

*Full Length Research Paper*

# Screening for anti-methicillin resistant *Staphylococcus aureus* (MRSA) bacteriocin producing bacteria

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**Methicillin resistant bacterial infections give a tough challenge in the selection of antibiotics. Traditional use of antibiotics is worsening the problem day by day. So, it is essential to sort out other strategies which can replace antibiotic therapy successfully. Bacteriocins are the proteinaceous compounds with a narrower spectrum of antimicrobial activity but its use as antibiotic is not common. No one has ever tried to use it for the treatment of infections. Presently, we have isolated bacteriocin producing bacteria effective against methicillin resistant bacteria. It will help in controlling MRSA infections as well as provide a new strategy to treat reemerging infections.**

**Key words:** Anti- MRSA, bacteriocins, infection treatment.

## INTRODUCTION

The effectiveness of currently available antibiotics is decreasing due to the increasing number of resistant strains causing infections. Methicillin Resistant *Staphylococcus aureus* (MRSA) is one of such bacteria. It causes a wide range of syndromes, from minor skin and soft tissue infection to life-threatening pneumonia and toxinoses such as toxic shock syndrome (Lowy, 1998). The only tool for combating this serious infection was vancomycin. During the late 1990s, emergence of vancomycin resistant strains became difficult challenge for clinicians (Hanaki and Hiramatsu, 1997; Hiramatsu et al., 1997; Smith et al., 1999). It becomes inevitable to discover new antimicrobial agents for combating such a problem.

Lactic acid bacteria (LAB) are famous as friendly bacteria for human health. Their natural habitat is intestine and vagina where these protect against the entrance and proliferation of pathogenic bacteria by using various strategies. One of these defense mechanisms is the production of bacteriocins (Brooks et al., 1998). Bacteriocins are ribosomally synthesized cationic peptides with a narrower spectrum of antimicrobial activity than most antibiotics (Brooks et al., 1998). These are produced by the bacteria that have adapted for competition against

other micro-organisms. Their antimicrobial activity has been found more effective against strains closely related to the organism that produced them, or against bacteria from the same ecological system (Settanni and Corsetti, 2008). Several bacteriocins from *Lactobacillus* spp. have been characterized with respect to their protein sequence, molecular mass, biochemical properties and antimicrobial activity spectrum (Settanni and Corsetti, 2008).

In the present work, MRSA was isolated and characterized biochemically. Its sensitivity was checked against various antibiotics. Moreover *Lactobacillus* strains were isolated from different sources and bacteriocin producer was screened out and characterized biochemically. The antimicrobial efficiency of bacteriocin was checked against MRSA by well diffusion method. Clear zone indicated the antibacterial potential of bacteriocin against MRSA.

## MATERIAL AND METHODS

### Isolation and characterization of MRSA

Methicillin resistant *S.aureus* (MRSA) was isolated from blood culture of a 90 years old male. Identification was done following Standard operating procedure of Clinical laboratory standard institute or NCCLS (National Committee of clinical laboratory standards). Other tests performed for confirmation of *S. aureus* includes Gram staining, colonial morphology on blood agar, and positive results for catalase, coagulase, mannitol agar and DNase tests following (Cheesbrough, 2001). Susceptibility testing was checked against augmentin, ampicillin, cephadrine, ciprofloxacin, gentamy-

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cin, ceftriaxone, cefuroxime, clindamycin, imepenum, oxacillin, teicoplanin and vancomycin.

### Isolation of lactobacillus bacteria

Samples were prepared by making  $10^3$  dilution of yogurt, fecal material of chick, fecal material of parrot, fecal material of hen, fecal material of cat and fecal material of human. MRS agar plates (De Man et al., 1960) were prepared and the 50  $\mu$ l of each sample was inoculated on each plate. These plates were incubated in anaerobic condition at 37°C for 48 h to obtain isolates.

### Screening of bacteriocin producer and its characterization

Isolates were grown in 5 ml MRS broth with pH 5 at 37°C for 48 h. After incubation of 48 h, 1.5 ml of culture was transferred from each tube to eppendorff. Cell free supernatant was obtained by centrifugation at 10,000 rpm for 5 min followed by neutralization to pH by the addition of 5 mol l<sup>-1</sup> of NaOH. The resulting cell free supernatant was used for estimating its inhibitory effects against MRSA by using well diffusion assay as described by Schillinger and Lucke (1989). Wells of 6 mm in diameter were cut and 80  $\mu$ l of the cell free supernatant of the bacteriocin-producing strains were placed into each well. MRSA was inoculated on each plate. All plates were then incubated at 37°C for 24 h and examined for formation of inhibition zones. Inhibition was scored positive if the width of the clear zone around the well was observed. Presence of bacteriocin in the cell free supernatant was confirmed by treating it with proteinase k for 2 h and effect on zone of inhibition. Disappearance of zone will show the presence of proteinaceous compound in the supernatant.

The culture of bacteriocin producer was identified according to their morphological, and biochemical properties. The used tests were: Gram reaction; growth at 15, 25 and 45°C; production of catalase, cytochrome oxidase; acid and gas production from glucose; acid production from carbohydrates (1% w/v) lactose, sucrose, mannitol, ribose, sorbitol and mannose in MRS broth devoid of glucose and beef extract with phenol red as indicator.

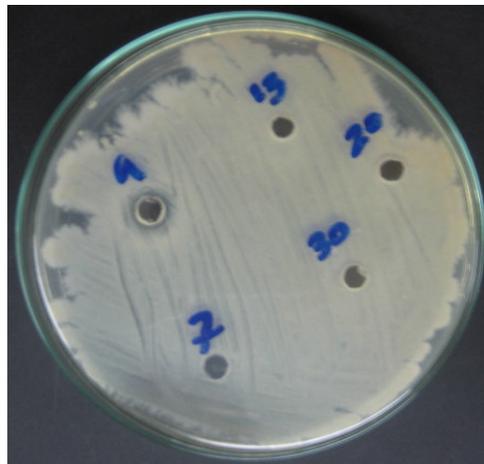
## RESULTS

### Isolation and characterization of MRSA

The isolate from the blood was gram +ve, coagulase +ve, Catalase +ve, Dnase +ve. These all characteristics show that isolate belong to the group of *S. aureus*. Sensitivity test shows that it is resistant to augmentin, ampicillin, cephradine, ciprofloxacin, gentamycin, ceftriaxone, cefuroxime, clindamycin, imepenum, oxacillin. It is sensitive to teicoplanin and vancomycin.

### Isolation and characterization of bacteriocin producers

A total of 50 non motile, gram positive, catalase and oxidase negative bacilli isolates harvested from  $10^3$  dilution of yogurt, fecal material of chick, parrot, hen, cat and of human female of age 22 were first examined for potential inhibitory substances against MRSA. Cell free supernatant of only one strain "9" exhibited zone of inhibition (Figure 1) which disappeared after the treatment of



**Figure 1.** Zone of Inhibition against MRSA by bacteriocin in well No. labeled as 9 having cell free supernatant of strain 9. No zone of inhibition was shown by the cell free supernatant of other strains.

proteinase k. Biochemical properties of isolate "9" showed +ve fermentation reactions for lactose, sucrose, mannitol and mannose, and ribose sugars. Acid and gas production were also detected. Growth was visible at 15, 25 and 45°C. These all results show that strain belong to the group of *Lactobacillus fermentum*.

## DISCUSSION

It is fact that antibiotics are the active controller of bacterial infections and occupy an important status in the field of medicines. These are used frequently not only in the infection treatment of human beings but also in agriculture for the improvement of livestock. Unfortunately, its beneficial uses were highlighted in such a manner that, people started to ignore its side effects. Non judicious use became common practice. Although the problem has been recognized for many years, imprudent use of antibiotics continues to be a major public health concern (Nash et al., 2002). This malpractice has resulted in the release of 1–10 million tons of antibiotics into the biosphere over the last 60 years (European Commission, 2005). This has exerted a very strong selective pressure for the appearance of resistant strains. One of these is methicillin-resistant *S. aureus* (MRSA).

*S. aureus* is a major cause of serious nosocomial infections colligated with morbidity and mortality (Noskin et al., 2005). Emergence of MRSA resistant to all  $\beta$ -lactam antibiotics and usually to several other antimicrobial classes has made infections difficult to treat. If factors like serious underlying diseases, longer prior hospitalization, more prior antimicrobial therapy and other adverse prognostic factors are considered, it becomes obvious that patients infected with MRSA suffer more than those infected with methicillin-susceptible *S. aureus* (MSSA) (Graffunder and Venezia, 2002; Engemann et al.,

2003; Cosgrove et al., 2003). Prevalence rates of MRSA strains vary between (and within) countries but have increased significantly since the early 1990s in England and Wales rising from 2% in 1990 and 1991 to a peak of 43% in 2002, with a slight decline thereafter (Johnson et al., 2005). Interestingly, only a few clones are spreading throughout the world and are responsible for the high resistant rates (Oliveira et al., 2002). Presence of the *mecA* gene, encoding the low affinity penicillin-binding protein 2A conferring resistance against methicillin and other  $\beta$ -lactam antibiotics is the common feature of reported MRSA strains (Cottagnoud, 2008). Vancomycin was the only effective treatment for severe MRSA infection strains till 1996 when an intermediate resistance to vancomycin (VISA: vancomycin-intermediate *S. aureus*), with MICs between 8 and 16 mg/L was reported from Japan (Hanaki and Hiramatsu, 1997; Hiramatsu et al., 1997) and since 1997 from the United States (Smith et al., 1999). This highlights the need of new strategies for stringent control over MRSA infections.

The use of bacteriocins can be promising. Their current applications are as food additives, whereas less research has been conducted on the therapeutic applications as antimicrobial agents (Riley and Wertz, 2002; Settanni and Corsetti, 2008). Due to their target specificity, susceptibility to proteolytic digestion, possibility of genetic transfer and manipulation, they may be considered as more beneficial as compared to the regular antibiotics (Kalmokoff et al., 1996).

Several groups of lactic acid producing bacilli (LAB) produce bacteriocins that have been evaluated for their ability to extend shelf life and to control pathogenic bacteria in food products (Settanni and Corsetti, 2008). LAB are also found associated with health benefits, including ability to reduce symptoms associated with diarrhea (Isolauri et al., 1991), irritable bowel syndrome (O'Mahony et al., 2005) and infant asthma (Kalliomaki et al., 2001) as well as reducing the duration of the common cold (de Vrese et al., 2005). LAB might be ideal in producing bacteriocin effective against MRSA. For this purpose, we collected the samples from natural habitat of LAB and used the selective media (MRS media). We obtained 50 isolates, out of which only one produced bacteriocin effective against MRSA. Its growth and biochemical characteristics show that it belongs to the group of *Lactobacilli fermentum* (Klein et al., 1998; Nair and Surendran, 2005). This study shows that *L. fermentum* can be used for the production of bacteriocin effective against MRSA. The isolated strain can also be used as probiotic after the positive reports of *in vivo* experimentation. This strategy can be applied for the control of other reemerging infections also.

## REFERENCES

Brooks GF, Butel JS, Morse SA (1998). Jawetz, Melnick, and Adelberg's Medical Microbiology. Appleton & Lange a Simon & Schuster Company, Princeton Hall, Upper Saddle River, New

- Jersey, USA, p. 269
- Cheesbrough M (2001). District Laboratory Practice in Tropical Countries. Part 2. Cambridge University Press, Cambridge.
- Cosgrove SE, Sakoulas G, Perencevich EN, Perencevich EN, Schwaber MJ, Karchmer AW, Carmeli Y (2003). Comparison of mortality associated with methicillin-resistant and methicillin-susceptible *Staphylococcus aureus* bacteremia: a meta-analysis. *Clin Infect Dis*. 36: 53-59.
- Cottagnoud P (2008). Daptomycin: a new treatment for insidious infections due to gram-positive pathogens. *Swiss Med Wkly*. 138(7-8): 93-99.
- De Man JD, Rogosa M, Sharpe ME (1960). A medium for the cultivation of lactobacilli. *J. Appl. Bact.* 23: 130-135.
- de Vrese M, Winkler P, Rautenberg P, Harder T, Noah C, Laue C, Ott S, Hampe J, Schreiber S (2005). other authors. Effect of *Lactobacillus gasseri* PA 16/8, *Bifidobacterium longum* SP 07/3, *B. bifidum* MF 20/5 on common cold episodes: a double blind, randomized, controlled trial. *Clin Nutr*. 24: 481-491.
- Engemann JJ, Carmeli Y, Cosgrove SE, Fowler VG, Bronstein MZ, Trivette SL, Briggs JP, Sexton DJ, Kaye KS (2003). Adverse clinical and economic outcomes attributable to methicillin resistance among patients with *Staphylococcus aureus* surgical site infection. *Clin Infect Dis*. 36: 592-598.
- European Commission (2005). Opinion of the FEEDAP Panel on the updating of the criteria used in the assessment of bacteria for resistance to antibiotics of human or veterinary importance. *EFSA J*. 223: 1-12.
- Graffunder EM, Venezia RA (2002). Risk factors associated with nosocomial methicillin-resistant *Staphylococcus aureus* (MRSA) infection including previous use of antimicrobials. *J. Antimicrob. Chemother.* 49: 999-1005.
- Hanaki H, Hiramatsu K (1997). Emerging infectious and drug-resistant diseases – vancomycin resistant gram-positive cocci. *Nippon Naika Gakkai Zasshi*. 86(11): 2075-2080.
- Hiramatsu K, Hanaki H, Ino T, Yabuta K, Oguri T, Tenover FC (1997). Methicillin-resistant *Staphylococcus aureus* clinical strain with reduced vancomycin susceptibility. *J. Antimicrob Chemother.* 40(1): 135-136.
- Isolauri E, Juntunen M, Rautanen T, Sillanaukee P, Koivula T (1991). A human *Lactobacillus* strain (*Lactobacillus casei* sp. strain GG) promotes recovery from acute diarrhea in children. *Pediatrics* 88: 90-97.
- Johnson AP, Pearson A, Duckworth G (2005). Surveillance and epidemiology of MRSA bacteraemia in the UK. *J. Antimicrob. Chemother.* 56: 455-462.
- Kalliomaki M, Salminen S, Arvilommi H, Kero P, Koskinen P, Isolauri E (2001). Probiotics in primary prevention of atopic disease: a randomised placebo-controlled trial. *Lancet* 357: 1076-1079.
- Kalmokoff ML, Bartlett F, Teather RM (1996). Are Ruminant Bacteria Armed with Bacteriocins? *J. Dairy Sci.* 79: 2297-2306.
- Klein G, Pack A, Bonaparte C, Reuter G (1998). Taxonomy and physiology of probiotic lactic acid bacteria. *Int J. Food Microbiol.* 41: 103-125.
- Lowy FD (1998). *Staphylococcus aureus* infections. *N. Engl. J. Med.* 339: 520-532.
- Nair PS Surendran PK (2005). Biochemical characterization of lactic acid bacteria isolated from fish and prawn. *J. Cult. Col.* 4: 48-52.
- Nash DR, Harman J, Wald ER, Kelleher KJ (2002). Antibiotic prescribing by primary care physicians for children with upper respiratory tract infections. *Arch. Pediatr. Adolesc. Med.* 156: 1114-1119.
- National Committee for Clinical Laboratory Standards: Performance standards for antimicrobial susceptibility testing. In NCCLS approved standard M100-S14.2004.NCCLS, Wayne, PA USA.
- Noskin GA Rubin RJ, Schentag JJ, Klyutmans J, Hedblom EC, Smulders M, Lapetina E, Gemmen E (2005). The burden of *Staphylococcus aureus* infections on hospitals in the United States: an analysis of the 2000 and 2001 Nationwide Inpatient Sample Database. *Arch. Int. Med.* 165: 1756-1761.
- Oliveira DC, Tomasz A, de Lencastre H (2002). Secrets of success of a human pathogen: molecular evolution of pandemic clones of methicillin-resistant *Staphylococcus aureus*. *Lancet Infect. Dis.* 2(3):

- 180-189.
- O'Mahony L, McCarthy J, Kelly P, Hurley G, Luo F, Chen K, O'Sullivan GC, Kiely B, Collins JK. (2005). other authors. *Lactobacillus* and *Bifidobacterium* in irritable bowel syndrome: symptom responses and relationship to cytokine profiles. *Gastroenterology*. 128: 541-551
- Riley MA, Wertz JE (2002). BACTERIOCINS: Evolution, Ecology, and Application. *Ann. Rev. Microbiol.* 56: 117-137.
- Schillinger U, Lücke KF (1989). Antibacterial activity of *Lactobacillus sake* isolated from meat. *Appl. Environ. Microbiol.* 55: 1901-1906.
- Settanni L, Corsetti A (2008). Application of bacteriocins in vegetable food biopreservation. *Int. J. Food Microbiol.* 121(2): 123-138.
- Smith TL, Pearson ML, Wilcox KR, Cruz C, Lancaster MV, Robinson-Dunn B, Tenover FC, Zervos MJ, Band JD, White E, Jarvis WR (1999). Emergence of vancomycin resistance in *Staphylococcus aureus*. Glycopeptide-Intermediate *Staphylococcus aureus* Working Group. *N. Engl. J. Med.* 340(7): 493-501.