IN VITRO INVESTIGATION ON ANTIBACTERIAL ACTIVITY OF SELECTED PROBIOTICS AGAINST SUPERBUG OF METHICILLIN RESISTANT STAPHYLOCOCCUS AUREUS (MRSA) ISOLATED FROM HOSPITAL POPULATIONS

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AIMS AND OBJECTIVES

Aim: This study aims to in vitro investigate the antibacterial activity of selected probiotics against superbug of Methicillin resistant Staphylococcus aureus (MRSA) isolated from Hospital populations by agar well diffusion assay. Objectives: In Vitro Investigation on Antibacterial activity of selected probiotics against superbug of Methicillin resistant Staphylococcus aureus (MRSA) isolated from Hospital populations. Abstract: Antibacterial activity of different parts of selected probiotics like Lactobacillus acidophilus and Lactobacillus casei against methicillin resistant Staphylococcus aureus (MRSA) were determined by agar well diffusion assay. The broth young culture of Lactobacillus acidophilus and Lactobacillus casei revealed highest inhibitory activity against all the tested strains of MRSA with zones of inhibition the range of 20.0 ± 1.0 to 26.0 ± 1.25 mm and 16.0 to 19.15 mm respectively. The results of the present study indicate that these probiotics can be used as new leads for the novel plant derived chemotherapeutic agents to confront the problem of nosocomial infections in particular, those involving MRSA.

KEYWORDS: Antibacterial, Methicillin resistant Staphylococcus aureus (MRSA), probiotics agar well diffusion assay.
INTRODUCTION
The problem of antimicrobial drug resistance is not new, but it has increased during the last decade, creating a serious threat to the treatment of infectious diseases (Conly, 2002) causing some important human pathogens that have recently been reported to have acquired antibiotic resistance are; *Mycobacterium tuberculosis, Staphylococcus aureus, Neisseria gonorrhoeae, Haemophilus influenzae, and Pseudomonas aeruginosa*.[28]

Pollivar *et al.*, 1982 reported the *Staphylococcus aureus* has been recognized as one of the most important gram positive bacterial pathogens contributing for infections and epidemic throughout the world. Resistance to Staphylococcus was reported in 1961, when first Methicillin (Penicillin) resistant *Staphylococcus aureus* (MRSA) was isolated in Europe, followed by Australia in 1966 and United States of America in 1968. MRSA continues to be a major cause of serious infection to man, both in hospitals and in the community. In the early 1980’s MRSA reports consisted of isolated cases but later in 1982 epidemic MRSA strains (EMRSA) were described as multi-resistant strains with special capacity to colonize patients and staff and cause widespread outbreaks of infections.[23] These epidemic MRSA strains have subsequently spread to various parts of the world.

Brigitte, (2002) reported the Resistance to antimicrobials is a natural biological phenomenon that can be amplified or accelerated by a variety of factors, including human practices. The use of an antimicrobial for any infection, real or feared, in any dose and over any time period, forces microbes to either adapt or die, in a phenomenon known as “selective pressure”. The microbes, which adapt and survive, carry genes for resistance, which can be passed on when bacteria replicates. The resistance mechanisms in microorganisms are based on four strategies

i) Inactivation of the drug,
ii) Prevention of the drug to reach its target,
iii) Reduction of target’s susceptibility, or
iv) Acquisition of new, insensitive target.[6]

Antibiotic drug resistance arises, either by mutation and selection for growth in presence of increasing concentrations of antibiotics, or by the acquisition of foreign resistance determinants. Resistance can spread either vertically, by dissemination of resistant clones, e.g. in case of MRSA or horizontally, by inter- and intra-species-specific gene transfer, such as by i) Transduction, ii) Conjugation, and iii) Transformation.[6]
The World Health Organization documented the *Staphylococcus aureus* is a prevalent bacterium carried by humans that can cause a number of problems, from mild skin infections to serious diseases including food poisoning, wound infections, pneumonia, and toxic shock syndrome.[28] The World Health Organization reported the emergence and spread of antimicrobial resistance are complex problems driven by numerous interconnected factors, many of which are linked to the misuse of antimicrobials and thus amenable to change. In turn, antimicrobial use is influenced by interplay of the knowledge, expectations, and interactions of prescribers and patients, economic incentives, characteristics of a country's health system, and the regulatory environment.[28]

Centers for Disease Control and Prevention (CCDR) was documented the selective pressure exerted by widespread antimicrobial use is considered to be a major factor in the emergence of resistance. In some countries, antimicrobial drugs are available over the counter and are present in folk remedies; in others, their abuse in prophylactic and empiric therapy and the indiscriminate use of broad-spectrum antimicrobial drugs in the community have been major contributors. Resistance factors can spread rapidly, not only locally but also, with greater movement of people around the world, globally. Microorganisms and their resistance factors may also be transferred from country to country in animals and commercially produced fruits and vegetables.[7] The World Health Organization was illustrated the hospitals are a critical component of the antimicrobial resistance problem worldwide. The combination of highly susceptible patients, intensive and prolonged antimicrobial use, and cross-infection have resulted in nosocomial infections with highly resistant bacterial pathogens. Resistant hospital-acquired infections are expensive to control and extremely difficult to eradicate.[28]

Chambers (1997) documented in many parts of the globe, particularly the developed countries, fluoroquinolones (pefloxacin, ciprofloxacin and ofloxacin) are recommended for serious infections associated with *Staphylococci*, although, occasional resistance among MRSA. Furthermore, in spite of recent reports of vancomycin resistant strains MRSA in some parts of the globe, vancomycin still remain the drug of choice for most MRSA-associated diseases.[25]

Nascimento, *et al.*, (2000) reported the problem of microbial resistance is growing and the outlook for the use of antimicrobial drugs in the future is still uncertain. Therefore, action must be taken to reduce this problem, for example, to control the use of antibiotic, develop
research to better understand the genetic mechanism of resistance, and to continue studies to develop new drugs, either synthetically or natural.\cite{22}

Probiotic microorganisms may release chemical substances that have a bactericidal or bacteriostatic effects on other microbial populations; they do so by altering interpopulation relationships like competition for chemicals or available energy rich compounds\cite{30}. The probiotic microorganisms produce inhibitory substances in the intestine of the host, on its body surface, or in culture medium where organism live and create a barrier against the proliferation of opportunistic pathogens.\cite{4}

Treatment with selected probiotic strains may be the ultimate answer to decolonization of MRSA because they do not increase the risk of multi-drug resistance of this pathogen.\cite{23, 27} The alarming increase in inappropriate antibiotic use along with bacterial resistance has led to renewed interest in ecological methods to prevent infections, which make probiotics a very interesting field for further research. For example, a patient in Japan with a decubitus ulcer colonized by MRSA was successfully treated with a probiotic Lactobacillus preparation (Alvarez-Olmoz and Oberherman 2001). One non-antibiotic strategy to combat the bacterial infections involves the selection and promotion of endogenous barrier flora to interfere with pathogenic bacterial adhesion.\cite{23, 26}

Lactic acid bacteria (LAB) strains are potentially promising because they generate bactericidal bioactive peptides (bacteriocins) and enzymes that are able to control biofilm formation and the growth of the pathogens. Nisin is the best defined bacteriocin (Huttunen et al. 1995) produced by species Lactococcus that has been approved for use in food products.\cite{12, 19}

Bacteriocins are also present in species of genus Lactobacillus. The Lb. acidophilus produce lactacin B or F, whereas Lb. casei B80 produce casein 80.\cite{24, 14} Certain LAB strains have been reported to be highly antagonistic to biofilm-forming S. aureus.\cite{2} The genus Lactobacillus has a long history of safe use, especially in the dairy industry, and it plays a major role in the transformation of fermented milk and other food products.

Over the past few decades, there has been increased impetus to introduce new Lactobacillus strains into foodstuffs with the goal of exerting a beneficial health effect when ingested by humans or animals.\cite{26, 29} Four types of LAB strains have been studied as competitive
inhibitors of pathogenic organisms. These strains are: Lb. casei 99p rhamnosus GG, Lb. casei Shirota, Bifido-bacteria brave Yacult and Lb. acidophilus Johnson-nii.

Beneficial effects conferred by Lactobacilli, including inhibition of gram negative and positive pathogenic bacteria, were described by Maragkou-dakis et al. 2006, Nomato 2005. Charlier et al. 2008 reported that Lactococcus (Lc.) lactis had a specific antagonistic effect against S. aureus.

Antimicrobial activity produced by LAB strains appears to be unrelated to the acidification of the medium. LAB strains were reported to exert a strong inhibitory effect on S. aureus growth in milk. Several suggestions have been proposed for inhibition of S. aureus by LAB. These include production of bacteriocins, hydrogen peroxide, and organic acids such as lactic and acetic acid.

In our previous study, using the European LAB strains, we were able to demonstrate their anti-MRSA activity. The purpose of this study was to evaluate the in vitro anti-bacterial activity of the LAB Lb. acidophilus and Lb. casei, against pathogenic MRSA from human clinical isolates.

To our knowledge, no data exist on a competitive interaction between clinical MRSA isolates and LAB cultures. Furthermore, we could not find any data on the survival of clinical MRSA isolates in co-cultures after being mixed with LAB strains.

In a recent study, researchers from the University of Quebec, Montreal, evaluated the in vitro antimicrobial activity of lactobacillus acidophilus and lactobacillus casei, against pathogenic MSRA from human clinical isolates. The results of this study showed that lactobacillus acidophilus and lactobacillus casei produce antimicrobial components that can inhibit the growth and eliminate MRSA cells. The most effective solution contained 64 percent lactobacillus acidophilus and 34 percent lactobacillus casei.

Our research approach is to discover probiotics as new leads, which could be developed for the treatment of infectious diseases. In the course of screening probiotics for antibacterial agents, we examined the inhibitory effects of a five crude probiotics against methicillinresistant Staphylococcus aureus. In this work, we report identification of the active principle from the probiotics and its antibacterial properties.
MATERIALS AND METHODS

Requirements

<table>
<thead>
<tr>
<th>Item</th>
<th>Supplier</th>
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<tbody>
<tr>
<td>TSB broth</td>
<td>HiMedia</td>
</tr>
<tr>
<td>Micropipette (100 μl)</td>
<td>Eppendorf India Limited</td>
</tr>
<tr>
<td>MRSA</td>
<td>ATTC 25923</td>
</tr>
<tr>
<td>Lactobacillus acidophilus</td>
<td>MTCC 5401</td>
</tr>
<tr>
<td>Lactobacillus casei</td>
<td>MTCC 1423</td>
</tr>
<tr>
<td>Manitol salt Agar</td>
<td>HiMedia</td>
</tr>
<tr>
<td>Muller Hinton Agar</td>
<td>HiMedia</td>
</tr>
<tr>
<td>Tryptic soya broth</td>
<td>HiMedia</td>
</tr>
<tr>
<td>Oxacillin</td>
<td>HiMedia</td>
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</tbody>
</table>

Test Organisms

The MRSA used for the experimental were isolated diabetic care centres and intensive care units of various corporate hospitals in East Godavari, West Godavari and Krishna districts of Coastal Andhra Pradesh, South India and MRSA (ATCC 33591) procured from ATCC (USA) purchase through HiMedia, Mumbai, India.

The microorganisms (Lactobacillus acidophilus MTCC 5401 Lactobacillus casei MTCC 1423 used for the experimental were procured from MTCC, purchase directly from IMTECH, Chandighar.

Antimicrobial agents

The antimicrobial agent used in the present study instead of Methicillin replaced by Oxacillin was used because of Methicillin was hydrolyzed on MHA, Oxacillin disc (1mcg standards for antimicrobial disk susceptibility tests) a product of HiMedia Laboratories Pvt. Ltd, India. And The Oxacillin powder form potency of 500 mg of powder a product of Cipla pharmaceutical Pvt. Ltd, India. Stored the drug in sealed containers in the dark at 4°C with a desiccant unless recommended by manufacturer. Prior to experiments these antibiotic powders/stock solutions of oxacillin should be brought to room temperature.

Source of clinical isolates and identification of MRSA

MRSA isolates were consecutively isolated from diabetic care centres and intensive care units of various corporate hospitals in East Godavari, West Godavari and Krishna districts of Coastal Andhra Pradesh, South India. Samples comprised of blood, urine, pus, ear swabs, eye
swabs and anterior nasal swabs. The swabs and body fluids of patient’s samples were inoculated onto blood agar plates, each plate inoculated with a sample of single patient. The inoculated plates were incubated at 37°C for 18-24 h. After inoculation on blood agar, the swabs were placed in brain heart infusion (BHA) with 7.5% sodium chloride, which were also incubated at 37°C for 18-24 h. Inoculated BHI broth was sub cultured onto blood agar plates. From these blood agar plates, the colonies which were opaque, circular, pigmented with β hemolytic were identified as *S. aureus* by the Grams staining, catalase and coagulase (slide and tube) test (3) Adequate controls were put up at every stage. A total of 153 coagulase positive *S. aureus* strains were isolated and identified from 478 clinical samples.

Antibiotic susceptibility testing was performed for the antibiotics; oxacillin (1μg) gentamycin (10 μg), erythromycin (15 μg), co-trimoxazole (25 μg), vancomycin (30 μg) (Hi-media) by Kirby-bauer disc diffusion technique with quality control strain of *S. aureus* ATCC 25923 as per National Committee for Clinical Laboratory Standards (NCCLS). Bacterial suspension matching 0.5 McFarland turbidity standards were inoculated on Muller-Hinton agar containing 4% NaCl and 6 μg/ml oxacillin. Isolates showing visible growth after 24h incubation at 33-35°C were identified as MRSA. Oxford strains of *S. aureus* (ATCC 25923) sensitive to methicillin and *S. aureus* (ATCC 33591) resistant to methicillin were used as control organisms. Final identification was made on detection of mecA gene by PCR. Finally 32 MRSA were identified out of 58 MRSA, 5 isolates were screened for inhibitory action of selected probiotics.

**LAB Strains and culture conditions**

All strains were stored at _20 1C in appropriate culture media with 12–15% glycerol and revived using basic microbiological techniques in strain-appropriate media. After being revived, the cultures were stored at 4 1C (1–4 days) and propagated twice before being used in antimicrobial tests. The identified Lactic Acid Bacteria (LAB) strains were of commercial origin and were provided by MTCC Chandigarh – 160036, INDIA

The Lactobacillus acidophilus and Lactobacillus casei strains were purified and earlier identified by standard biochemical test. The commercial product that contained the mixture of these strains LAB in a fermented milk-based or soya-based medium was also used in this study. The basic growth media for LAB were Man-Rogosa-Sharpe (MRS; Difco), M17 (Difco) and Peptonized Milk Nutrient (PMN; Sigma).
Agar well diffusion method
The antibacterial activity of probiotic cultures against ATCC MRSA and MRSA clinical isolates from hospitalized patients were evaluated by using agar well diffusion method according to the National Committee for Clinical Laboratory Standard (NCCLS). Briefly, 3-5 morphologically identical colonies from each agar plate were lifted with a sterile wire loop and transferred into a tube containing 5 ml of tryptic soya broth (TSB). The turbidity of each bacterial suspension was adjusted to reach an optical comparison to that of 0.5 McFarland standards, resulting in suspension containing approximately 1 to 2 x 10^8 CFU/ml. Muller-Hinton agar plates were inoculated by streaking the swab over the entire sterile agar surface. This procedure was repeated by streaking two more times, rotating the plates approximately 60° each time to ensure even distribution of the inoculum. As a final step the rim of the agar plate was also swabbed. After allowing the inoculum to dry at room temperature, 6mm diameter wells were bored with sterile metal borer. Each probiotic was checked for antibacterial activity by introducing 20µl of liquid culture of probiotic into duplicate wells by using sterile micro pipette. The plates were allowed to stand at room temperature for 1 h to allow diffusion of extract into the agar. Then, plates were incubated at 37°C for 18 h in upright position. The presence of zone of inhibition was regarded as the indicator of antimicrobial action and the antimicrobial activity of probiotic was expressed in terms of average diameter of zone inhibition measured in millimeters. Each test was carried out in triplicate.

RESULT
Table 1. Agar well diffusion method for selected two probiotics (20µL) against methicillin resistant S. aureus ATCC and clinical isolates of MRSA (n=5) Diameter of Zone of inhibition (mm)

<table>
<thead>
<tr>
<th>Microorganisms ATCC MRSA and MRSA clinical isolates (n=5)</th>
<th>Lactobacillus acidophilus</th>
<th>Lactobacillus casei</th>
<th>Oxacillin</th>
</tr>
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<tbody>
<tr>
<td><strong>ATCC MRSA</strong></td>
<td>19.0 ± 1.0</td>
<td>22.0 ± 1.80</td>
<td>00.0 ± 0.00</td>
</tr>
<tr>
<td><strong>MRSA1</strong></td>
<td>16.0 ± 1.25</td>
<td>21.0 ± 1.72</td>
<td>00.0 ± 0.00</td>
</tr>
<tr>
<td><strong>MRSA2</strong></td>
<td>17.0 ± 1.25</td>
<td>24.0 ± 1.52</td>
<td>00.0 ± 0.00</td>
</tr>
<tr>
<td><strong>MRSA3</strong></td>
<td>19.0 ± 1.5</td>
<td>20.0 ± 1.0</td>
<td>00.0 ± 0.00</td>
</tr>
<tr>
<td><strong>MRSA4</strong></td>
<td>19.0 ± 1.0</td>
<td>26.0 ± 1.25</td>
<td>00.0 ± 0.00</td>
</tr>
<tr>
<td><strong>MRSA5</strong></td>
<td>17.0 ± 0.5</td>
<td>22.0 ± 1.5</td>
<td>00.0 ± 0.00</td>
</tr>
</tbody>
</table>

Note: Values of the observed zone of inhibition (in mm diameter) including the diameter of well (6 mm) after 24 hours incubation against different bacterial species when subjected to two probiotics in agar well diffusion assay. Assay was performed in triplicate and results are
the mean of three values ± Standard Deviation. In each well, the sample size was 10µl. Inhibition observed in extracts due to solvent were assessed through negative controls. “0.00” No Inhibition Zone was observed. “Ox.” - Oxacillin (5 µg ml-1) was used as standard antibiotic.

Fig. 1: Zones of inhibition produced by of broth probiotic culture 1. Lactobacillus acidophilus, 2. Lactobacillus casei, 3. Lactobacillus acidophilus, 4. Oxacillin and 5. Sterile broth against clinical isolates MRSA1 and 2

RESULT

The continuous development of antibiotic resistance of pathogenic microorganisms is a major health concern worldwide. The screening of two different probiotics like Lactobacillus acidophilus and Lactobacillus casei for new antimicrobial compounds represent an important source for new effective medicines. In this study, two selected lactobacillus probiotics were used to evaluate its inhibitory properties on selected clinical isolates of methicillin resistant Staphylococcus aureus (MRSA) were consecutively isolated from diabetic care centres and intensive care units of various corporate hospitals in East Godavari, West Godavari and Krishna districts of Coastal Andhra Pradesh, South India. Standardized techniques was used to determine the qualitative (Agar well diffusion) bacterial activity.

In the present study results from the antibacterial activity of probiotics by agar well diffusions summarized in table 1 respectively. Among two probiotics particular in Lactobacillus casei strong inhibitory activity at 20µL concentration against ATCC MRSA and MRSA clinical isolates than Lactobacillus acidophilus was found to be most active against all MRSA clinical isolated including ATCC MRSA. It showed inhibition zones of up to 20.0 ± 1.0 to 26.0 ± 1.25 mm (showed fig 1), and Lactobacillus acidophilus showed inhibition zone of up to 16.0 ± 1.25 to 19.0 ± 1.0 mm (showed fig 2).
DISCUSSION

MRSA as a hospital pathogen presented many clinical problems in India. In Indian hospitals, MRSA is one of the common cause of hospital-acquired infections and different hospitals have reported about 30% to 80% methicillin resistance based on antibiotic sensitivity tests. The identification of hospital isolates bacterial strains is very important to confirm the presence of MRSA infection in patients. During identification coagulase test was carried out (showed fig. 4). This is an important test to differentiate S. aureus from other species especially from S. epidermidis and screening to oxacillin disc test was conducted. The choice of drugs to be used against MRSA is shrinking day by day as susceptibility of MRSA to drugs is decreasing by target site alteration, enzyme modification and permeability changes.

MRSA has gained much attention in the last decade, as the MRSA is a major cause of hospital acquired (nosocomial) infections. β-lacatm antibiotic are the preferred drugs against Staphylococcus aureus infections, although, antibiotics due to the production of chromosomal or plasmid mediated β-lacatmases or by producing Pencillín Binding Proteins (PBP₃). All the Staphylococcus aureus strains have four PBPs (PBP₁ to PBP₄), but MRSA express a special PBP (PBP₂ or PBP₂a) from mecA gene. PBP₂a takes over the biosynthetic function of normal PBP₃ in the presences of inhibitory concentration of β-lacatms because PBP₂ has a decreased binding affinity to β-lacatms (Bachi and Rohrer, 2002). This has resulted in the development of multi-drug resistance against β-lacatm and other antibiotics. Moreover, increased incidence of vancomycin-resistant MRSA has also been reported.[10] Although strategies have been proposed in an attempt to control the spread, the searches for new ways to treat MRSA infections stimulate the investigation of natural compounds as an alternative treatment of these infections.

The World Health Organization (WHO) is encouraging, promoting and facilitating the effective use of herbal medicine in developing countries for health programs. The potential of higher plants as a source of new drugs is still largely unexplored. Hence, last decade witnessed an increase in the investigations on plants as a source of new biomolecules for human disease management.[10, 2]

Multiple drug resistance in human and animal pathogenic microorganisms have been commonly reported in recent years from all over the world, particularly in developing countries, due to indiscriminate use of commercial antibiotics in the treatment of infectious diseases. Though, the resistance development by microbes cannot be stopped, appropriate
action will reduce the mortality and health care costs by using antibiotic resistant inhibitors of plant origin.\[25\] Moreover, traditional remedies utilizing plants still occupy a central place among rural communities of developing countries for curing various diseases in the absence of an efficient primary health care system.\[1\] Medicinal plants represent a rich source of antimicrobial agents. Plants are used medicinally in different countries and are a source of many potent and powerful drugs.\[2\]

This study shows that the Lactobacillus acidophilus and Lactobacillus casei produce antimicrobial components that can inhibit the growth and eliminate of the MRSA cells. This phenomenon was also observed when tests were performed with commercialised food products, which were fermented by the patented Lactobacillus acidophilus and Lactobacillus casei.

To our knowledge, these results provide the first direct evidence that MRSA strains collected from a variety of patients were vulnerable to the anti-microbial action expressed by LAB when tested in vitro. The interaction between Lactic acid bacteria and MRSA, in mixed liquid culture, can be bactericidal for a pathogenic microorganism such as MRSA.

The objective of our research was not a comparative study of different pure LAB strains but rather to increase our knowledge on bactericidal effect produced by mixed LAB cells against MRSA when these two species are cultivated together in the same environment in liquid medium. In this situation, they can produce multiple antibacterial components, which are absent when each species has been cultivated separately as pure monoculture. In order to prevent variability in the results, homogenous MRSA and LAB strains were chosen.

In addition, for the agar diffusion spot test, we standardized and optimized bacterial cultures and the inoculation method. This method included a preparation of conditions and cell concentrations for LAB, which can be used for elimination of the pathogenic cells from mixed co-culture.\[15\]

In the course of this study, the antibacterial activities against MRSA produced by a mixture of LAB strains cultivated on milk or soya-based medium were analyzed. The analysis of the anti-MRSA activity of these patented strains increased our knowledge on the use of natural media, such as milk or soya, for multiplication of LAB strains.
The dimensions of the inhibitory zones are related to the concentration of LAB cells. They were different in milk from that seen in soya-based medium. In soya-based medium, the cell concentration, as measured by our method, was smaller than in milk-based medium. Both of these natural media were effective in the production of anti-bacterial agent activity.\textsuperscript{[15]} reported that a probiotic mixture containing multi-ple strains with different properties, resulted in more effective activity in the prevention of infection with pathogenic bacteria.

We also observed that the proportion of cells added to co-cultures during the preparation of a solution formed by LAB strains influenced their anti-MRSA activity (Table 1). These results (Table 1) clearly demonstrate that the Lb. acidophilus (64\%) rather than Lb. casei (34\%) in mixed solution expressed the best antibacterial effects. The results presented in Table 1 compare the two types of lactic acid bacteria and their antimicrobial activity against ten strains of MRSA clinical isolates.

Table 1 also shows the level of sensitivity of each one of the ten MRSA strains to the antibacterial action of LAB Lb. casei or Lb. acidophilus. When the diameters of MRSA-inhibited zones on Petri dish assays are compared, it is evident that they are similar for all 10 different MRSA strains, which leads to the conclusion that the sensitivity of all these strains to LAB inhibitory activity is also similar.

The agar diffusion method was primarily used to study the effects on the production of anti-MRSA compounds by LAB co-cultures, which were mixed with co-cultures of ten MRSA isolates. This study demonstrated that whether all ten MRSA isolates or a single (#43) isolate were tested with cultures of LAB strains, the sensitivity of MRSA towards antimicrobial LAB activity was similar (as comparing for ratio 1:1 of Lb. acidophilus and casei mixture). As described, these experiments prove the bactericidal activity of LAB mixture against MRSA strain. This phenomenon can have a practical application if it can be performed in vivo.

It is difficult to compare our results with results of other studies concerning the co-culture of LAB with MRSA, since the majority of information available is on the co-culture of LAB with the S. aureus sensitive to methicillin (MSSA) (Huletsky et al. 2004; Millette et al. 2006). Our situation is very different.

The global activity of mixed microbial population is determined by the presence and function
of each species, which are strongly influenced by interactions among the different partners. However, current knowledge of microbial physiology is generally based on pure culture studies and conditions, that are different from those encountered in a complex ecosystem. Consequently, performing mixed culture studies is essential to get closer to the reality of complex populations that exist in hospitalized humans.

**CONCLUSION**

Our findings lend support to the assertion that there are components produced by lactic acid bacteria (LAB) that can inhibit growth and eliminate MRSA. These effects open the possibility for a protective use of LAB against infections with MRSA. While using natural media and adapted cell concentrations of LAB and MRSA strains, we have standardized conditions to determine the efficiency of LAB to express their anti-MRSA activity. For this purpose, strains of MRSA from clinical human isolates and strains of Lactobacillus currently used by the dairy industry were selected for study. These two microbial species are part of the flora that live on humans and animal tissues.

We believe the study will provide an opportunity to explore this natural process by letting the antagonism between LAB and MRSA play out to the advantage of human health. We hope that our research will lead to in vivo studies of the inhibitory reactions similar to those described in vitro. This approach has merit supported by the finding that Clostridium difficile diarrhea can be controlled by administering mixed cultures of Lactobacillus. At this time, there is an urgent need for a new program to control the rapidly progressing dissemination of MRSA in the human and animal populations. Currently, the use of antibiotics destroys a patient’s microbial ecology, which was established during the evolutionary process and thus allowed for the adaptation of humans and animals to life on our planet. For this reason, the application of new food additives to dairy and other food products, which can stimulate growth of lactic acid bacteria and increase their antagonistic activity expressed by LAB (probiotic) toward most pathogenic organisms, opens new perspectives for research. This new preventive approach offers great possibilities to put a stop to selection and dissemination of antibiotic-resistant microbial infections. What becomes very interesting, as demonstrated by our research, is that one can choose mixtures of various probiotics to better adapt their common action and obtain surprising results.
REFERENCES


