Case Report

Effect of Anti Bacterial Skin Secretion of *"Rana ridibanda"* Frog on Methycillin Resistant Staph Aureus

Mohammad Amin Abbasi, M.D., Nariman Mosaffa, D.V.M., Ph.D.[:], Sodabe Taheri, M.Sc. Khosro Molla Jaafari, M.Sc.

Immunology and Microbiology Department, Shaheed Beheshti University of Medical Sciences

[:] *Corresponding Address: P.O.Box: 19395-4719, Immunology and Microbiology Department, Shaheed Beheshti University of Medical Science,Tehran, Iran*
Email: mosaffan@sbmu.ac.ir

Abstract

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The dorsal skin of the Iranian frog, *Rana ridibanda*, is associated with numerous prominent granular glands which extract their secretions in the response to stressor or invading pathogens .The secretions have broad spectrum antimicrobial effects. In this research the effect of antimicrobial skin secretions from Iranian frog (*Rana ridibanda)* has been examined against gram positive *Methycillin Resistant Staph Aureus (MRSA)*, under sterile conditions. To show this effect, 1cm² of frog skin was cultured in complete tissue culture medium containing RPMI, FBS and FUNGISON for the period of 10 days. Immediately after, *MRSA* was exposed to frog skin secretion (medium culture) and Vancomycin. The results showed that the frog skin secretions has significant antimicrobial effect against *MRSA*. The range of inhibition zone for *MRSA* was the same as (20mm) Vancomycin in DISK method. In Minimum Inhibitory Concentration method, for *MRSA*, the tube 1/8 was positive.

Keywords: Methycillin Resistant Staph Aureus (MRSA), Minimum Inhibitory Concentration, Rana Ridibanda, Peptide, Anti Bacterial Effect

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Introduction

Animals, similar to plants, defend themselves against invading pathogenic microorganisms by producing and secreting antimicrobial peptides. Natives of Argentina sometimes tie a certain kind of live frog onto wounds as a remedy. Amphibians have rich chemical arsenals in their skin glands forming an integral part of their defense system, and also assisting with the regulation of dermal physiological action. In response to a variety of stimuli, host defense compounds are secreted from specialized glands onto the dorsal surface and into the gut of the amphibian (1). Extensive studies have been conducted on amphibian antimicrobial peptides of frogs belonging to the genus Rana. In otherwise MRSA, a gram positive cocci, is the most important pathogen of ventilator acquired pneumonia (VAP), which may lead to septicemia and death. One of the most important problems in treating patients is resistance to antibiotics. Recently, infections caused by multidrug-resistant pathogens have been frequently diagnosed (2). For this reason, it has become critical to identify agents to treat multidrug-resistant gram-positive infections with novel mechanisms of activity (3).In recent years, Amphibians have shown rich chemical arsenal of antimicrobial peptides (4). The secretions from amphibian's skin have become a pivotal model for the discovery of new peptide antibiotics originating from non myeloid cells of vertebrates (1). The bioactive peptides are released into the skin secretions in a holocrine fashion upon stressor injury and protect them against invasion by pathogenic microorganisms (4). It is believed that the simultaneous presence of antimicrobial molecules acting in synergy, provides frogs with a better shield against a wide range

of harmful invaders bacterial, fungal and protozoa (1, 4).

The dorsal skin of the Marsh frog (*Rana ridibanda*) is associated with numerous glandular glands (5, 6) which synthesize polypeptides with a broad spectrum of antimicrobial activities (2, 7) on gram positive bacteria such as *MRSA* which isolated from the patients suffering Ventilator Acquired Pneumonia (VAP).

Materials and Methods

Collecting of skin secretions

Two adult specimens of *(Rana ridibanda)* frogs in both sex (one male and one female),about 150 gr in each sex, were captured from Sorkhe Hesar (the province of Tehran) and moved to department of zeology of science faculty in order to comfirm the genus of frogs. Then they were moved to Immunology Research Center of Shaheed Beheshti University and housed in a cage under natural lighting conditions with regular diet. The frogs were decelebrated and their skins were removed of the ventral and dorsal part of the frog under sterile condition and were cut into small pieces (1cm²).The excised pieces were put in a complete tissue culture medium, containing (RPMI 1640 , FBS 12٪ and FUNGISON) to extract skin secretions. After a ten-day incubation, the tissue culture process was terminated when the upper layer of tissue culture was discolored to yellow. Then the suppernatantes was moved to Muller- Hinton culture in the microbiological research center to carry out antimicrobial laboratory tests (8).

Antimicrobial assay

The antimicrobial testing on skin secretions was carried out by the microbiology department of the institute of medical science. In this phase, three gram positive bacteria, *Methycillin Resistant Staphylococcus aureus (MRSA)*, *Streptococcus beta hemolytic* and *Etrococcus fecalis,* and three gram negative bacteria, *E.coli, Klebsiella* and *Salmonella typhi* were chosen with standard methods. The secretion of frog skin was adjusted with the bacteria in two groups (secretions of ventral skin and dorsal skin, male/female samples) in order to evaluate the antibacterial effect with Minimal Inhibitory Concentration (MIC) and DISK methods. Both methods are defined as follow:

In disk method

In this phase, a colony of (1.5×10 bacteria) from each bacterium was isolated within 0.5 Macfarlan tube by a standard method. The colonies were cultured in Solid Brain Heart Infusion Agar (BHI) used sterile swaps sterilized in 180˚c for 45 min.

Then a disk concluded the secretion of frog skin was put in the Solid culture and incubated for 24 hours within 37˚c. We also used Vancomycin and Canamycin for *staph aureus*, Penicillin and Ampicillin for *strep beta hemolytic*, Gentamycin and Ciprofloxacin for gram negatives. At the end of incubation period the range of inhibition zone for each bacteria was measured and compared with the antibiotic used (8). In Minimal Inhibitory Concentrations (MICs) method was assayed according to the procedures outlined by the National Committee for Clinical Laboratory Standards (measured by a standard micro dilution method) and were taken as the lowest concentration whereupon no visible growth was observed (9): a dilution of 1/2, 1/4, 1/8, 1/16 for each bacterium was prepared within 0.5 Macfarlan tube then the frog skin secretions were added to the culture containing bacteria in a ratio of 1 to 9. After 24 hours the growth inhibition was measured.

Results

The results of the study indicated that:

1.the frog skin secretions inhibited the growth of MRSA and streptococcus, and has antibacterial effect as Vancomycin and other conventional antibiotics.

2. An interesting finding was that only the dorsal skin secretions showed a significant antimicrobial effect against *MRSA*.

3. The secretions of ventral frog skin did not show any antibacterial effect (Table1).

4. In DISK method, the range of inhibition zone for MRSA was the same as Vancomycin and Canamycin (20mm). The

Table 1: Showes the antibacterial effect of frog ridibanda on MRSA in comparison with some conventiation antibiotics (Disk Method)

ANTIBIOTIC BAC	Penicilin	Ampilicin	Canamycin	Vancomycin	Gentamycin	Dorsal skin secretion	Ventral skin	Ciprofloxicin
MRSA	-	-	20mm	20mm	-	20mm	-	-
Strepb hemolytic	30mm	21mm	-	-	-	21mm	-	-
Enterococcus	30mm	21mm	-	-	-	21mm	-	30mm
fecalis	-	-	-	-	21mm	Resistant	-	30mm
E.coli	-	-	-	-	20mm	Resistant	-	
Klebsiella pneumoniae								

range of inhibition zone for *strep.B hemolytic* (21mm) was the same as Ampicillin (21mm) and less than Penicillin (30mm).

The range of inhibition zone for *Entrococus* (18mm) was less than those for Ampicillin (21mm) and Penicillin (30mm). There was no range of inhibition zone for *E.coli, Klebsiella Pneumoniae* and *salmonella typhi*, but they were sensitive to Ciprofloxacin and Gentamycin. In minimum inhibitory concentration method Positive range of growth for MRSA, the tube 1/8 was In MIC method: Positive range of growth for *Strep.beta hemolytic* tube 1/8 was positive. Positive range of growth for *Enrococcus fecalis* tube 1/16 was positive. No positive range of growth for gram negative bacteria was detected.

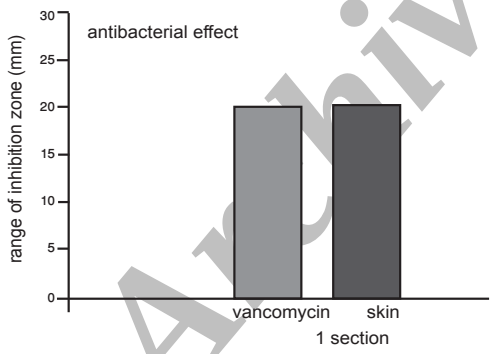

Fig 1: Showes the range of inhibition zone of frog Rana ridibanda skin secretion and Vancomycin on MRSA.

Discussion

Pneumonia is one of the life threatening diseases in elderly, especially in patients who are admitted in hospital and intensive care units. Many organisms can cause Pneumonia. *MRSA* is one of the most important pathogens causing pneumonia in patients admitted in intensive care units (ICU).it is the main pathogen isolated from patients with ventilator acquired pneumonia. Nowadays the most important problem dealing with *MRSA* is that, it is becoming resistant to most of the conventional antibiotics and causes death in many patients who are admitted in hospital and ICU. So it is necessary to find novel antibiotics with high potency and lower risk of resistance against them. The present study showed that isolation from dorsal extract of *Rana ridibanda* skin tissue has the ability to inhibit the growth of gram positive bacterium such as *MRSA* but there was no antimicrobial effect of ventral skin secretions as mentioned in previous studies (3, 10) with high potency which was comparable with Vancomycin those effects was not sex dependent. There are many peptides isolated from amphibian skin secretions like the caerins, maculatins, citropins, aureins and uperins are membrane active antibiotic species (2, 3). Because of their non-specific mechanism of producing cell death, pathogens develop resistance to the antimicrobial peptides at much lower rates than to conventional antibiotics. However, despite showing broad-spectrum activity against strains of antibiotic-resistant bacteria and potent activity against certain pathogenic bacteria, their therapeutic potential remains to be realized (11, 12). These peptides act completely unlike traditional antibiotic agents and may open novel prospectives in the conception of new therapeutic treatments of nosocomial infections and also infections of multi drug resistant pathogens no anti-infective peptide based upon their structures has yet been adopted in clinical practice. Vancomycin has also adverse side effects, such as allergic reactions, Red man syndrome and bonemarrow suppression.

Thus, these extarcs, if purified, might be used as new antibiotics in future. New clinical applications need to be found if progress in the field is to continue.

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