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Cultural Sensitivity of Septic Wound in Animals

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Abstract: Bacteriological examination of septic wounds in animals was conducted in the Veterinary Clinic, Bangladesh Agricultural University, Mymensingh, Bangladesh during the period of January 2001 to July 2002. A total of 250 samples were collected for the identification of bacteria from the wound and 227 (90.8%) were found infected with bacteria. *Staphylococcus aureus* was the most common organism (33.92%) and then *Escherichia coli* (18.94%) and the lowest prevalence was the *Streptococcus* spp. (9.25%). *In vitro* drug sensitivity study revealed that Gentamycin, Cephalosporin, Neomycin and Streptomycin are potent antibiotic for the treatment of infected wound and Penicillin and Sulphamethaxazole are less sensitive to contaminated wound. For better conception and accurate treatment of complicated wounds, it is necessary to have cultural isolation of the microorganisms and antibiotic sensitivity of each isolate before the treatment.

Key words: Isolation, culture, microorganisms, wound, antibiotic-sensitivity

Introduction

Wound is one of the most dangerous affections in the body and it is aggravated by the invasion of pathogenic organisms. Various types of organisms are involved for the ailing of animals and human being. Postoperative wound infection is still one of the major problems in our country and also a source of morbidity to animals.

Most common organisms cause wound infection and lingering the healing process are *Staphylococcus aureus, Escherichia coli, Pseudoonas aureginosa, Proteus vulgaris, Klebsella pneumonie, Streptococcus* spp. The wound sometimes gets infection with either by single organism or by mixed organisms, which deteriorate the affections. Among them *Staphylococcus aureus* are found to be associated with suppurative, pyogenic and food poisoning disorders in human and animals (Das and Khanna, 1995). Now-a-days different kinds of antibiotics are indiscriminately used by quack and layman in incomplete course and doses, which ultimately enhance the emergence of new generation resistant organisms in animals. It is a very alarming situation both for human and animal kingdom. Antimicrobials have had an integral role in livestock production as well as in the treatment and prevention of bacterial infections in human and animals for many years. So isolation and then identification of micro-organisms are necessary for the use of antibiotic as treatment and preventive measure. Antibiotic sensitivity study is prime important in clinical management of ailing cases caused by various pathogenic organisms. Several attempts were directed to find out the causal agent of wound and their eradication by antibiotic therapy in man (Saha *et al*., 1995). Specific causal agents of other infectious diseases of animals were diagnosed (Lee and Kapatkin, 2002) and researches of non-traumatic infection conducted using this technique and effective result found in Bangladesh (Kamal *et al*., 2001; Taleb *et al*., 2001). But no such work has yet been carried out for the wound treatment in animals in Bangladesh. So the present work was undertaken to study the specific causal agents of wound complication and their sensitivity *in vitro* antibiotic therapy.

Materials and Methods

A total of 250 samples of pus, exudate and dirt materials from septic wound of patients attending in the Veterinary Clinic, Bangladesh Agricultural University, Mymensingh were collected. Instruments (scalpel, forceps, scissors, probe etc) and glasses ware (petridishes, conical flask, test tube) were thoroughly cleaned with soap water and with methylated spirit. When these were dried up then sterilized by autoclave. The surroundings of wounds were cleaned with 95% methylated spirit to prevent the mixing of organisms with surrounding ones. Pus and exudate were directly collected with cotton bud from the centre of

the wound and scratches materials were collected with the help of sterile scalpel and forceps. Then the cotton bud with swabs were dipped in sterile nutrient broth and kept in refrigerator at 4^0C. Afterwards the broth was incubated for 24 h at 37^0C.

Culture media and broth were prepared according to the method of Dey and Dey (1986). After mixing of culture media with distilled water, they were subjected to autoclave for half an hour. Thereafter the autoclaved liquid media were poured on the sterilized petri dishes with strict aseptic measure and in the laminar flow. After pouring of autoclaved media on plate and nutrient broth in conical flask were kept in room temperature. At this temperature, the media were solidified on the plate and broth (solution) in conical flask in room temperature. The media and the broth were placed in incubator for 24 h at 37^0C to check the growth of organisms on the plate and broth, if any. It was now ready for culture of bacteria and stored for a few days in refrigerator to avoid evaporation of moisture and extraneous contamination.

The swabs were seeded on blood agar and nutrient agar media and again incubated at 37^0C for 24 h. A single colony from the solid media was identified on the basis of morphology and cultural characters and was then transferred on to the same media for propagation and separation. Then the selective medium was used for inoculation of specific organisms and incubated for 24 h at 37^0C. Identification of the organism was made on the basis of colony characteristics and biochemical tests. The purity of the inoculums was tested by Gram staining of the culture broth as well as by colony characteristics of the selective medium.

Drug sensitivity of the isolates was conducted out to assess the extent of bacterial resistance and efficacy of treatment based on antibiotic sensitivity against 10 antibiotics using standard disc diffusion techniques. Pouring of specific organism densely and uniformly on blood agar and nutrient agar media. The plates were dried in the incubator until the surface was free from visible moisture. The antibiotic discs were then applied at adequate spacing (4 cm apart) to the surface of the plate with sterile fine pointed forceps and pressed gently to ensure full contact with the medium. The plates were incubated at 37^0C for 24 h and the diameter of the zone of inhibition of growth was measured as per the chart of the manufacturer. The organism was considered resistant to a particular drug when zone of inhibition was absent and sensitive when a clear zone around the disc was present.

Results and Discussion

Out of 250 cases 227 (90.8%) were found infected by microorganisms. Among the infected cases 85 (37.44%) were found mixed infection and 142 (62.55%) cases were single type infection. Multiple types of infection were found in recent and clean type of wound. Similar types of investigation carried out by other investigators (Ashraf, 1973; Aman, 1982; Saha *et al.*, 1995). This may be due to the rapid attack of wound by the normal inhabitant bacteria in skin. These organisms do not cause harm to the patient without any breakage in the body. Non-infected wounds were also found as these wounds were treated with antibiotic before the sample collection.

The infected wound samples were subjected to subculture and the specific organisms were isolated. The culture of wound specimens revealed that most of the isolates are *Staphylococcus aureus* 77 (32.92%), then the *E. coli* 43 (18.94%) and the least infection by *Streptococci* 21 (9.25%) infections (Table 1). This type of results had been correlated with the previous work of Pecnik and Butinar (2000) who described that *Staphylococcal* sp. and *Pseudomonas aeruginosa* were the most frequent isolates from the wound. Dog bite wound also represented the most common infection due to *Staphylococcal* sp. (Griffin and Holt, 2001). It is also reported that *Staphylococcus* organism is the most common etiological agent in case of chronic wound infection of ear (Pandey *et al.*, 1998). It may be due to the frequent presence of *Staphylococcus aureus* in skin and any damage to skin lead to wound also favour the organisms to grow and multiply. Similar statement also cited by Hazarika *et al.* (1991) who found that *Staphylococcus* organism is the most common isolates from the cutaneous wound. *E. coli* is an enteric organisms but also ubiquitous in distribution in other infection. It is one of the pus forming organisms in the wound. But some author replaced the Staphylococcus by *E.coli* as the principal causal agent of incidence in human being (Saha *et al.*, 1995).

In vitro drug sensitivity test revealed that all the organisms isolated from the wound were not equally sensitive or resistant to most antimicrobials (Table 2). Some were found highly sensitive and some totally resistant. Altogether 10 antibiotics were tested in the culture media by disc diffusion test. Sensitivity to individual antibiotic was varied and highest degrees of sensitivity were found in Cephalexin, Gentamycin and Streptomycin. The least antibiotics sensitive to bacterial agents were Penicillin and Sulphamethaxazole. Similar

Table 1: Incidence of bacterial infection in septic wounds of animals

Types of bacteria	Number of isolates	% of affection
Staphylococcus aureus	77	33.92
Escherichia coli	43	18.94
Kl. pneumoniae	38	16.74
Ps. aeruginosa	22	9.69
Proteus vulgaris	26	11.45
Streptococcus sp.	21	9.25

Table 2: Drug sensitivity pattern of bacterial isolates from septic wounds in animals

Bacteria (number)	TE (%)	CL (%)	N (%)	C (%)	AML (%)	AMP (%)	P (%)	CN (%)	SXT (%)	S (%)
Staphylococcus aureus (77)	32	62	72	75	32	35	15	77	12	77
	(41.55)	(80.51)	(93.50)	(97.40)	(40.25)	(45.45)	(19.48)	(100.0)	(15.58)	(100.0)
Escherichia coli (43)	17	20	39	40	18	16	15	43	8	41
	(39.53)	(46.51)	(90.69)	(93.02)	(41.86)	(37.20)	(34.88)	(100.0)	(18.60)	(95.34)
Klebsella pneumonie (38)	10	30	31	18	20	23	4	36	1	38
	(26.31)	(78.94)	(81.57)	(47.36)	(52.63)	(60.52)	(10.52)	(94.73)	(2.63)	(100.0)
Proteus vulgaris (26)	1	24	25	15	2	3	0	26	0	26
	(3.48)	(92.30)	(96.15)	(57.69)	(7.69)	(11.53)	(0)	(100.0)	(0)	(100.0)
Psedomonous aureginosa(22)	5	20	21	10	14	16	2	20	0	21
	(22.72)	(90.99)	(95.45)	(45.45)	(63.63)	(72.72)	(9.09)	(90.90)	(0)	(95.45)
Streptococcus Sp. (21)	15	21	20	9	17	15	0	20	2	18
	(71.42)	(100.0)	(95.23)	(42.85)	(80.95)	(71.42)	(0)	(95.23)	(9.52)	(85.7)

TE= Tetracycline, CL=Cephalexin, N=Neomycin, C=Chloramphenicol, AML=Amoxycillin, AMP= Ampicillin, P=Penicillin, CN=Gentamycin, SXT=Sulphamethaxazole, S=Streptomycin

results have also been described earlier (Hazarika *et al.*, 1991; Bajpai *et al.*, 1999; Malik *et al.*, 2000). Penicillin was the first antibiotic discovered for the treatment of bacterial infection and its indiscriminate use causes emergence of resistant organisms. Currently some important organisms are developing resistance rapidly, including those that cause skin and bloodstream infections, *S. aureus* (Torrence, 2001). Though the resistance varies among geographic regions and within communities, up to 30% of *S. pneumonie* has been found in some case to get resistant to penicillin. Most of the strains of *S. aureus* in the United States are resistant to Penicillin (Panlilio *et al.*, 1992). In our study most of the organisms were relatively resistant to sulphamethazone, amoxycillin, ampicillin, penicillin and tetracyclin. Similar results also stated by Hazarika *et al.* (1990) that the *Staphylococcus* organisms were highly sensitive to most of the antibiotics but less sensitive to streptomycin and neomycin. Although laboratory culture should not be expected to provide a diagnosis of infection, they merely guide the treatment of an infected patient, revealing the presence of organisms resistant to a particular antibiotic. So the treatment of wound should be done after culture and sensitivity test of the affection. In filed condition where facilities are absent for cultural and sensitivity tests, gentamycin, neomycin, cephalexin and chloramphenicol should be used instead of penicillin and sulphur drug.

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