Studying the antibiotics sensitivity test of *Lactobacillus* on two different media

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الخلاصة:

الدراسة تقارن فحص الحساسية لاثني وعشرين نوعا مختلفا من المضادات الحياتية على ثلاث عزلات من بكتريا حامض اللاكتيك المعزولة من نساء سليمة المهبل بأستخدام طريقة الانتشار على الاطباق في وسطي Muller–Hinton agar و De Mann–Rogosa–Sharp (MRS) agar لتحديد الوسط الامثل لعمل فحص الحساسية و تأثير المضادات الحياتية المستخدمة لعلاج الاصابات المختلفة على بكتريا حامض اللاكتيك ,حيث لوحظ مقاومة البكتريا للسيفتازديم والسبروفلاكساسين وكنميسين وحامض النالديكسك وحامض البابيمديك والستربتوميسين والميترونايدوزول وكانت حساسة لبقية المضادات المستخدمة وهذا يدل على وجود تأثير للمضادات المستخدمة في علاج الامراض المختلفة على توازن بكتريا حامض اللاكتيك داخل الجسم.

Abstract:

The purpose of this paper is to study the antimicrobial susceptibility of twenty two different antimicrobial agents on three different isolates of *Lactobacilli* isolated from the vagina of healthy female by using disc diffusion method on Muller-Hinton agar and De Mann-Rogosa-Sharp (MRS) agar to determine the appropriate medium for susceptibility test and the pattern of resistant and sensitivity of *Lactobacilli* to different antimicrobial agents used for treatment of different infections. *Lactobacilli* were found resistant to Ceftazidime, Ciprofloxacin, Kanamycin, Nalidixic acid, Pipemidic acid, Streptomycin and Metronidazole, while they were sensitive to the rest of antibiotics used in this paper and this indicates that some antibiotics had effect on the presence of *Lactobacilli* (normal flora) inside the body.

Introduction:

Bacteria of the genus *Lactobacillus* have been proposed as probiotic microorganisms to restore the ecological equilibrium of the intestinal and urogenital tracts [1].This type of bacterial replacement therapy has been widely used as fermented milks to prevent diarrhea in humans and animals [2].They have also been increasingly considered for their use in women to prevent genital and

urinary tract infections [3].A large variety of methods to determine antibiotic susceptibilities of non- enterococcal lactic acid bacteria (LAB) belonging to the genera *Lactobacillus, Pediococcus, Lactococcus* and *Bifidobacterium* based on either agar disk diffusion [4,8], E-test[9], agar dilution [10] or broth dilution [11,12] has been described. Due to the fact that many of these organisms require special growth conditions in terms of medium acidity and nutrient supplementation, conventional media such as Mueller-Hinton agar and Iso-Sensitest (IST) agar or broth are not uniformly suitable for susceptibility testing of non enterococcal LAB [13]. Having in mind that a method to study antimicrobial susceptibility of genus *Lactobacillus* has not been standardized yet, different techniques were assayed. The results obtained by using the disc diffusion method with culture media different from Müller-Hinton agar proposed by the NCCLS (National Committee for Clinical Laboratory Standards) are described and also knowing the behavior of *Lactobacillus* under the effect of antimicrobial substances are to have an approach of the response of *lactobacilli* administered to patients subjected to some kind of antibiotic therapy and to consider the concomitant use of *Lactobacilli* and an antibiotic to restore the disrupted ecological environment[14].The main aim of this study is to compare between two media Muller-Hinton agar & MRS agar for antibiotic susceptibility test and to determine the behavior of *Lactobacillus* genus toward different antimicrobial agents administered to patient as antibiotic therapy.

Materials and Methods:

Isolation of *Lactobacilli*

Lactobacili were collected from vagina of healthy females,specimens transferred by MRS broth, then cultured on MRS agar in 37°C for 28 hrs under anaerobic conditions. The *Lactobacilli* were initially identified by their ability to grow on the selective MRS agar, gram positive staining, rod shape and catalase-negative phenotype. Biochemical analyses, including sugar fermentation profile and gas production in MRS broth [15].

Antimicrobial agents

Twenty two different antimicrobial agents were used for inhibition tests including: Ampicillin (10mcg), amoxicillin cavulanic acid (30mcg), azithromycin(15mcg), ceftriaxone(30mcg), ceftazidime(30mcg), cefotaxime (30mcg), chloramphenicol (30mcg), clarithromycin (15mcg), ciprofloxacin (5mcg), erythromycin(15mcg), imipenem (10mcg), kanamycin (30mcg), metronidazole (5mcg), nalidixicacid (30mcg),nitrofurantoin(300mcg), oxacillin(1mcg), pipemidicacid (20mcg), piperacillin (100mcg), rifampin (5mcg), streptomycin (10mcg) and trimethoprim-sulfamethoxazole (25mcg). (Commercial discs from Bioanalyse,Turkey) .

Disc diffusion method:

Antimicrobial susceptibility was studied by employing the method described by Bauer *et.al* [16], Two different base agar media were used: Muller-Hinton agar and MRS agar.

The procedure included:

1. Each *Lactobacillus* isolate spreaded on both media by streaking through using sterile loop.
2. The plates were allowed to dry for 5-15 min.
3. Antibiotic discs were placed on the surface of each agar and the plates were incubated for (24 – 48) hrs at 37C° under microaerophilic condition using a candle jar.
4. After the incubation the diameter of inhibition zones were measured and recorded.

Result and Discussion:

In the present study, the conventional methodology described by Bauer *et. al*. [16] was first applied. Müller-Hinton base medium was employed to test the effect of the antimicrobial agents routinely used for the treatment of different infection on *Lactobacillus* isolates. Growth of *lactobacilli* in Müller-Hinton agar was poor and when any type of growth was detected on the agar, it was irregular and the halos were undefined, this agree with Danielson [17] result and it was not possible to measure diameter of the inhibition halos, while in MRS agar medium the growth was appropriate only for some *Lactobacillus* isolates but not to all of them as shown in (Figures-1 and 2).

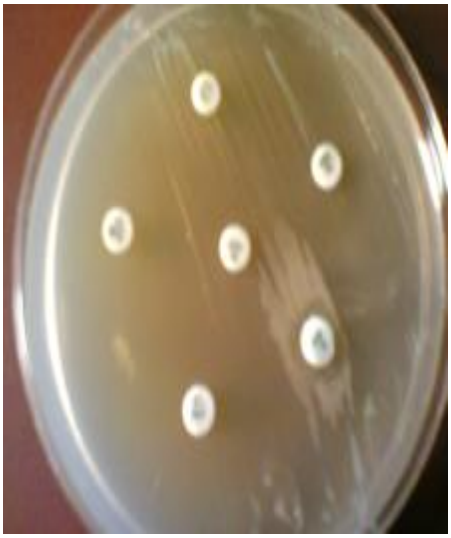

Figure-1:Irregular growth of *Lactoacilli* on Muller-Hinton agar

Figure -2: Regular growth of *Lactobacilli* on MRS agar

Charteris *et.al.* [18] have also used MRS for the disc diffusion and the *E*-test under anaerobic incubation conditions in both cases. Based on size of the

halos, the mentioned authors have classified the microorganisms into susceptible, moderate susceptible, and resistant. However, the reasons by which they consider the published ranges for the susceptibility category are not explained. Considering that the size of the halos depends on the diffusion media. Felten *et. al* [5]. Müller Hinton with 5% of sheep blood, and Klein *et.al*.[19] who have used the same base media with horse blood (3%). More recently, Klare *et. al*. [20] proposed a mixed formulation of Iso-Sensitest broth and MRS with or without supplementation with L-cysteine.On other side antimicrobial susceptibility of exogenously applied microorganisms needs to be known for treating eventual collateral effects [21]. In this regard, the performance of antimicrobial susceptibility testing may be considered as both a necessary selection criterion for probiotic cultures and an effective guide for specific antimicrobial therapy [18]. The available standard techniques and the guidelines for the disc diffusion method have been provided by the NCCLS only for selected aerobic and anaerobic bacteria or yeasts related with laboratory clinical diagnostic. However, many researchers have developed modifications of the semi quantitative disc assay for *lactobacilli* [22]. Different base media and type strains have been employed but reference data are still not available.

The results showed also that *Lactobacilli* were resistant to Ceftazidime, Ciprofloxacin, Kanamycin,Nalidixic acid, Pipemidic acid,Streptomycin and Metronidazole and have variable sensitivity to the rest antibiotics as shown in table-1.

Bacteria / Antibiotics	***Lactobacillus 1*** **Inhibition**	***Lactobacillus 2*** **Zone(mm)**	***Lactobacillus 3***
Ampicillin(10mcg)	20	23	19
Amoxicillin Clavulanic acid(30mcg))	27	32	23
Azithromycin(15mcg)	18	22	23
Aztreonam(30mcg)	-	25	-
Ceftrixone(30mcg)	15	20	17
Ceftazidime(30mcg)	-	-	-
Cefotaxime(30mcg)	-	19	20
Chloramphenicol(30mcg)	26	30	28
Clarithromycin(15mcg)	25	30	32
Ciprofloxacin(5mcg)	-	-	-
Erytromycin(15mcg)	25	25	30
Imipenem(10mcg)	30	20	25
Kanamycin(30mcg)	-	-	-
Metronidazole(5mcg)	-	-	-
Nalidixic acid(30mcg)	-	-	-

Nitrofurantoin(300mcg)	17	28	28
Oxacillin(1mcg),	13	17	14
Pipemidic acid(20mcg)	-	-	-
Piperacillin(100mcg)	30	39	35
Streptomycin(10mcg)	-	-	-
Rifampin(5mcg)	26	30	28
Trimethoprim-sulfamethoxazole(25mcg)	12	13	10

Table -1: Sensitivity of *Lactobacilli* to different antimicrobial agents.

This indicates that many antimicrobial agents effected on ecological equilibrium of *Lactobacilli* inside the body during antibiotics cores taken by patients and probiotics also should be taken along with antibiotics therapy due to the fact that antimicrobial agents effected the present of the normal flora including *Lactobacilli*.Metronidazole for example are the most commonly used antibiotic for the treatment of bacterial vaginosis. *Lactobacillus* isolates were able to grow at the presence of metronidazole. These results suggest that selected strains could be used for a restoration therapy together with the antimicrobial bacterial vaginosis treatment. Simoes *et. al.*[23] have also studied the effect of metronidazole on the growth of vaginal *lactobacilli*. These authors have observed partial and complete inhibition at concentration above 1000 μg/mL while they have reported a stimulating effect at concentrations between 128 μg/mL and 256 μg/mL,while *Lacobacilli* were sensitive to most antibiotics used for treatment of urinary tract infection(UTI) such as Amoxicillin-Clavulanic acid, Chloramphenicol, Nitrofurantoin and Trimethoprim-sulfamethoxazole and were resistant to Ciprofloxacin and Nalidixic acid which helps in the selection of proposed probiotics which should be taken by patients during antimicrobial therapy.

Conclusion:

More consideration must be taken when antibiotic are administrated to patients having in mind the effect of these antibiotics to ecological environment inside the body and further study should be made to define the best method and media for antimicrobial susceptibility test for *Lactobacillus* genus, information acquired to know if the administrated probiotics have no pathogenic or virulence properties or it may contain antibiotic resistance genes.

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