Title: IDENTIFICATION OF INFECTIOUS ORGANISMS FROM ANIMAL WOUNDS AND CHARACTERIZATION OF TOXIN BY IN VITRO AN DIN VIVO METHODS

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Keywords

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Original Article

Identification of Infectious Organisms From Animal Wounds And Characterization of Toxin by *In Vitro and In Vivo Methods*

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Abstract

Isolation of infectious organisms from animal bite wounds, mostly dog and cat bite, which shows polymicrobial organism, aerobic and anaerobic, characterization of the toxin by *in vivo* and *in vitro* methods. Study was carried out at anti rabies clinic of Pasteur Institute of India dispensary, Coonoor, Nilgiri, Tamil nadu during December 2009 to March 2010. After isolation and toxin characterization, antibiotic sensitivity pattern was also done.

The result shows that most of the organisms are *Staphylococcus Aureus,* resistant to Penicillin and Ciprofloxacin but sensitive to Offloxacin, Cephazolin, Tetracycline, Cotrimoxale, Errythromycin, Gentamycin, Levofloxacin, Amikacin, Ceftriaxone, Cephotaxime and Linezolid.

Introduction

Human contact with animals can result in bite injuries. To be eligible for enrolment, patient had to meet one of the three major criteria for infection of a bite wound such as flare, abscess and associated erythema or fierce minor criteria such as wound associated erythema, tenderness and swelling, purulent discharge and leucocytosis. Majority of the bite wounds are caused by dogs and cats, are usually polymicrobial, aerobic or anaerobic. Untreated deep punctured wounds will lead to osteomyelitis, meningitis, endocarditis, pneumonia, abscess formation etc. Early recognition of warning signs and appropriate treatment are key in minimizing potential problems from the bites. Consequences of infection range from mild discomfort to life threatening complications. At least 64 species of bacteria are found in canine mouth causing nearly all infections to be mixed. Common bacteria involved are mentioned below.

1. Dog

- Staphylococcus
- Streptococcus
- Eikenella
- Pasturella
- Proteus
- Klebsiella
- Hemophilus
- Enterobacter
- Capnoctophaga canimorsus
- Bacteroides
- Moraxella
- Corynebacterium
- Neisseria
- Fusobacterium
- Prevotella

2. Cat

- Pasturella
- Actinomyces\
- Propionibacterium
- Bacteroides
- Fusobacterium
- Clostridium
- Wolinella
- Peptostreptococcus
- Staphylococcus
- Streptococcus

Among dog saliva pathogens, Capnocytophaga canimorsus are gram negative rods, rarely causing soft tissue infection but can lead to fulminant sepsis and meningitis which is highly fatal. Brucella canis causes local wound infection as well as non-specific symptoms associated with brucellosis. Among cat saliva pathogens, pasturella is a common infection. Corneal infection with *C. canimorsus* leads to cats tooth keratitis. Bartonella henselae infection is common in cases of cat scratch.

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Rabies

Rabies is a disease of antiquity. It is 100% fatal but 100% preventable by vaccination. It is caused by a bullet-shaped virus, Rhabdoviridae found in saliva of infected animals. All mammals including humans, dogs, cats, cows, horses etc can act as carrier for rabies. Rabies is contracted by exposure to saliva of a rabid animal.

Materials and Methods

For the study, 25 samples from animal bite patients with wound infection, were collected from dispensary of Pasteur Institute of India, Coonoor. The samples were taken from the wounds in different age groups.

Bacterial Isolation and Identification

Specimens collected aseptically were swabbed on blood agar, MacConkey agar and nutrient agar plates and incubated at 37°C for 24 – 48 hrs. Pure culture of organisms can be obtained from mixed culture by isolating the colonies. Colonies are identified by gram-staining, growth on selective media, biochemical tests and antimicrobial sensitivity

Table No - 1
Characterization of the samples

Sample number	Class of wound	Growth on blood agar	Growth on MacConkey agar	Gram staining
1.	II	α-hemolytic	No growth	Gram +ve cocci in clusters
2.	II	1. □-hemolytic	No growth	Gram +ve short chain
		2. □-hemolytic	No growth	Gram +ve cocci
3.	III	γ-hemolytic	No growth	Gram –ve coccobacilli
4.	II	γ -hemolytic	No growth	Gram +ve spore bearing bacilli
5.	II	α-hemolytic	No growth	Gram +ve cocci in clusters
6.	I	No growth	No growth	-
7.	II	γ -hemolytic	No growth	Gram +ve cocci in clusters
8.	II	β-hemolytic	No growth	Gram +ve cocci in clusters
9.	III	1. □ –hemolytic	No growth	Gram –ve coccobacilli
		2. □ –hemolytic	No growth	Gram +ve cocci
		3. hemolytic colonies	Growth	Gram –ve rods
		4. wrinkled non hemolytic colonies	No growth	Yeast colony
		5. green paste like smooth oily colonies	No growth	Fungal colony
10.	II	β-hemolytic	No growth	Gram +ve cocci in short chains
11.	III	Hemolytic colonies	No growth	Gram +ve cocci in short chains
12.	II	γ -hemolytic	No growth	Gram +ve spore bearing bacilli
13.	II	α-hemolytic	No growth	Gram +ve cocci in tetrads
14.	I	No growth	No growth	-
15.	II	γ -hemolytic	No growth	Gram +ve spore bearing bacilli
16.	II	γ -hemolytic	No growth	Gram +ve cocci in clusters
17.	II	γ -hemolytic	Creamy colonies	Gram –ve short rods
18.	II	α-hemolytic	No growth	Gram +ve cocci
19.	I	No growth	No growth	-
20.	II	γ -hemolytic	No growth	Gram +ve cocci in clusters
21.	II	1. □-hemolytic	No growth	Gram +ve spore bearing bacilli
		2. □ -hemolytic	No growth	Gram –ve coccobacilli
22.	II	1. □-hemolytic	No growth	Gram +ve cocci in clusters
		2. □-hemolytic	No growth	Gram +ve cocci in chains
23.	II	γ -hemolytic	No growth	Gram +ve spore bearing bacilli
24.	II	Iβ-hemolytic	No growth	Gram +ve cocci in short chains
25.	III	γ -hemolytic	No growth	Gram +ve cocci in clusters

patterns. Biochemical tests used were IMViC test, catalase test, coagulase test, TSI test and urease test.

Results and Discussion

Twenty five samples were taken for study during the 3 months period. staphylococcus aureus was the major isolate. exotoxin from staphylococcus had been involved directly in the bacterial infection. the crude exotoxin was separated by centrifugation and gel filtration chromatography eluted sample purified by polyacrilamide gel electrophoresis band formed indicated the molecular weight of the protein present in the toxin. the plasmid, extrachromosomal circular DNA that codes for resistance was isolated by lysis method and followed by Agarose gel electrophoresis. band formed indicated the presence of plasmid DNA. virulence test of staphylococcus aureus exotIoxin analysed in animal and in vero cell lines. In this study reveals that some strain of staphylococcus aureus are resistant to penicillin and methicillin but sensitive to ofloxacin, cephazolin and linezolid. Staphylococcus was the major isolate from the animal bite wounds of our study which is gram-postive cocci in grape like clusters. It forms hemolytic colonies on blood agar, pink color colony on MacConkey agar and golden yellow colony on nutrient agar. It is Coagulase test positive. On the biochemical tests, IMViC + + +-, TSI no gas, urease negative. Characterization of staphylococcal exotoxin was done by isolation of plasmid, separation of plasmid by Agarose gel electrophoresis, gel filtration chromatography. On protein identification by SDS-PAGE, toxin band was obtained in the range of 43 kDa and 63 kDa so molecular weight of toxin was 43 kDa. The virulence of staphylococcus was analyzed *in vitro* by vero cell. Cytotoxic effect was observed.

Antibiotic sensitivity test were done on pure 24 hr old cultures of all the aerobic bacteria using Muller Hinton media. The organism was tested for its ability to grow on artificial nutrient media containing different antibiotic disc. Antibiotics diffuse outward from each disc into the surrounding agar and produce a diminishing gradient of concentration. On incubation, the bacteria grow on areas of the plate except those around the drugs to which they are sensitive. The width of each growth free "Zone of inhibition" is a measure of their sensitivity to the drug.

Table No-2
Isolation of organisms

Organism	Number of colonies obtained
Staphylococcus	12
Streptococcus	4
Bacillus	4
Pasteurella	3
Klebsiella	1
Pseudomonas	1

Summary and Conclusion

Staphylococcus aureus was the major isolates from the animal bites of our study. The *S. aureus* exotoxin is involved directly in the bacterial infection. The virulence test of *S. aureus* toxin was analyzed by in vivo and in vitro methods. *In vivo* studies involve toxin dilution and 0.3 ml of each dilution was injected in mice and 0.5 ml in guinea pigs. Animals were observed for 7 days and autopsy showed hemorrhagic spots in liver and lungs. In vitro studies involve use of Vero cell lines to establish virulence of

Table 3:
Drug Sensitivity Pattern

Drug	Diameter of Zone(mm)	Resul
Amikacin	25	S
Azithrimycin	No zone	R
Cefazolin	30	S
Cefpodoxime	20	I
Cefixime	19	S
Ceftriaxone	22	S
Cefuroxime	24	S
Cephalexin	16	S
Cefotaxime	25	S
Chloramphenicol	24	I
Ciprofloxacin	16	S
Clindamycin	28	I
Cloxacillin	15	S
Co-trimoxazole	23	S
Erythromycin	27	S
Gentamicin	23	S
Levofloxacin	19	S
Linezolid	30	S
Methicillin	19	S
Netilmicin	25	S
Ofloxacin	25	S
Penicillin	7	R
Teicoplanin	No zone	R
Tetracycline	20	S
Vancomycin	15	I

R- Resistant; S-Sensitive, I- Intermediate

Fig. 1: Growth on Neutrient Agar Media

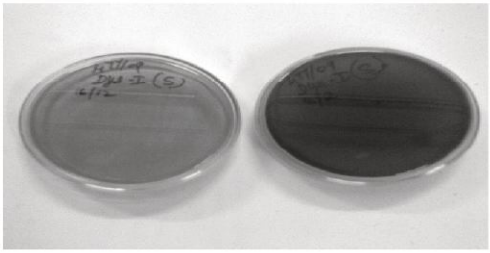

Fig. 2: On Maconkey Agar Media

S. aureus toxin at different dilutions. The treatment of choice for *S. aureus* is Penicillin but due to widespread resistance, first choice drugs are penicillinase resistant penicillins (oxacillin or flucloxacillin). Gentamicin can be used in endocarditis but can cause kidney damage. The organism is sensitive to Offloxacin, Cephazolin, Tetracycline, Cotrimoxale, Errythromycin, Gentamycin, Levofloxacin, Amikacin, Ceftriaxone, Cephotaxime and Linezolid.

Prevention of spread of *S. aureus* infection can be done by hand washing and use of disposable mask and gloves by staff since vancomycin resistant strains are also reported.

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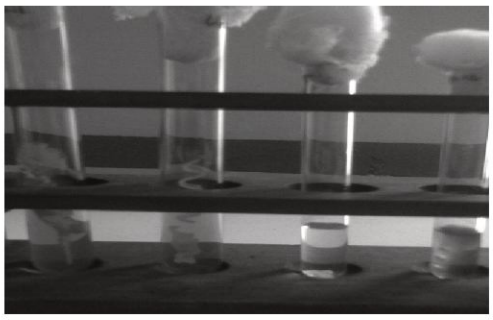

Fig. 3: Biochemical Tests

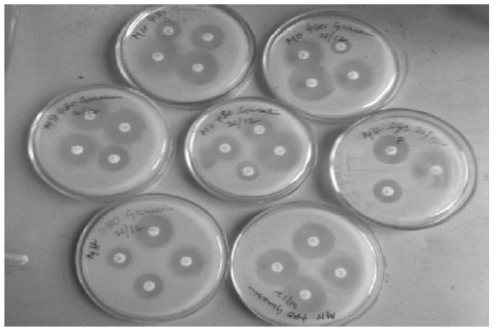

Fig. 4: Antibiotic Sensitivity Test

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Announcement

The APCRI Journal is published twice a year. Once in January and again in July. The APCRI Journal invites Contributions from the Scientific Community, on All aspects of Rabies and Related Matter, in the form of Original Articles and Review Articles, Brief Reports, Case Reports, Personal Viewpoint, Letters to the Editor, Notes and News, Your Questions and Book Review.

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Announcement

The APCRI Newsletter is published every six monthly, in October and in April. APCRI members and the members of the Scientific Community are requested to contribute News Clippings, Photographs and Reports on Scientific activity on Rabies and Related matter for publication in the Newsletter.

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