while it ranks first in terms of hospital-related infections , It constitutes about 40% of hospital-acquired infections It affects a large percentage of the community[2].

E. coli is one of the most important members of the intestinal family, Bacilli, gram-negative, positive for the catalase test and negative for the oxidase test, positive for methyl red test, producing indole [3]. It is found within the natural flora within the human body at the same time, it is an Opportunistic pathogens thus it causes many diseases such as diarrhea , Sepsis , Meningitis [4] .

E.coli that cause urinary tract infections possess a variety of virulence factors that allow them to cause disease[5], including the ability to form a biofilm, which play an important role in adhesion of cells to non-living surfaces ,these biofilms are a group of

bacterial cells that adhere to solid surfaces and have a role in infecting the host, increases the ability of bacteria to resist antibiotics, and protects cells from the defense mechanisms of the host's body. It also helps bacteria survive in conditions that are not suitable for growth[6].

The bacteria that cause urinary tract infection have the ability to resist antibiotics, which results in the emergence of multiple resistant strains that cause infections, Although antibiotics are available to treat many diseases that are caused by bacteria , there are some types of bacteria that have become resistant to many antibiotics, Antibiotic resistance is the most important health problems that has gradually increased in all countries of the world, prompting researchers to discover new antibiotics to overcome resistant bacterial strains[7].

Materials and Methods

Isolation and Phenotypic Detection of *E.coli*

A total of 150 urine samples were collected from patients suffering from urinary tract infections of different ages and for both genders from October 2021 to January 2022 from private laboratories in Diyala and Baghdad governorates , Iraq. The isolates were identified as *E.coli* by culture tests and it was by using MacConky agar medium and Eosin methylene blue medium ,the dishes were incubated at a temperature of 37°C for 24 hours,Microscopy with a gram stain was used , In addition to biochemical tests includes Catalase test, oxidase test, indole test, methyl red test, voges-proskauer test, citrate test[8,9].

Detection of the ability of *E. coli* bacteria to form a biofilm

Detection of biofilm formation using the Congo red agar method

The medium included 37 gm brain-heart infusion broth, 50 gm sucrose, 10 gm agar-agar, 0.8 gm Congo red stain. The medium was inoculated with isolates and incubated at 37°C for 48 hours , this medium is used to detect the ability of bacteria to produce biofilm [10].

Detection of biofilm formation using the Microtiter Plate method

The Microtiter Plate method was used to quantitatively detect the biofilm as stated [11]. Optical density (O.D) was measured at 630 nm.

Antibiotic sensitivity test

The disc diffusion method was applied on Mueller-Hinton agar to determine antibiotic susceptibility as described in[12]. The results were classified as susceptible, intermediate or resistant according to what was stated in[13].

International Journal of Studies in Natural and Medical Sciences
Volume 02 Issue 01, January, 2023
ISSN (E): 2949-8848
Scholarsdigest.org

Statistical Analysis

The genetic relationship was found among all the bacterial isolates under study by Antibiogram by converting the results that appeared in the characterization table when the result is positive put the number 1 and if the result is negative put 0, This data was entered into Software Past in order to obtain antibiogram.

Results and Discussion

Phenotypic Identification of *E.coli*

E.coli isolates were identified by culture tests as it was planted on the MacConky agar medium the colonies appeared in a pink color(fermentation of lactose), soft and shiny. while the colonies appeared on the eosin methylene blue medium with green metallic sheen. As for the microscopic diagnosis, it appeared negative for the Gram stain, a short rod.

The isolates showed that they were positive for catalase, indole and methyl red and negative for oxidase and voges-proskauer, citrate test , 73 isolates of *E.coli* were obtained.

Biofilm formation

The results of detection of biofilm formation of 73 isolates using Congo red agar methods revealed that 72.60 % isolates biofilm producer , the colonies appeared black while 27.39% isolate not biofilm-producing ,the colonies appeared red .

The results of the current study were close to the results Alwan and Katongole ,The percentage of *E.coli* isolates from the biofilm 66%,62.5% respectively[14,15].

As for the results of detection of biofilm formation of 73 isolates using Microtiter plate method revealed that 83.56% isolates biofilm producer included 6.55% isolates were a strong producer for biofilm,34.42 % isolates were moderate producer for biofilm , 59.01% isolates were weak producer for biofilm,while 16.43% isolates were not biofilm producing(Fig.1).

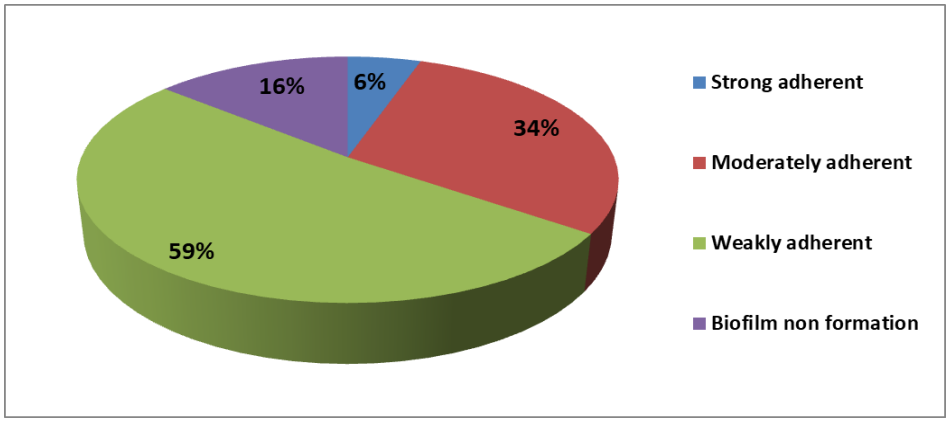

Figure 1. The ability of *E. coli* to produce biofilm

The results of the current study were close to the results Ahmed and Poursina, The percentage of *E. coli* isolates from the biofilm 83.3%,80% respectively[16,17].

Antibiotic Susceptibility

The results of the antibiotic sensitivity test showed that the studied isolates showed a clear difference in their resistance to all types of antibiotics (Fig.2).

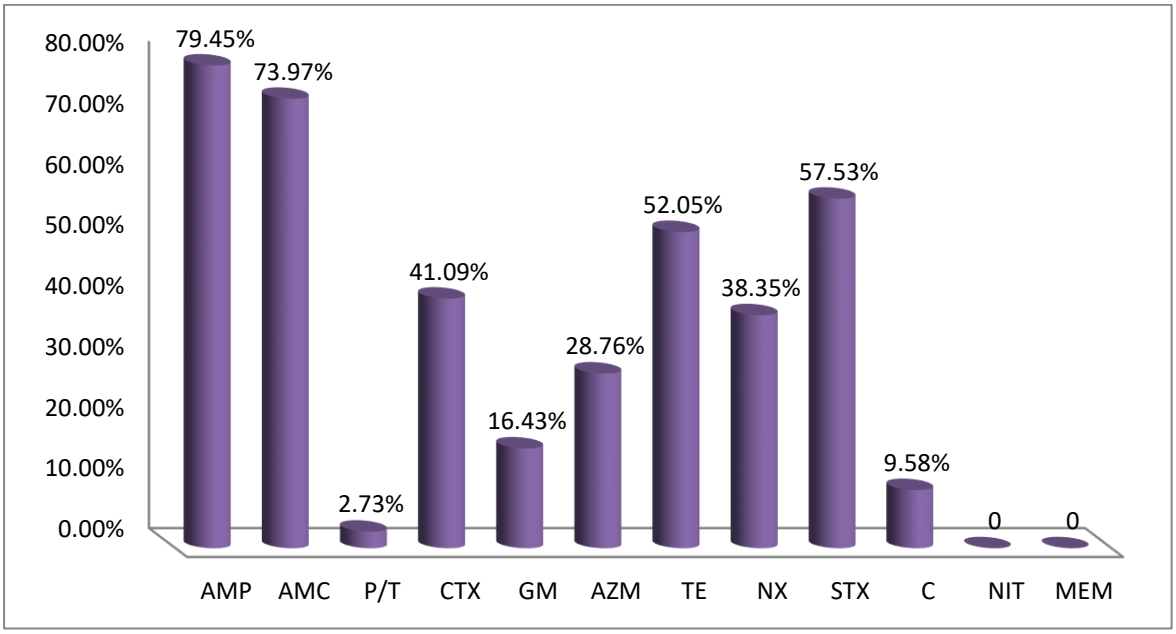

Figure2. Percentage of *E.coli* resistance to different antibiotics using the Kirby - Bauer method

73 of *E.coli* were subjected to 12 antibiotics . The results were interpreted according to the recommendation of CLSI (2021). The isolates were the highest rate of resistance against to Ampicillin 79.45% ,the reason for the resistance is that ampicillin, the first of the broad-spectrum penicillin group of beta-lactam antibiotics, acts as an inhibitor of the bacterial cell wall by inhibiting the enzyme transpeptidase[18], the results of the current study were close to the results Dehkordi , the resistance ratio 79.54%[19].

The isolates showed resistance to the β-lactam combination of both Amoxicillin-clavulanate and Piperacillin-Tazobactam. The percentage of resistance to the antibiotic Amoxicillin-clavulanate 73.97% and antibiotic Piperacillin-Tazobactam resistance rate is very weak 2.73%, the results of the current study were close to the results Mandawat, the resistance rate 75.8%,10% respectively[20] . The reasons for the resistance of *E.coli* to beta-lactam antibiotics is a change in the permeability of the cell membrane of the bacteria and its possession of efflux pumps[21].

The isolates showed resistance towards the third generation cephalosporins represented by Cefotaxime 41.09 % this result is close to the result Karam, the

resistance rate 49.1%[22] , Cephalosporins contain a beta-lactam ring, as these antibiotics inhibit cell wall synthesis.

The isolates showed resistance towards aminoglycosides represented by Gentamicin 16.43% this result is close to the result Taseen ,the resistance rate 12%[23],the reason for the resistance of *E. coli* to a group of aminoglycosides is the possession of bacteria flow systems and a change in the permeability of the outer membrane of the cell[24].

Bacteria resistance to Azithromycin belonging to the group of macrolides28.76%this result is close to the result Aldamarchi, the resistance rate 23%[25], the reason for the resistance of bacteria to antibiotics belonging to the group of macrolides as a result of hydrolysis, which works to degrade the antibiotic and inhibit its effectiveness[26].

The isolates showed resistance to the Tetracyclin 52.05% this result is close to the result Raeispour, the resistance rate 50%[27] , the reason for the resistance of *E. coli* to tetracyclines is that the bacterium possesses a efflux pump that expels the tetracycline antibiotic to the outside, and a change in the permeability of the cell membrane[28].

Bacteria resistance to Norfloxacin belonging to the group of fluoroquinolones 38.35% this result is close to the result Karam ,the resistance rate 40.9%[22], the reason for the resistance of *E.coli* to fluoroquinolones is due to a mutation in the DNA gyrase enzyme or the inhibition of DNA synthesis by stopping the action of the DNA gyrase enzyme[29]. Bacteria resistance to Sulfamethoxazole - Trimethoprim belonging to the group of Folate 57.53% this result is close to the result Tewawong , the resistance rate54.3 %[30] . Bacteria resistance to Chloramphenicol belonging to the group of Phenicol 9.58 % this result is close to the result Dehkordi, the resistance rate 6.81%[19].

E.coli showed the highest sensitivity to Nitrofurantoin, which was 100% this result is close to the result Shahbazi , the sensitivity of bacteria to this antibiotic was 95.8%[31], this antibiotic is one of the best and oldest antibiotics that should be used to treat patients with urinary tract infections due to the weak resistance shown by bacterial isolates of *E. coli* towards this antibiotic. Antibiotic Meropenem belonging to the group of Carbapenems, no isolates showed resistance to this antibiotic, this result is close to the result Alzahrani ,the sensitivity of bacteria to this antibiotic was 100%[32] ,Carbapenem is one of the most effective antibiotics for the treatment of infections caused by Gram-negative bacteria due to its stability against hydrolysis by beta-lactamase enzymes in addition to its high permeability through the outer membrane of bacteria[33].

The study showed that the multidrug resistant (MDR) which is resistant to three classes of antimicrobials increased among these organisms making it difficult to choose appropriate suitable antimicrobial therapy, and the increased resistance of to *E.coli*

numerous antibiotics. The results showed that 76.71% of *E.coli* isolates had multiple antibiotic resistance, and the resistance ranged from three to eight antibiotics.

Antibiogram of antibiotic susceptibility test results for *E.coli*

(Figure3) shown antibiogram for antibiotic resistance of *E.coli* isolates under study, and 12 antibiotics were used which included (AMP, AMC, CTX, T/P , GM, AZM, TE, NX, SXT, MEM , C, NIT) .

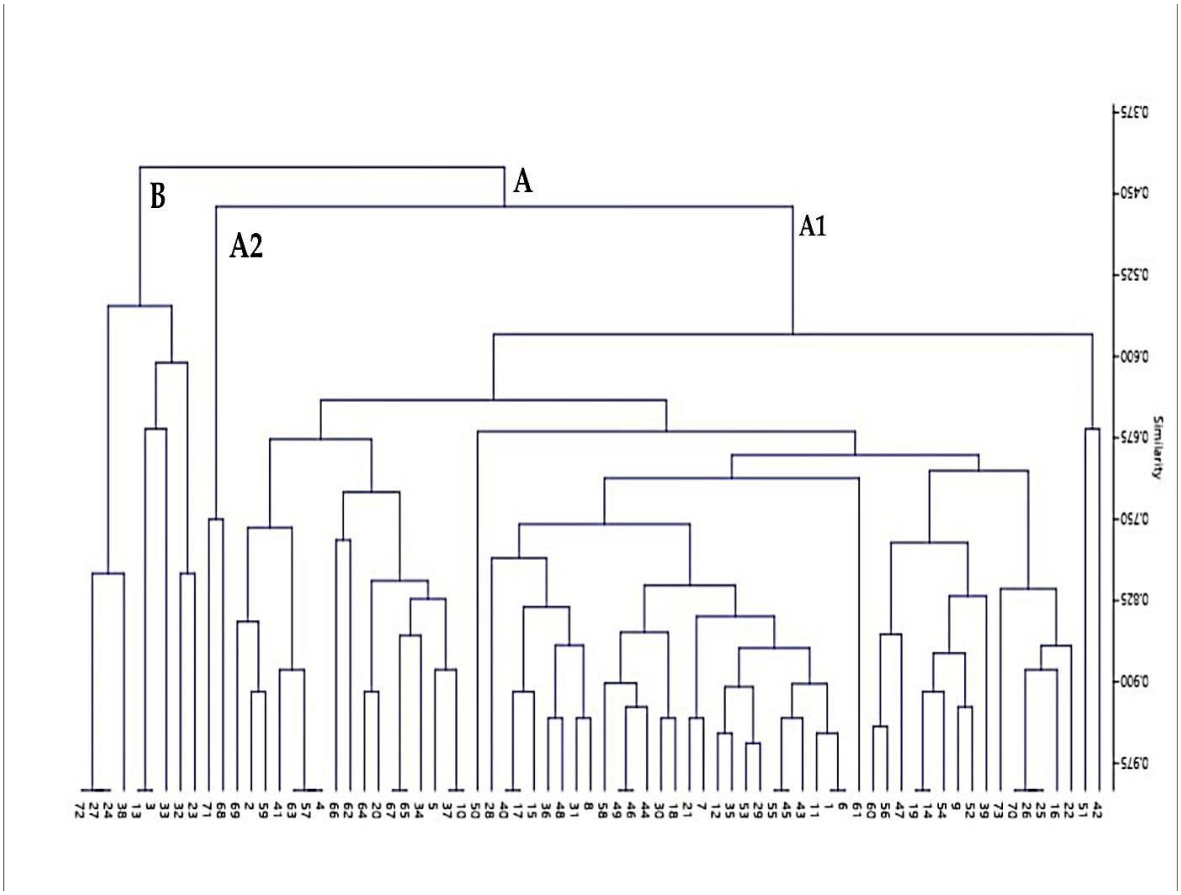

Figure3: Antibiogram of antibiotic susceptibility test results for *E.coli*

There are two main groups A and B, Group A included 64 isolates and was divided into A1, which included 62 isolates, and A2 which included two isolates.

Group A1 consists of two main groups, the first group consisted of two isolates No. 42 and 51 resistant to four antibiotics which included(AMP, CTX, MEM, AZM) and sensitive to other antibiotics.

The second group included 9 clones and 40 isolates, the first clone included 25,26,70 isolates, resistant to eight antibiotics which included(AMP, AMC, CTX, GM, AZM, TE, NX, SXT) and sensitive to other antibiotics.The second clone included two isolates 14,19 resistant to seven antibiotics which included (AMP, AMC, CTX, GM, TE, NX, SXT) and sensitive to other antibiotics.The third clone included isolates 1,6 resistant

to three antibiotics which included(AMP, AMC, CTX) and sensitive to other antibiotics.The fourth clone included two isolates 45, 55 resistant to four antibiotics which included(AMP, AMC, CTX, SXT) and sensitive to other antibiotics.

The fifth clone included two isolates 46 , 49 resistant to six antibiotics which included (AMP, AMC, CTX, TE, NX, SXT) and sensitive to other antibiotics.

The sixth clone included isolates 17 , 40 resistant to seven antibiotics which included (AMP, AMC, CTX, AZM, TE, NX, SXT) and sensitive to other antibiotics.The seventh clone included two isolates 10 , 37 resistant to seven antibiotics which included (AMP, AMC, CTX, GM, TE, C, SXT) and sensitive to other antibiotics.The eighth clone included two isolates 65 , 67 resistant to eight antibiotics which included (AMP, AMC, CTX, GM, AZM, TE, C, SXT) and sensitive to other antibiotics.The ninth clone included isolates 4, 57 ,63 resistant to seven antibiotics which included (AMP, AMC, CTX, TE, NX, SXT, C) and sensitive to other antibiotics. While the 40 isolates showed varying phenotypes of antibiotic resistance.

Group A2 included two isolates 68 and 71 resistant to six antibiotics which included(AMP, CTX, GM, TE , NX,C) and showed different phenotypes for other antibodies.

Group B consists of two clones and 4 isolates, The first clone included two isolates 3 and 13 resistant to eleven antibiotics which included (AMP, AMC, T/P, CTX, MEM, AZM, GM, TE, NX, SXT, C) and sensitive to NIT antibiotic. The second clone included isolates 24, 27, 72 resistant to ten antibiotics which included (AMP, AMC T/P, CTX, GM, AZM, TE, NX, SXT, C) and sensitive to MEM ,NIT antibiotics. While the 4 isolates showed varying phenotypes of antibiotic resistance.

Conclusions

Most of the low biofilm-producing and non-producing isolates showed greater sensitivity to the antibiotics under study compared to the strong and moderate biofilm-producing isolates.

References

1-Zare, F., Mohammadzadeh Rostami, F., and Shahsafi, M. (2018). Prevalence and pattern of antibiotic resistance of gram-negative bacteria isolated from urinary tract infections in patients referring to Neka Laboratories-Iran. International Journal of Biomedicine Public Health, 1(1), 30-36.

2- Hamad Edham, D. (2017). Bacteriological study of urinary tract infection to children in Kirkuk city. *Kirkuk University Journal-Scientific Studies*, *12*(3), 713-736.

3-Pant, D.R., Chaudhary, N. and Upadhyaya, S., 2015. Isolation and identification of Escherichia coli (E. coli) from children suspecting urinary tract infection (UTI). *International Journal Of Health Sciences And Research*, *5*(2), pp.163-8.

4- Shweikh, Rahim Saber and Jassem Farah Ali Hameed. (2016). Types of bacteria causing urinary tract tumors and their extent to antibiotics in some Baghdad hospitals. Al-Qadisiyah Journal of Pure Sciences. 21 (4): 25-32.

5-Malekzadegan, Y., Khashei, R., Sedigh Ebrahim-Saraie, H. and Jahanabadi, Z., 2018. Distribution of virulence genes and their association with antimicrobial resistance among uropathogenic Escherichia coli isolates from Iranian patients. BMC infectious diseases, 18(1), pp.1-9.

6- Soto, S.M., 2014. Importance of biofilms in urinary tract infections: new therapeutic approaches. *Advances in biology*, *2014*.

7- Basak, S., Singh, P. and Rajurkar, M., 2016. Multidrug resistant and extensively drug resistant bacteria: a study. *Journal of pathogens*, *2016*.

8-Levinson, W. (2016). Review of Medical Microbiology and Immunology. 14thed. McGraw-Hill education, Inc. PP 821.

9- MacFaddin, J.F., 2000. Biochemical tests for identification of medical bacteria, williams and wilkins. *Philadelphia, PA*, *113*.

10-Sahra, K. (2019). The Methods for Detection of Biofilm and Screening Antibiofilm Activity of Agents, Antimicrobials, Antibiotic Resistance, Antibiofilm Strategies and Activity Methods, Sahra Kırmusaoğlu, IntechOpen

11-Shrestha, R., Khanal, S., Poudel, P., Khadayat, K., Ghaju, S., Bhandari, A., Lekhak, S., Pant, N.D., Sharma, M. and Marasini, B.P., 2019. Extended spectrum β-lactamase producing uropathogenic Escherichia coli and the correlation of biofilm with antibiotics resistance in Nepal. *Annals of clinical microbiology and antimicrobials*, *18*(1), pp.1-6.

12- Magiorakos, A.P., Srinivasan, A., Carey, R.B., Carmeli, Y., Falagas, M.E., Giske, C.G., Harbarth, S., Hindler, J.F., Kahlmeter, G., OlssonLiljequist, B. and Paterson, D.L.(2012).Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. Clinical microbiology and infection, 18(3):268-281.

13- CLSI (2021) Performance Standards for Antimicrobial Susceptibility Testing. CLSI Publishing

14- Alwan, A.H., Hassan, A.S., Khwen, N.N., Kareem, H.A. and Sahib, R.A., 2016. New Medium For Identification of Low and Moderate Density Biofilm Production of Escherichia coli Isolates From UTI Patients. *Advances in Environmental Biology*, *10*(12), pp.180-188.

15-Katongole, P., Nalubega, F., Florence, N. C., Asiimwe, B., & Andia, I. (2020). Biofilm formation, antimicrobial susceptibility and virulence genes of Uropathogenic Escherichia coli isolated from clinical isolates in Uganda. BMC Infectious Diseases, 20(1), 1-6.

16-Ahmed,A.Q.(2021). Molecular detection of some virulence genes in E. coli isolated from women with urinary tract infections. PhD Thesis. Department of Biology. College of Science. University of Diyala.

17-Poursina, F.; Sepehrpour, S. and Mobasherizadeh, S. (2018). Biofilm Formation in Nonmultidrug-resistant Escherichia coli Isolated from Patients with Urinary Tract Infection in Isfahan, Iran. Adv Biomed Res., 27(7):40.

18- Sharma, S.K., Singh, L. and Singh, S., 2013. Comparative study between penicillin and ampicillin. *Scholars Journal of Applied Medical Sciences*, *1*(4), pp.291-294.

19- Dehkordi, F.S., Tavakoli-Far, B., Jafariaskari, S., Momtaz, H., Esmaeilzadeh, S., Ranjbar, R. and Rabiei, M., 2020. Uropathogenic Escherichia coli in the high vaginal swab samples of fertile and infertile women: virulence factors, O-serogroups, and phenotyping and genotyping characterization of antibiotic resistance. New Microbes and New Infections, 38, p.100824.

20- Mandawat, B. K. Detection and Antibiogram of Extended Spectrum Beta Lactamase (ESBL) producing Escherichia coli isolated from urine samples at Goverment Medical College, Kota, Rajasthan.

21- Al-Shuwaikh, Rana Mujahid Abdullah (2016). Antibiotics and their uses. Degla House. Jordan . Page 112.(in Iraq).

22- Karam, M.R.A., Habibi, M. and Bouzari, S., 2018. Relationships between virulence factors and antimicrobial resistance among Escherichia coli isolated from urinary tract infections and commensal isolates in Tehran, Iran. Osong public health and research perspectives, 9(5), p.217.

23- Taseen, A. (2019). Antibiotic resistance pattern of Escherichia coli isolated from patients with urinary tract infection (Doctoral dissertation, Brac University).

24- Paltansing, S., 2015. *Antimicrobial resistance in Enterobacteriaceae: characterization and detection* (Doctoral dissertation, Leiden University).

25- Aldamarchi, A. A. T., Alnaily, D. G., & Alsalhy, R. M. (2017). Detection of virulence factors of E. coli in patients with acute UTI. Al-Qadisiyah Medical Journal, 13(23), 63-73.

26- Zaman, S. B.; Hussain, M. A.; Nye, R.; Mehta, V.; Mamun, K. T. and Hossain, N. (2017). A Review on Antibiotic Resistance: Alarm Bells are Ringing. Cureus. 9(6): 1-9.

27- Raeispour, M. and Ranjbar, R., 2018. Antibiotic resistance, virulence factors and genotyping of Uropathogenic Escherichia coli strains. Antimicrobial Resistance & Infection Control, 7(1), pp.1-9.

28- Kapoor, J.; Saigal, S. and Elongavan, A. (2017). Action and Resistance Mechanisms of Antibiotics: A Guide for Clinicians. J Anaesthesiol Clin Pharmacol. 33(3):300-305.

29- Kireçci, E. and Kareem, R.D., 2014. Antibiotic susceptibility patterns of

Pseudomonas aeruginosa strains isolated from various clinical specimens. Sky J Microbiol Research, 2, pp.13-17.

30- Tewawong, N., Kowaboot, S., Pimainog, Y., Watanagul, N., Thongmee, T., & Poovorawan, Y. (2020). Distribution of phylogenetic groups, adhesin genes, biofilm formation, and antimicrobial resistance of uropathogenic Escherichia coli isolated from hospitalized patients in Thailand. PeerJ, 8, e10453.

31- Shahbazi, S., Karam, M. R. A., Habibi, M., Talebi, A., & Bouzari, S. (2018). Distribution of extended-spectrum β-lactam, quinolone and carbapenem resistance genes, and genetic diversity among uropathogenic Escherichia coli isolates in Tehran, Iran. Journal of global antimicrobial resistance, 14, 118-125.

32- Alzahrani, M. A., Ali, M. S., & Anwar, S. (2020). Bacteria causing urinary tract infections and its antibiotic susceptibility pattern at tertiary hospital in Al-Baha region, Saudi Arabia: a retrospective study. Journal of Pharmacy & Bioallied Sciences, 12(4), 449.

33- Hawkey, P. & Munday, C. (2004). Multiple resistance in Gram-negative bacteria. Lippincott Williams and Wilkins. Rev. Med. Microbiol. 15(2).