Journal University of Kerbala , Vol. 17 No.1 Scientific . 2019

Study to determination minimum inhibitory concentration (MIC) values of different antibiotics against the common isolates of *Enterococcus* that isolated from pregnant women urine by agar dilution method.

دراسة لتحديد قيم التركيز المثبط الأدنى لبعض المضادات الحيوية ضد العزلات البكتيرية الشائعة لبكتريا *Enterococcus* المعزولة من إدرار النساء الحوامل بإستخدام طريقة التخفيف بالأكار.

Atyaf Ali Sahib and Meead Hamza Xigheer
University College AL- Safwa - Department pharmacy

Abstract

Bacterial invation of any part of the urinary tract is known as Urinary Tract Infection (UTI). 70 urin specimen was taken from pregnant women who had been referred to laboratories of AL- Hussein teaching hospital and Maternity teaching hospital, Karbala, between (1 December - 30 February). Isolates were identified phenotypically and species assigned according to results of biochemical tests. The results showed *Enterococcus faecium* was predominant, its comprising (10 %) compare with other enterococcal species, and among 5 enterococcal isolates, 2 (comprising 40%) and 1 (comprising 20%) were β and α haemolytic respectively and 2 (comprising 40%) were no haemolytic activity, gelatinase was produced by 4 isolates (comprising 80%) and 1 isolate (comprising 20%) no gelatin liquefaction. In addition to that the results showed that the number of moderate biofilm and non-biofilm producers were 2 (40%) , and weak biofilm was 1 (20%) among 5 enterococcal isolates.

The MIC values of the different antibiotics (Vancomycin, Teicoplanin, Ampicillin, Erythromycin, and Ciprofloxacin) were determined against the four *E . faecium* isolates. The MIC values of the vancomycin against *E . faecium* 1, *E . faecium* 2, *E . faecium* 3, and *E . faecium* 4 was found to be (8 , 8 , 30.6 , and 15.3 µg/ml) respectively. The MIC values of (teicoplanin, ampicillin, erythromycin, and ciprofloxacin) against the four *E . faecium* isolates was found to be (32 , 16 , 32 , and 16 µg/ml) for teicoplanin , (8 , 8 , 8 , and 15.3 µg/ml) for ampicillin , (4 , 8 , 8 , and 8 µg/ml) for erythromycin , and (2 , 1 , 1 , and 2 µg/ml) for ciprofloxacin.

On the other hand, the results observed that the activity of the ciprofloxacin against the four *Enterococcus faecium* isolates was more effective than existing drugs. Its showed very good activity against *Enterococcus faecium* 2 and *Enterococcus faecium* 3. Teicoplanin has very low activity against the four enterococcal isolates compared with the existing drugs in our study.

Key Words: UTI , *Enterococci* , Virulence factors , MIC.

المستخلص

يعرف إلتهاب المجاري البولية على أنه الأجتياح البكتيري لأي جزء من اجزاء القناة البولية. إذ تم جمع (70) عينة إدرار من النساء الحوامل التي ترتاد مختبرات مستشفى الحسين (ع) التعليمي ومستشفى الولادة التعليمي في محافظة كربلاء المقدسة للفترة مابين (1 كانون الأول - 30 شباط)، شخصت العينات مظهرياً وأكدت الأنواع طبقا إلى الأختبارات الكيموحيوية.

أظهرت نتائج الدراسة بأن بكتيريا *Enterococcus faecium* هي الأكثر شيوعا إذ شكلت نسبة (10 %) مقارنة مع الأنواع الأخرى.

أوضحت الدراسة الحالية مابين (5) عزلات من بكتريا *Enterococcus* كان انتاجها لعامل الضراوة الهيمولايسين بنسب 2 (40 %) و 1 (20 %) منتجة للنوع بيتا وألفا هيمولايسين على التوالي وعزلتين فقط لم تظهر أي فعالية تحليلية في حين بلغت نسبة العزلات المنتجة لأنزيم محلل الجيلاتين 4 (80 %) وعزلة واحدة (20 %) فقط غير منتجة للأنزيم. إضافة إلى ذلك أظهرت النتائج بأن عدد المنتجات المتوسطة أو غير منتجة للغشاء الحيوي ثلاث عزلات كانت منتجة للغشاء الحيوي وعزلتين غير منتجة للغشاء الحيوي من بين (5) عزلات من بكتريا *Enterococcus*.

تم تحديد قيم التركيز المثبط الأدنى لمضادات حيوية مختلفة شملت الفانكومايسين ، التيكوبلانين ، الأمبيسيلين ، الأريثرومايسين ، و السبروفلوكساسين ضد العزلات الأربعة من بكتيريا *E. faecium*. إذ بلغت قيم التركيز المثبط الأدنى للمضاد الحيوي الفانكومايسين ضد العزلات الأربعة 1*E . faecium* ، 2 *E . faecium* ، 3 *E . faecium* ، و 4 *E . faecium* هي كما يلي (8 ، 8 ، 30,6 و 15,3 مايكروكرام / ملليتر) على التوالي ، في حين بلغت قيم التركيز المثبط الأدنى للمضادات الحيوية (التيكوبلانين ، الأمبيسيلين ، الأريثرومايسين ، والسبروفلوكساسين) هي كما يلي ضد العزلات الأربعة من بكتيريا *E. faecium* على التوالي (32 ، 16 ، 32 و 16 مايكروكرام / ملليتر) للتيكوبلانين و (8 ، 8 ، 8 و 15,3 مايكروكرام / ملليتر) للأمبيسيلين و (4 ، 8 ، 8 و 8 مايكروكرام / ملليتر) للأريثرومايسين و (2 ، 1 ، 1 و 2 مايكروكرام / ملليتر) للسبروفلوكساسين.

من جانب اخر، أظهرت النتائج إن فعالية المضاد الحيوي السبروفلوكساسين ضد عزلات *Enterococcus* الأربعة كانت اعلى فعالية من بين المضادات المستخدمة، إذ سجل أعلى فعالية ضد العزلتين 2 *E . faecium* و 3 *E . faecium* في حين أقل فعالية كانت للمضاد الحيوي التيكوبلانين ضد العزلات الأربعة المستخدمة في الدراسة

الكلمات المفتاحية : إلتهاب المجاري البولية ، المكورات المعوية، عوامل الضراوة ، التركيز المثبط الأدنى.

Introduction

Urinary Tract Infection (UTI) is considered a common disease between male and female but the happening is more among female due to their anatomical structure. Bacteria are the prime cause of the infection among humans but the role of fungi and viruses cannot be over looked. [1 , 2]. The prevalence of bacteria is higher among pregnant women, because the pregnancy enhances possibility of infection among women [3]. Numerous studies are trying to discover the relationship between pregnancy and UTI, sexual coupulation, and family date has a major role in UTI. The anatomical position of the woman urethra to the vagina create it susceptible to lesion during sexual connection; the wet medium of the females perineum predisposes her to bladder bacterial contamination . *E. coli* is considered the major cause of infection which comprising up to 80 to 85% , in addition to other agents like *Pseudomonas*, *Klebsiella*, *Staphylococcus*, and *Enterococcus* [4]. Although *Enterococcus spp.* less common, has been recognized as an important uropathogen. At first it is known as *streptococci*, but with the introduction of serological typing system in 1930 and molecular methods, they were given the genus name *Enterococci* [5]. *Enterococcus faecium* and *Enterococcus faecalis* are believed the main types of *Enterococcus* genus and mostly responsible for UTI. [6 , 7]. Depending on the results of the studies accomplished in Turkey, *E. faecalis* accounts for 39-85.2%, and *E. faecium* accounts for 9-61% of enterococcal infections [8]. These values can difference according to regions and hospitals. *Enterococci* are known to cause various clinical infections like endocarditis, and pelvic infections, due to the virulence factors that described in enterococci including cytolysins (haemolysin) , gelatinase, biofilm formation, and extracellular surface protein [9]. *Enterococci* have both an intrinsic and acquired resistance to antibiotics, making them important nosocomial pathogens. *Enterococci* are able to acquire drug resistance either by gene transformation or transport of mobile elements such as plasmids or transposons containing genetic sequences that confer resistance in other bacteria [10]. The aim of this study was determinated minimum inhibitory concentration (MIC) values of different antibacterial agents against the most common isolates of *Enterococci* by agar dilution method.

Materials and Methods

70 urine specimen was taken from pregnant women into a sterile container from Materinary hospital, Karbala, between (1 December - 30 February). The specimens were transported to the laboratory and processed within two hours of collection.

The bacterial isolates was identified depending on Bergey's Manual [11] and by methods used by [12] and [13]. Bacterial specimens cultured on enrichment media and selective media as blood agar and macConkey agar, respectively for G (-ve) bacteria isolation. Then microscopic examination was accomplished by using gram stain as well as the morphological and biochemical tests such as catalase enzyme production, cytochrome oxidase production, coagulase enzyme production of *S. aureus*, haemolysin production, growth in triple sugar iron agar, methyl red test, voges proskauer test, the movement test, urea analysis enzyme, citrate utilization test, indol test, growth in eosin methyelen blue agar, and growth in mannitol salt agar media. Salt tolerance for *E. faecalis* by growing the isolates in media containing 6.5% NaCl , and heating at 60 C^0 for 30 min of both *E. faecalis* and *E. faecium*, and growth in bile esculin agar media.

Hemolysis was determined by streaking enterococcal isolates on 5% human blood agar plates, incubated at 37°C for 24 hours. Then the plates were examined for haemolysis, a clear zone of hemolysis around the streak on human blood agar was considered to be a positive indication of hemolysin production. While gelatin liquefaction can be tested by stabbing the enterococci onto nutrient gelatin deep tubes, following incubation at 37 °C, the cultures are placed in refrigerator at 4 °C until the bottom are solidify. If gelatin has been hydrolyzed, the medium will remain liquid after refrigeration. If gelatin has not been hydrolyzed, the medium will resoldify during the time it is in refrigerator [14].

While in the case of biofilm formation, the isolates was determined by tube method, a loopful of the organisms was inoculated in 5 ml of nutrient broth with 1% glucose. The tubes were incubated at 37 °C for 24 h. After incubation, the tubes were poured and washed with normal saline and dried, then tubes were stained with crystal violet (0.1%). Increase stain was washed with deionized water. All tubes were dried in inverted position. Biofilm formation was considered positive when a visible film filled the wall and the bottom of the tube. The amount of biofilm formed was recorded as:- (weak / none , moderate and high / strong).

The procedure was performed in triplicate [15].

Agar dilution method was used to calculate the MIC for (Vancomycin, Ampicillin, Erythromycin, Ciprofloxacin and Teicoplanin), that bring from pharmacy as powder. Stock solution was prepared by dissolving 0.1 gm of the antibacterial agents in a few amount of distile water, then completed the volum to 100 ml. The concentration of stock solution became 1000 µg / ml. Other concentrations was prepared from the stock solution. (0.24 , 0.48 , 0.96 , 1.92 , 3.84 , 7.68 , 15.3 , 30.6 , 61.2 , 122 µg / ml) for Vancomycin and Ampicillin. (0.25 , 0.5 , 1 , 2 , 4 , 8 , 16 , 32 , 64 , 128 µg / ml) for Erythromycin and Ciprofloxacin. (0.5 , 1 , 2 , 4 , 8 , 16 , 32 , 64 , 128 , 256 µg / ml) for Teicoplanin. Appropriate volume of antibacterial agent was mixed with Mueller-Hinton agar is the recommended medium for testing most bacteria. Then poured into sterile plastic petri plates, and the agar is allowed to solidify.

The final inoculum for agar dilution was $1.5 * 10^8$ cfu / ml. Five colonies were taken from overnight growth on agar medium and inoculated into 5 ml of nutrient broth, then the broth was incubated at 37°C, and then the suspension was diluted until it matches the turbidity of a 0.5 McFarland standard (0.05 ml barium – chloride 1 % and 9.95 ml sulfuric acid 1 %) , after that 100 µl of the suspension was transported to the agar surface and spread it by glass rod L- shape [16].

The lowest concentration that inhibits obvious growth, was preserved as the MIC. These quantitative results should be reported and interepted as (susceptible, intermediate, or resistant) using the criteria published by CLSI [17].

Results and Discussion

70 urine specimen was taken from pregnant women who had been referred to laboratories of AL- Hussein teaching hospital and Maternity teaching hospital, Karbala. The prevalence of bacteria in 70 urine specimen were shown in the bacterial isolates were found in 40 specimen (57 %), and 30 specimen (43 %) were negative in bacterial growth.

Table (3-1) showed to the positive for gram-stain was (13) isolate comprising (33 %) of the total isolates that gave growing in culture media, while the number of bacterial isolates were negative for

gram-stain was (27) isolate comprising (68 %) of the total number of isolates which isolated from urine.

Table (1) The Numbers and Percentages of Bacterial Isolated From UTI.

Bacterial isolates	No.	The percentage
G (+ ve) bacteria	13	33 %
G (- ve) bacteria	27	68 %
The total isolates that gave growing	40	57 %
The total isolates that negative in growing	30	43 %

Table (2) Shows Isolation Ratio of Bacterial Species From UTI

Bacterial sp.	No.	The percentage
E .coli	15	38 %
Klebsiella pneumonia	7	18 %
Pseudomonas aeruginosa	5	13 %
Staphylococcus saprophyticus	7	18 %
Enterococcus faecium	4	10 %
Enterococcus faecalis	1	3 %
Staphylococcus aureus	1	3 %

On the other hands results showed in table (3-2), bacteria *E.coli* was the commonest isolate (15 isolates ; comprising 38 %) followed by *Klebsiella pneumonia* (7 isolates ; comprising 18 %) , *P. aeruginosa* (5 isolates ; comprising 13 %) , *Staphylococcus saprophyticus* (7 isolates ; comprising 18 %) , *Enterococcus faecium* (4 isolates ; comprising 10 %) , and *Enterococcus faecalis , Staphylococcus aureus* (1 isolates ; comprising 3 %) . Research has indicated that *E. coli* is prevalent among bacterial isolates which isolated from UTI and many studies have certain the importance of the pathogen in the UTI , followed by *Staphylococcus sp* and other pathogens like *Enterococcus sp.* [18 , 19]. The study showed about 13% enterococcal isolates were obtained from women with UTI. This was supported by a survey done by the Center for Disease Control and Prevention (CDC) on UTI, in which *Enterococcus* comprising 14% [20]. In the present study *E. faecium* was predominant among *Enterococcus species* followed by *E. faecalis*, this agreement with the recent studies which indicated that there is an increase of *E. faecium* isolation, as in the study accomplished by [21] stated that *E. faecium* was the most commonly isolated strain. In this study, 2 (40%) and 1 (20%) of 5 enterococcal isolates were β and α haemolytic respectively, and 2 (40%) isolates were no hemolysin production, table (3-3). A study by [22] showed 2 of 44 *E. faecalis* and 1 of 4 *E. faecium* produced haemolysin. In addition to a study by [23] showed 33 (16.5%) clinical isolates produced haemolysin. In contrast, haemolysin was produced by 82 % of the enterococcal isolates according to a study by [24]. The factor gelatinase was produced by 4 isolates (comprising 80%) and 1 isolate (comprising 20%) no gelatin liquefaction. By tube method (TM)), The results showed that the number of moderate and non-biofilm producers were 2 (40%) , and weak biofilm

was 1 (20%) among 5 enterococcal isolates. Many studies have reported a decrease in biofilm formation of *Enterococcus* genus [25].

Table (3) The Results of Diagnostic Tests For *Enterococcus sp.* Isolates

Diagnostic tests	*E. faecalis*	*E. faecium*
Cells morphology	Cocci	Cocci
Gram stain	+	+
Catalase test	-	-
Oxidase test	-	-
Voges Proskauer Test	+	+
Esculin hydrolysis	+	+
Ability to growth at 6.5% NaCl	+	-
heating at 60 C^0 for 30 min	+	+
Haemolysis test	No haemolysis	2 β haemolysis 1 α haemolysis 1 No haemolysis
Gelatin liquify	+	3 (+) & 1 (-)
Biofilm production	Weak	2 moderate 2 non biofilm
Motility test	-	-

Table (4) Refer to Diagnostic Tests For Bacteria That Isolation From UTI.

Diagnostic tests	*E .coli*	*Klebsiella pneumonia*	*P. aeruginosa*
Cells morphology	short bacilli	short bacilli	rods
Gram stain	-	-	-
Catalase test	+	+	+
Oxidase test	-	-	+
IMViC	+ + - -	- - + +	- - - +
TSI	A / A	A / A	K / A
H_2S production	-	-	-
Gas production	+	+	+
Motility test	+	-	+

Urea hydrolysis	-	+	-
Haemolysin Test	1 (7%) β haemolysis 3 (20%) α haemolysis 11 (73%) No haemolysis	(100%) No haemolysis	2 (40%) α haemolysis 3 (60%) No haemolysis
Lactose fermenter	+	+	-
ability to growth at 42 C^0	-	-	+

(+) : positive result (-) : negative result (A / A) : Alkaline (K / A) : Acidic

The MIC values of the different antibiotics (Vancomycin, Teicoplanin, Ampicillin, Erythromycin, and Ciprofloxacin) were determined against *E . faecium* by the agar dilution method and the results are depicted in Table (3-5).

Table (5) MIC Values of Different Antimicrobial Agents Against *Enterococcus faecium*

Isolate Number	Minimum Inhibitory Concentration MIC µg /ml				
	Vancomycin	Teicoplanin	Ampicillin	Erythromycin	Ciprofloxacin
***E . faecium* 1**	8	32	8	4	2
***E . faecium* 2**	8	16	8	8	1
***E . faecium* 3**	30.6	32	8	8	1
***E . faecium* 4**	15.3	16	15.3	8	2

The MIC value of the vancomycin against the four *E . faecium* isolates was found to be (8 , 8 , 30.6 , and 15.3 µg/ml) respectively. The MIC of teicoplanin against the four *E . faecium* isolates was found to be (32 , 16 , 32 , and 16 µg/ml) respectively. According to reference cited in [26] , the MIC of vancomycin against some enterococcal strains was found to be 30 µg/ml, whereas for teicoplanin was at 32 µg/ml. The MIC value of the ampicillin was at (8 , 8 , 8 , and 15.3 µg/ml) , and of the erythromycin was at (4 , 8 , 8 , and 8 µg/ml) , where as for ciprofloxacin was at (2 , 1 , 1 , and 2 µg/ml) for *E . faecium* 1, *E . faecium* 2, *E . faecium* 3, and *E . faecium* 4 respectively.

From the above results we can conclude that the activity of the ciprofloxacin against the four *E . faecium* isolates was more effective than existing drugs. It showed very good activity against *E . faecium* 2 and *E . faecium* 3. Erythromycin showed less activity than ciprofloxacin. It showed good activity against *E . faecium* 1 only. Ampicillin and Vancomycin nearly has the same activity against the four enterococcal isolates, except *E . faecium* 3 isolate that showed the activity of vancomycin against it is low compared with the other isolate. Teicoplanin has very low activity against the four enterococcal isolates compared with the existing drugs in our study.

Depending on the criteria published by CLSI [17]. Among four *E. faecium* isolates, only one (25%) isolate was resistant to vancomycin (had MIC value ≥ 32) , and 3 (75%) isolates were intermediate to vancomycin with a MIC of 8 µg/ml. Two (50%) isolates were resistant to teicoplanin (had MIC values ≥ 32), and two (50%) isolates were intermediate to teicoplanin with a MIC of 16 µg/ml. The studies accomplished in the other countries report varying resistance rates, these variations may be according to the regions. According to reference cited in [21] reported 4% ,

and 3% of enterococcal isolates were resistance for vancomycin, and teicoplanin respectively.
In an Indian study [27] reported 8% resistance rates for vancomycin; they did not report resistance to teicoplanin. Vancomycin resistance *Enterococci* is mainly caused by the change of peptidoglycan precursors on the cell wall of enterococci, which leads to the failure of vancomycin to prevent the cell wall synthesis of *Enterococci* [28]. In addition to the common use of the glycopeptides in the present years compared with the few past years.
Three (75%) isolates were sensitive to ampicillin (had MIC values ≤ 8) , and one (25%) isolate was resistant to ampicillin with a MIC of ≥ 16 µg/ml. This result disagreement with the study accomplished by [29] reported 81.5% resistance rates for ampicillin in *E. faecium*. While the research [30] reported 57.7% resistance rates for ampicillin in *Enterococci*. The reference [31] reported that the resistance to β-lactam antibiotics by enterococci is caused by the production of β-lactamase, or transformation in the penicillin-binding proteins (PBPs).

Three (75%) isolates were resistant to erythromycin (had MIC values ≥ 8) , and one (25%) isolate was intermediate to erythromycin with a MIC of 4 µg/ml. Erythromycin resistance among our isolates was high, probably reflecting the increased use of it in our hospital over the past few years. Two (50%) isolates were sensitive to ciprofloxacin (had MIC values ≤ 1) , and two (50%) isolate was intermediate to ciprofloxacin with a MIC of 2 µg/ml. This result disagreement with the study accomplished by [32] who observed about 50% of *Enterococcus* isolates was resistance to ciprofloxacin. From these results the study observed the erythromycin resistance rate among the *Enterococcus* isolates was high 75% compared with the other antibacterial agents in our study followed by teicoplanin 50% and vancomycin 25%. In addition to that enterococcal isolates was more susceptible to ampicillin comprising 75% followed by ciprofloxacin comprising 50%.

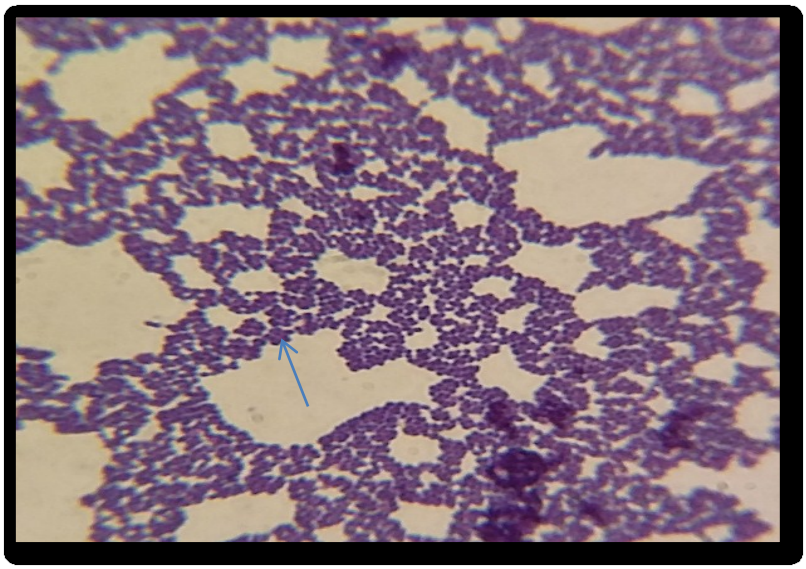

Figure (1). The arrow showed the microscopically picture of *Enterococcus faecium*

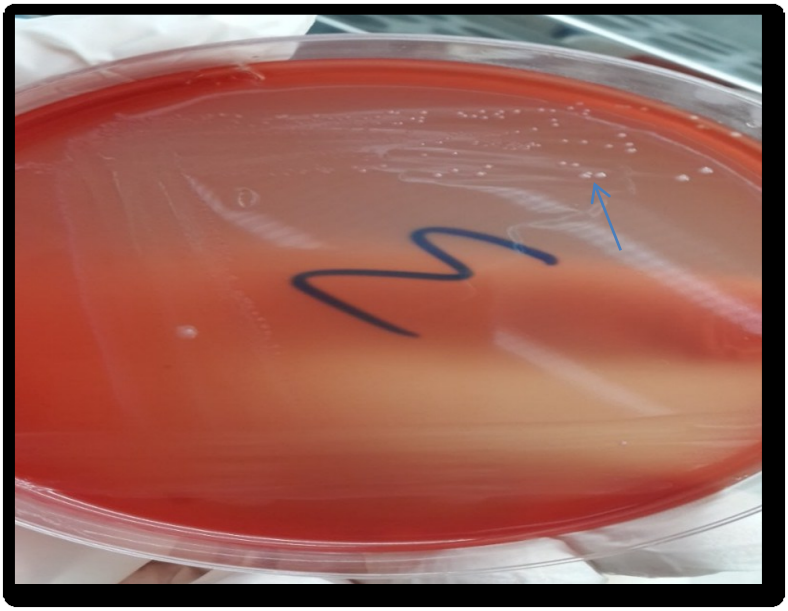

Figure (2). The arrow showed the morphology of *Enterococcus faecium* colony on blood agar

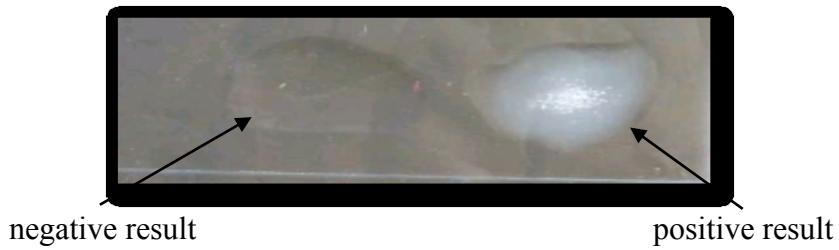

Figure (3). The arrow showed catalase test of *Enterococcus faecium*

Appendix (1)

Figure (4). The arrow showed voges proskauer test of *Enterococcus* on MR-VP media

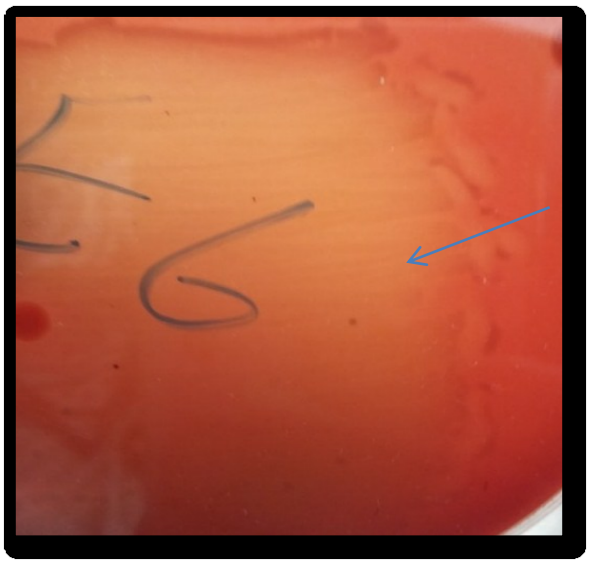

Figure (5). The arrow showed α hemolysis produced by *Enterococcus faecium* on blood agar

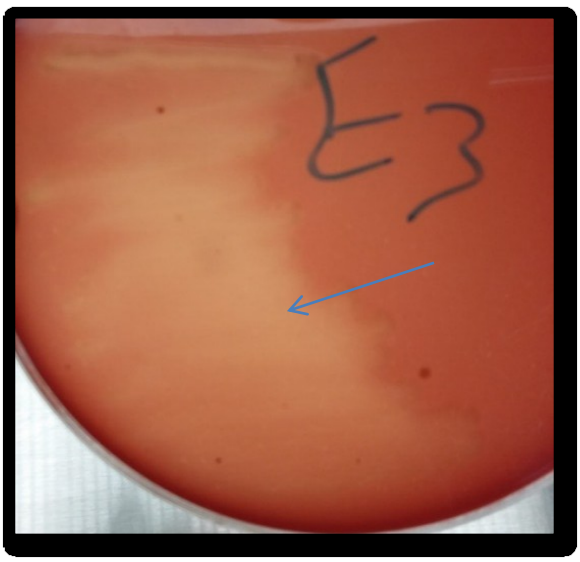

Figure (6). The arrow showed β hemolysis produced by *Enterococcus faecium* on blood agar

Appendix (2)

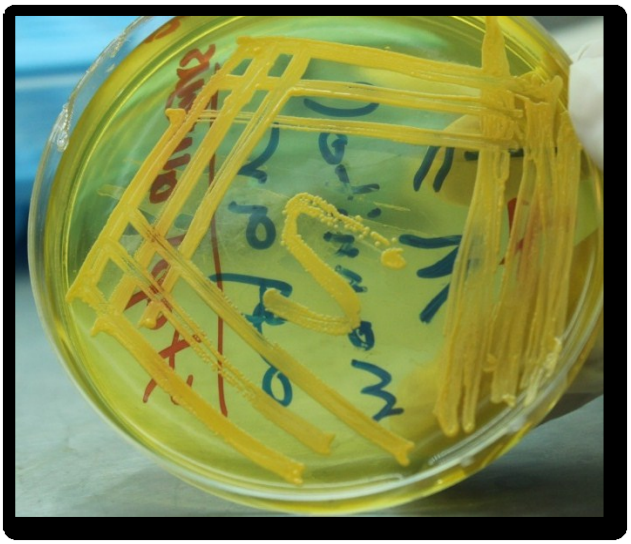

Figure (7). The arrow showed morphology of *Staphylococcus aureus* on mannitol salt agar

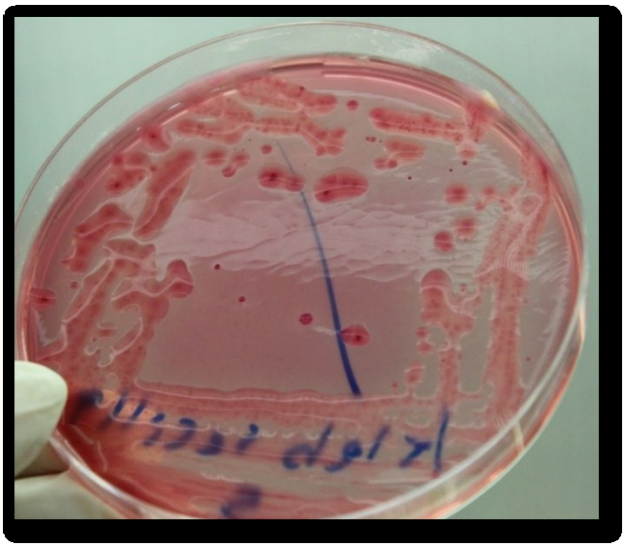

Figure (8). The arrow showed morphology of *Klebsiella pneumonia* colony on MacConkey agar

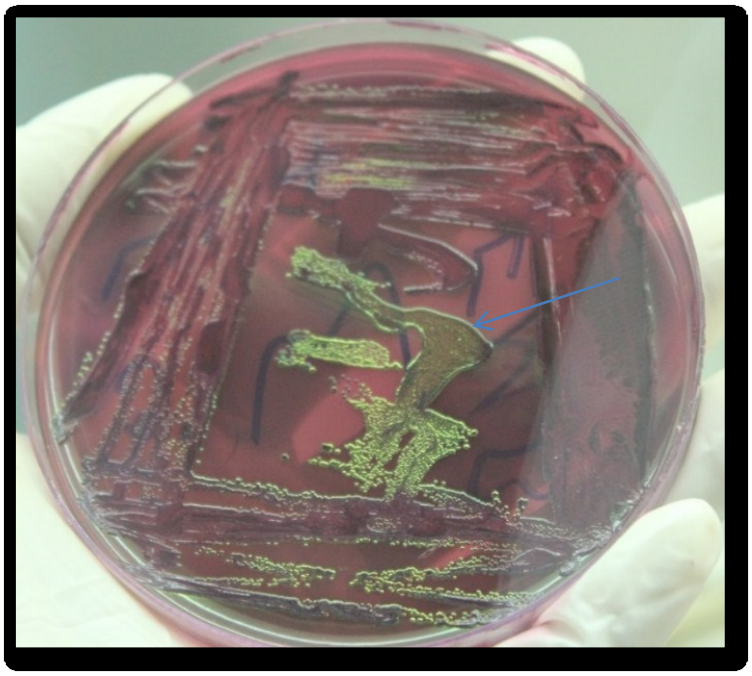

Figure (9). The arrow showed morphology of *E. coli* colony on EMB

Appendix (3)

The references

[1] Demilie, T. ., Beyene, G. ., Melaku, S. and Tsegaye, W. (2012). Urinary bacterial profile and antibtic susceptibility pattern among pregnant women in north west ethiopia. Ethiop J Health Sci ; 22 (2): 121-128.

[2] Parveen, K. ., Momen, A. ., Begum, A.A. and Begum, M. (2011). Prevalence of urinary tract infection during pregnancy. J Dhaka National Med Coll Hos; 17 (2): 8-12.

[3] Dafnis, E. and Sabatini, S. (1992). The effect of pregnancy on renal function physiology and pathophysiology. Am J Med Sci; 303 (3): 184-205:

[4] Ebie, M.Y. ., Kandakai-Olukemi, Y.T. ., Ayanbabejo, J. and Tanyigna, K.B. (2001) .Urinary tract infections in a Nigerian Military Hospital. Nigerian Journal of Microbiology; 15: 31-37.

[5] CetInkaya, Y. ., Falk, P. and Mayhall, C. G. (2000). Vancomycin - resistant enterococci. Clin Microbiol Rev; 13: 686-707.

Successful . 2008) (**[6]** Shea, K. ., Hilburger, E. ., Baroco, A. and Oldfield, E. treatment of vancomycin - resistant *Enterococcus faecium* pyelonephritis with daptomycin during pregnancy. Ann Pharmacother ; 42: 722-725.

[7] Wang, J.L. and Hsueh, P.R. (2009) Therapeutic options for infections due to vancomycin-resistant enterococci. Expert Opin Pharmacother ; 10: 785-796.

[8] Aktepe, O.C. ., Aşık, G. ., Çiftçi, İ.H. and Çetinkaya, Z. (2011). Klinik örneklerden izole edilen enterokok suşlarının antibiyotik direnç oranları. Türk Mikrobiyol Cem Derg; 41: 86-90.

[9] Barbosa, J. ., Gibbs, P. A. and Teixeira, P. (2010). Virulence factors among enterococci isolated from traditional fermented meat products produced in the North of Portugal. Food Control ; 21: 651- 656.

[10] Clark, N.M. ., Hershberger, E. ., Zervosc, M.J. and Lynch, J3. (2003). Antimicrobial resistance among gram-positive organisms in the intensive care unit. Curr Opin Crit Care ; 9 (5): 403–12.

[11] Holt, J. G. ., Kreig, N.R. ., Sneath, P.H.A. ., Staley, T.T. and Willi Williams, S.T. (1994). Bergey's Manual of Determinative Bacteriology. 9 th ed. USA.

[12] Macfaddin, J.F. (2000). Biochemical tests for identification of medical bacteria. 3rd ed . USA.

[13] Collee, J.G. ., Fraser, A.G. ., Marmion, B.P. and Simmons, A. (1996). Mackie and McCartney Practical Medical Microbiology.14th ed. New York ; 413-423.

[14] Vergis, E.N. ., Shankar, N. ., Chow, J.W. ., Hayden, M.K. ., Snydman, D.R. and Zervos, M. J. (2002). Association between the presence of enterococcal virulence factors gelatinase, haemolysin and enterococcal surface protein and mortality among patients with bacteremia due to *Enterococcus faecalis*. Clin Infect Dis; 35: 570-5.

[15] ManpreetKour, I. and Soni, R. S. (2014) . Assessment of biofilm formation by *Enterococcus faecalis* causing nosocomial infections and their statistical analysis. Indian journal of applied research; Volume: 4 Issue: 1.

[16] Clinical and Laboratory Standards Institute. (2012). Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. 9th ed.

[17] Clinical and Laboratory Standards Institute CLSI. (2016). Performance standards for antimicrobial susceptibility testing. 26th ed.

[18] Sibiani, S.A.A. (2010). Asymptomatic bacteriuria in pregnant women in Jeddah. JKAU Med Sci ; 17 (1): 29-42.

[19] Al- Jiffri, O. ., El- Sayed Z.M.F. and Al- Sharif, F.M. (2011). Urinary tract infection with *Esherichia coli* and antibacterial activity of some plants extracts. Int J Microbiol Res ; 2 (1): 1-07.

[20] Desai, P.J. ., Pandit, D. ., Mathur, M. and Gogate, A. (2001). The prevalence ,identification and the distribution of various species of enterococci which were isolated from clinical samples,

with special reference to the urinary tract infections in catheterized patients. India J Med Microbial; 19:132-37.

[21] Aral, M. ., Paköz, N.İ.E. ., Aral, İ. and Doğan, S. (2011). Çeşitli klinik örneklerdeni zole edilen *Enterococcus faecalis* ve *Enteococcusfaecium* suşlarının antibiyotik direnci. Türk Hij Den Biyol Derg ; 68: 85-92.

[22] Vittal, P. ., Prakash, R. and Subash, C. (2005). Emergence of unusual species of enterococci causing infections. BMC Infectious Diseases; 5:14.

[23] Giridhara Upadhyaya, P.M. ., Umapathy, B.L. and Ravikumar, K.L. (2010). Comparative Study for the presence of enterococcal virulence factors gelatinase, hemolysin and biofilm among clinical and commensal isolates of *Enterococcus faecalis*. J Lab Physicians; 2 (2):100–104.

[24] Sanal, C. ., Fernandes, and Dhanashree, B. (2013). Drug resistance & virulence determinants in clinical isolates of *Enterococcus species*. Indian J Med Res; 137 (5): 981–985.
[25] Thomas, V. C. ., Thurlow, L. R. ., Boyle, D. and Hancock, L. E. (2008). Regulation of autolysis dependent extracellular DNA release by *Enterococcus faecalis* extracellular proteases influences biofilm development. Journal of Bacteriology; 190: 5690-5698.

[26] Baldır, G. ., Engin, D. Ö. ., Küçükercan, M. ., İnan, A. ., Akçay, S. ., Özyürek, S. and Aksaray, S. (2013). High-level resistance to aminoglycoside, vancomycin, and linezolid in enterococci strains. Journa of Microbiol and infec disea; 3 (3): 100-103.

[27] Shah, L. ., Mulla, S. ., Patel, K. G. and Rewadiwala, S. (2012). Prevelance of enterococci with higher resistance level in atertiary care hospital. Nat J Med Res; 2: 25-27.

[28] Arthur, M. ., Reynolds, P. and Courvalin, P. (1996). Glycopeptide resistance in enterococci. Trends Microbiol; 4: 401–407.

[29] Aktepe, O.C. ., Aşık, G. ., Çiftçi, İ. H. and Çetinkaya, Z. (2011). Klinik örneklerden izole edilen enterokok suşlarının antibiyotik direnç oranları. Türk Mikrobiyol Cem Derg; 41: 86-90.

[30] Farzaneh, F. ., akhi, M. T. and Mahvash, O. (2010). Antibiotic resistance of enterococci isolated from clinical specimens. Med J Tabrez Uni Med Sci; 32: 34-37.

[31] Zapun, A. ., Contreras, C. and Vernet, T. (2008). Penicillin-binding proteins and β-lactam resistance. FEMS Microbiol; 32: 361–385.

[32] Sreeja, S. ., Sreenivasa, P. R. and Prathab, A. G. (2012). The prevalence and characterization of the *Enterococcus species* from various clinical samples in a tertiary care hospital. J Clin Diagn Res; 6 (9):1486–88.