Synergistic Inhibitory Effect of Some Probiotic Fiterates with Ampicillin and Clindamycin against Acne Pathogens
Kawther H. Ibrahem AL-Bajelan - Dept. of Microbiology. College of Science. Al-Mustansiriya University

ABSTRACT
The study included detecting the synergistic inhibitory ability of probiotic (Lactobacillus acidophilus, Bifidobacterium bifidium, Saccharomyces boulardii and Saccharomyces cerevisiae) filterates with Ampicillin and Clindamycin against acne pathogens especially (Propionibacterium acnes, Staphylococcus aureus and Staphylococcus epidermidis).

Susceptibility test of bacterial isolates to some antibiotic showed a considerable resistance to ampicillin and clindamycin (100%) and erythromycin (77.7%). Whereas these isolates were mainly sensitive to cefotaxime, ofloxacin, ciprofloxacin and piperacillin tazobactam.

Synergistic Inhibitory Effect of Some Probiotic Filterates with Ampicillin
Kawther H. Ibrahem AL-Bajelan

Minimum inhibitory concentration (MIC) of ampicillin and
clindamycin were determined by using tube dilution test. To detect synergistic inhibitory effects probiotic isolates were grown in (Glucose Yeast Extract Pepton and Man Rogosa Sharpe )broth media for 48 hrs., then their filterates were concentrated by dry freeze method.Concentrated filterates of probiotic after mixing with antibiotic were applied against bacterial isolates.

Results of mixing the minimum inhibitory concentrations(MIC) of ampicillin,clindamycin,individually with probiotic (Lactobacillus acidophilus, Saccharomyces boulardii and Saccharomyces cerevisiae) filterates, declared that there was synergistic observable inhibitory effect against the bacterial isolates in comparison to use each of the probiotic filterate and antibiotic alone.

INTRODUCTION

The use of antimicrobial therapy is associated with the risk of increased incidence of drug resistance and opportunistic infections, which can be avoided if the use of the antimicrobial agents can be minimized(1).

A great attention was made to use microorganisms or their metabolites in treatment of some disease, so bacteria and yeast considered as the first two types of microorganisms used. In this approach among bacterial group is the lactobacillus spp. which have a great role in probiotic due to its ability to produce inhibited materials such as organic acid, H₂O₂, CO₂, amino acid, diacetyl, acetaldehyde and bacteriocins(2).

Non pathogenic yeast, saccharomyces was the most widely tested in experimental and clinical assays.

FAO and WHO has adopted the definition of probiotics as "live microorganisms which when administered in adequate amounts confer a health benefit on the host" (3). There are a large number of probiotics currently used and available in dairy fermented foods, especially in yogurts. The probiotic microorganisms should not be pathogenic, have no connection with diarrhoeagenic bacteria and no ability to transfer antibiotic resistance genes(4).

Probiotics are marketed as a capsules, powders, enriched yogurts, yogurt-like products and milks. These agents are now being reconsidered as alternatives to antibiotics because of the increase of antibiotic-resistant strains of bacteria and they are relatively cheap and may have lower risk of resistance due to multifaceted mechanisms of action(5).

The aim of this study was to use probiotics lyeast and latic acid
bacteria as a good combination with same antibiotics for acne treatment

EXPERIMENTAL MATERIAL AND METHODS

Bacterial Isolates:
A total of (22) bacterial isolates of gram +ve bacteria were collected from medical city hospital. Out of 22 isolates, nine isolates achieved which distributed as follows: *Propionibacterium acnes* (4 isolates), *Staphylococcus aureus* (3 isolates), and *Staphylococcus epidermidis* (2 isolates).

Clinical isolates were identified at the lab. by classical microbiological tests (Cultural, morphological and biochemical). To confirm our results we used API STAPH system for *Staphylococcus* isolates and API20A system for *Propionibacterium* isolates according to (6).

Probiotic isolates:
A commercial brand of ready powdered of probiotics were used: *Saccharomyces boulardii*, *Saccharomyces cerevisiae* (baker's yeast), *Lactobacillus acidophilus* and *Bifidobacterium bifidium* from college of science, Al- Mustansiriya university.

Antimicrobial agents Susceptibility test:
Antimicrobial agents susceptibility test by disk diffusion was performed by the procedure recommended by the National Committee for Clinical Laboratory standards (NCCLs)(7).

Interpretative chart for disk diffusion tests were these described in the current National Committee for Clinical Laboratory standards documents (8). The following disks were used:

- Clindamycin (2 μ/disc), Erythromycin (15 μ/disc), Ciprofloxacin (5 μ/disc), Ofloxacin (5 μ/disc), Piperacillin-tazobactam (100 μ/disc), Cefotaxime (30 μ/disc) and Ampicillin (10 μ/disc) (a representative disks from Bioanalyse / Turkey).

Determination of the inhibitory effect of probiotics
Man Rogosa Sharpe broth and Glucose Yeast Extract Pepton broth were inoculated by 1% of *Lactobacillus acidophilus, Bifidobacterium bifidum, Saccharomyces boulardii* and *Saccharomyces cerevisiae* (baker yeast) cultures then incubated anaerobically and aerobically at 37°C for 48 hrs (9).

Synergistic Inhibitory Effect of Some Probiotic Fiterates with Ampicillin
Kawther H. Ibrahim AL- Bajelan

After incubation the culture were centrifuged at 4500 r.p.m. to get supernatant which filtered through milipore filter unit (0.22 mm), then the well diffusion method that mention by (10), was used on nutrient agar. The plate was inoculated with 0.1 ml of pathogenic bacteria by
using spreader. Wells were made in nutrient agar by cork Borer (5mm) and filled by the filtrate of probiotic isolates before incubated at 37ºc for 24hrs. The inhibition area around the well was measured by (mm). The filtrate was concentrated by the freeze-dryer and the well diffusion method was repeated to detect the effect of concentrated filtrate against the pathogenic bacteria.

Minimum inhibitory concentration (MIC) determination:

Minimum inhibitory concentrations were determined by tube dilution susceptibility tests using a serial two fold dilutions of Ampicillin and Clindamycin following the method described by (11). The results were compared with control tubes.

Determination synergistic inhibitory effect of probiotic filtrates with some antibiotics Ampicillin and Clindamycin:

Muller Hinton broth was inoculated by 1% of bacteria broth culture, then inoculated aerobically and anaerobically at 37ºc for 24 hrs respectively.

After incubation, the well diffusion method was used on Muller Hinton agar. The plate was inoculated with 0.1 ml of pathogenic bacteria by using sterilized spreader. Then by cork borer (5mm) wells were made in Muller hinton agar and filled by (100μl ) concentrated filtrates of probiotic and (100μl ) of MIC (Ampicillin or Clindamycin) individually before incubated at 37ºc for 24 hrs. The inhibition zone around the well was measured by (mm) and compared with control which contain (100μl ) of each antibiotic only and (100μl ) of probiotic filtrates.

RESULT and DISCUSSION

Out of twenty two of gram +ve bacteria obtained (9) isolates were identified including (4) Propionibacterium acnes, (3) Staphylococcus aureus, (2) Staphylococcus epidermidis.

The antimicrobial agents susceptibility test of the isolates were performed against (7) different agents including :Ampicillin (AMP), Clindamycin (DA), Erythromycin (E), Cefotaxime (CTX), Ofloxacin (OFX), Piperacillin- Tazobactam (TPZ), and Ciprofloxacin (CF).

Our results have been shown (table 1), that all bacterial isolates under this study were susceptible to ofloxacin, cefotaxime, piperacillin, tazobactam and ciprofloxacin, while they were mainly resistance to Clindamycin, Ampicillin and Erythromycin.

Table (1): Antibiotic Susceptibility Test of Bacterial isolates

<table>
<thead>
<tr>
<th>Antibiotics</th>
<th>OFX</th>
<th>CF</th>
<th>TPZ</th>
<th>DA</th>
<th>CTX</th>
<th>AMP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Active</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inactive</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Al- Mustansiriya J. Sci Vol. 18, No 1, 2007

29
Bacteria

\[ \begin{align*}
P..acnes1 & : S S R S R S \quad S..acnes2 : S S R S R S \quad S..acnes3 : S S R S R S \\
P..acnes4 & : S S R S R S \quad S..aureus1 : S S R S R S \quad S..aureus2 : S S R S R S \\
S..aureus3 & : S S S S R S \quad S..epidermidis1 : S S R S R S \quad S..epidermidis2 : S S S S R S \\
\end{align*}\]

OFX=ofloxacin, CF=ciprofloxacin, E=erythromycin, TPZ=piperacillintazobactam, DA=clindamycin, CTX=cefotaxime, AMP=ampicillin, P..acnes=Propionibacterium acnes, S..aureus=Staphylococcus aureus, S..epidermidis=Staphylococcus epidermidis

Ampicillin and clindamycin were selected as a more antibiotics resistance by bacterial isolates.

In order to study synergistic inhibitory effects between these two antibiotics and probiotic filterates after MIC determination for each bacterial isolate.

Our results showed (table 2) that equal quantities (100 μl) of antibiotic and filterate were mixed, in some cases synergistic effect appeared by forming a large inhibition zone around the well against isolated bacteria compared with inhibition zone formed by using probiotic filterate or antibiotic alone. (23-36)(25-30)mm inhibition zone confirmed by using (AMP/L filterate), (DA/L filterate) respectively had a better activity and greater degree of synergy against P..acnes isolates.

In the control and treatment of infectious diseases include the development of antibiotic resistance, increased frequency of opportunistic infections in patients and emergence of new types of pathogens there was a need to develop strategies for treatment and prevention of infectious diseases (12).

Synergistic Inhibitory Effect of Some Probiotic Filterates with Ampicillin
Kawther H. Ibrahem AL- Bajelan

"Natural" therapies are viewed favourably by many patients, chiefly because they believed often correctly that they were associated with fewer detrimental effects than most antibiotics (13). Barefoot and klaenhammer (14) reported that probiotic bacteria produced substances called bacteriocins, which act as natural antibiotics to kill undesirable microorganisms. As well as, (AMP/L filterate) and (DA/L filterate) synergistic activity were observed when diameters of inhibition zone measured (28-34), (27-30) mm respectively against S..aureus isolates. While, (AMP/L filterate) (DA/L filterate) formed (28-34) (20-27) inhibition zone
against *S.epidermidis*.
Gotz et al. (15) reported that several lactobacillus preparations have been evaluated for the prevention of antibiotic associated diarrhea (AAD). A commercial mixture of *L.acidophilus* and *L.bulgaricus* was given to 79 patients receiving ampicillin, no patients developed AAD.
Silva et al. (16) said that lactobacillus has been shown to produce amicrocin in vitro towards a broad spectrum of gram + and gram – pathogens and hydrogen peroxide which was bactericidal.
In the other hand, no synergistic effect was performed (table3) by using (AMP/B filterate) and (DA/B filterate) mixture against testing bacteria. In opposite to (AMP/S.b filterate)(DA/S.b filterate) which had the best synergistic inhibitory effect (table4) against all bacterial isolates by forming inhibition zone (15-35)(18-30) respectively. While (AMP/S.c filterate) was the least affective one (table5) despite ( DA/S.c filterate) had a better inhibitory effect against bacterial isolates under study compared with DA alone.
Yeast was resistant to antibiotic, sulfamides and other antibacterial agents. This resistant was natural and genetical and not susceptible to be modified or transmitted to other microorganisms (17).
Further more, Mcfarland et al. (18) reported that combination treatment of a standard antibiotic (either vancomycin or metronidazole) with *Saccharomyces boulardii* appeared to be safe and improved therapy .
Mansour-Ghanaei et al. (19) reported that adding *Saccharomyces boulardii* to antibiotics in the treatment of acute amebiasis decreased the duration of clinical symptoms and cyst passage.
Clindamycin is an antibiotic applied to the skin to treat acne, while only a small percentage of topical clindamycin was absorbed through skin, side effects such as diarrhea controlled studies have shown that taking probiotic microorganisms such as *lactobacillus acidophilus*, *bifidobacteri*
a or *Saccharomyces boulardii* helped prevent antibiotic-induced diarrhea (20).

Table (2): Inhibitory effect of probiotic filterate (*L.acidophilus*) with Antibiotics

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>Ampicillin</th>
<th>Clindamycin</th>
</tr>
</thead>
<tbody>
<tr>
<td>F</td>
<td>MIC MIC+F</td>
<td>MIC MIC+F</td>
</tr>
<tr>
<td>P.acnes1</td>
<td>28 15 30 10 30</td>
<td></td>
</tr>
<tr>
<td>P.acnes2</td>
<td>26 15 36 11 27</td>
<td></td>
</tr>
<tr>
<td>Bacteria</td>
<td>Ampicillin</td>
<td>Clindamycin</td>
</tr>
<tr>
<td>--------------</td>
<td>------------</td>
<td>-------------</td>
</tr>
<tr>
<td>P.acnes1</td>
<td>31</td>
<td>15</td>
</tr>
<tr>
<td>P.acnes2</td>
<td>28</td>
<td>20</td>
</tr>
<tr>
<td>P.acnes3</td>
<td>21</td>
<td>10</td>
</tr>
<tr>
<td>P.acnes4</td>
<td>21</td>
<td>14</td>
</tr>
<tr>
<td>St.aureus1</td>
<td>27</td>
<td>20</td>
</tr>
<tr>
<td>St.aureus2</td>
<td>16</td>
<td>15</td>
</tr>
<tr>
<td>St.aureus3</td>
<td>20</td>
<td>15</td>
</tr>
<tr>
<td>St.epidermidis1</td>
<td>15</td>
<td>10</td>
</tr>
<tr>
<td>St.epidermidis2</td>
<td>25</td>
<td>10</td>
</tr>
</tbody>
</table>

Synergistic Inhibitory Effect of Some Probiotic Filterates with Ampicillin
Kawther H. Ibrahim AL- Bajelan

Table(4): Inhibitory effect of probiotic filterate (S.boulardii) with Antibiotics
Bacteria Ampicillin Clindamycin isolates
F (filterate) MIC MIC+F MIC MIC+F
P.acnes1 16 15 18 10 18
P.acnes2 19 15 35 11 25
P.acnes3 16 10 18 10 20
P.acnes4 15 14 20 10 20
St.aureus1 15 20 30 14 26
St.aureus2 13 15 27 14 30
St.aureus3 13 15 15 12 20
St.epidermidis1 13 10 26 10 18
St.epidermidis2 15 10 26 10 18

Table(5): Inhibitory effect of probiotic filterate (S.cerevisiae) with Antibiotics
Bacteria Ampicillin Clindamycin isolates
F (filterate) MIC MIC+F MIC MIC+F
P.acnes1 15 15 15 10 15
REFERENCES

12-Elmer,G.W.;Surawicz,C.M. and Mcfarland , L.V.Biotherapeutic agents.aneglected modality for the treatment and
Synergistic Inhibitory Effect of Some Probiotic Fierates with Ampicillin
Kawther H. Ibrahim AL- Bajelan