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PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSI*net*

Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Bacteriological Studies on Surgical and Non-surgical Wounds Located on Body Surface of Animals

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Abstract: Eleven different species of bacteria were identified from surgical and non-surgical wound samples located on body surface of buffaloes, cattle, sheep and goats. The species were: *Streptococcus pyogenes*, *Streptococcus uberis*, *Staphylococcus aureus*, *Staphylococcus intermedius*, *Corynebacterium diphtheriae*, *Corynebacterium pyogenes*, *Escherichia coli*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Micrococcus luteus* and *Stomatococcus mucilaginosus*. Of the 100 samples studied, 15 were from buffaloes, 30 from cattle, 29 from sheep and 26 from goats and the percentage prevalence of infection in wound samples of the above animals was 93.3, 90, 100 and 100 respectively. The incidence of bacterial species in opened and un-opened wounds was demonstrated. The incidence of bacterial infection in opened wounds of buffaloes and cattle was 92.85 and 86.36% respectively and in sheep and goats was 100%. The incidence of infection in un-opened wounds of the above four species of animals observed as 100%. A survey on mixed infection was also carried-out during the present study. The mixed infections in samples of buffaloes, cattle, sheep and goats were common and recorded as 80, 90, 86.20 and 80.76% respectively. The occurrence of individual bacterial species in wound samples were also investigated in this study. The frequency of incidence of *Strept. pyogenes*, *Strept. uberis*, *Staph. aureus*, *Staph. intermedius*, *Coryn. diphtheriae*, *Coryn. pyogenes*, *E. coli*, *Prot. vulgaris*, *Pseud. aeruginosa*, *Micro. luteus* and *Stomat. mucilaginosus* in wounds of animals was 44.19, 16.66, 17.70, 10.4, 17.70, 13.54, 31.25, 8.3, 15.6, 41.66 and 6.25% respectively. Four of the 100 samples examined were found to be organisms free while pure infections were found in only 15. However, mixed infections 2-4 species were recorded in individual samples. While in a single sample 4 different species were detected. The wound samples examined in our study contained 1(15%), 2(49%), 3(31%) and 4(1%) species.

Key words: Bacteriological, surgical, non-surgical, wounds, body surface, animals

Introduction

All kinds of wounds on skins and hides usually occur due to breach with very sharp instruments, imperfect brand marks, rubbing against coarse surfaces or incisions made by doctors during surgical operations. Breach in skins and hides, various kinds of wounds and injuries get contaminated by a big population of bacterial flora such as the pyogenic bacteria especially, *Streptococcus pyogenes*, *Staphylococcus aureus* and the *Coliform* group together with *Proteus vulgaris* and *Pseudomonas pyocyanea* and the spore forming anaerobes viz. *Clostridium welchii* type A, *Clostridium septique* and *Clostridium diphtheriae*, which lead further damages to the qualities of leather which reflect in sense of losses to the leather industry and ultimately economy of this country. All kinds of abscesses on skins and hides are the main cause of organisms as well as parasites and become reason of contamination (Saini *et al.*, 1992).

The bacterial organisms which cause wounds on skin and hide of domestic animals have been investigated throughout the world (El-Sanousi *et al.*, 1989; Peregudon *et al.*, 1985). As far as the animals of Sindh province are concerned, no such type of study has been carried-out on identification, prevalence and Incidence of bacterial organisms in wounds of animals. The aim of the present investigation was planned to recognize bacterial species alongwith their prevalence and incidence in wounds. The identification of bacterial flora are also of great importance that they will provide proper guideline to the veterinarians in treating the wounds of different nature present on skin and hide of domestic as well as wild animals.

Materials and Methods

One hundred samples were collected during 1999 from surgical and non-surgical wounds located on the skins and hides of sheep, goats cattle and buffaloes respectively. The samples were collected from Veterinary hospital Sindh Agriculture University, Tando Jam, Civil Veterinary hospital Hyderabad and Red Sindhi Cattle Breeding Farm Tando Mohammed Khan. Wound samples were processed and examined at Central Veterinary Diagnostic Laboratory Tando Jam for isolation and identification of bacterial species.

The specimens were collected by different means:

1. Direct blood from wounds.
2. Pus from wounds by cotton swabs
3. Pus from subcutaneous lesions or abscesses

For collection of the above specimens different sterile measures were taken:

Blood samples: The surrounding of severely injured wounds of animals were cleaned and blood was taken into sterile bijoux bottle.

Pus from wounds by cotton swabs: Before collection, wounds and cutaneous lesions were properly cleaned with antiseptic (spirit) and then swabs were taken by removal of extraneous contaminant organisms.

Pus samples from subcut lesions or abscesses: The surface of abscesses were cleaned carefully with antiseptics (spirit) and then incision was made and samples collected into bijoux bottle as well as by cotton swabs.

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Before processing the wound samples, new as well as used glassware such as Petri dishes, pipettes and flasks etc were kept in 1% HCl solution overnight and washed well with distilled water for several times then dried in oven at 65°C. After that sterilization was carried-out in hot air oven at 180°C for one and half an hour.

The media were prepared and used for detailed investigation of bacterial organisms as described by Cruickshank (1968). Both the media, solid and liquid were used. In solid: nutrient, blood and MacConkey's agars and while in broth: nutrient broth was prepared, cultured and colony characteristics were recorded. A pure colony from cultured dishes was picked-up and smeared on a cleaned glass slide and stained by Gram's method of staining and morphological characteristics of the bacterial organisms were observed.

A few biochemical tests were also carried-out to confirm their specific chemical properties. For this purpose, oxidase, coagulase, indole, Voges Proskauer, urease, methyl red, gelatin liquefaction, Simmon's citrate, H₂S production, catalase and TSI tests were conducted (Cruickshank, 1970).

For sugar fermentation properties, eight different sugars of 1% were prepared and used for each isolated bacterium as described by Cruickshank (1970). The sugars were glucose, sucrose, lactose, maltose, mannitol, inositol, arabinose and raffinose.

Results and Discussion

The prevalence of positive wound samples infected with bacterial species of domestic animals are presented in Table 1. Of the 100 samples, 15 from buffaloes, 30 from cattle, 29 from sheep and 26 from goats were collected and examined. The prevalence of positive samples in buffaloes, cattle, sheep and goats recorded during the present survey was 93.3, 90, 100 and 100%, respectively. While the negative samples in buffaloes, cattle, sheep and goats was 6.6, 10, 0 and 0% respectively. The highest prevalence, however was recorded in sheep and goats which indicates that both the species are very prone to bacterial organisms. This might be due to immune response to different organisms from host species.

A similar findings regarding prevalence of micro-organisms in wounds of sheep and goats were recorded by Tadayon *et al.* (1980) who reported 52 and 48% prevalence in above animals. Khan and Barya (1970) carried-out an investigation on wounds of cattle and buffaloes in Pakistan and reported 50% in both the species. It is clear from this study that our results about the percentage prevalence of bacterial species in wounds of domestic animals are in line with the findings of above workers, but we recorded somewhat higher prevalence as compared the above workers. This whole might be due to environmental factor.

The bacterial species identified from surgical and non-surgical wounds of different animals during this survey are given in Table 2. The bacterial organisms recognized from wound samples of buffaloes were *Strept. pyogenes* (4), *Strept. uberis* (3), *Staph. aureus* (3), *Staph. intermedius* (4), *E. coli* (3), *Pseud. aeruginosa* (20), *Micro. luteus* (8), and *Stomatmucllaginosu.s* (2) and their prevalence in samples were 26.6, 20, 20, 26.6, 20, 13.3, 53.3 and 13.3% respectively. Whereas in cattle, a total of 60 bacterial flora were detected. The species identified from wounds were *Strept. pyogenes* (20), *Strept uberis* (6), *Staph. aureus* (1), *Staph. intermedius* (2), *Coryn. pyogenes* (6), *E. coli* (8), *Micro. luteus* (16) and *Stomat. mucilaginosus* (1) and their prevalence was 66.6, 20, 3.3, 6.6, 20, 26.6, 53.3 and 3.3% respectively.

While in sheep, a total of 68 bacterial flora were recognized from 29 samples examined (Table 2). The species were *Strept. pyogenes* (11), *Strept. uberis* (2), *Staph. aureus* (7), *Staph. intermedius* (4), *Coryn. diphtheriae* (11), *Coryn. pyogenes* (2), *E. coli* (9), *Prot. vulgaris* (5), *Pseud. aeruginosa* (8), *Micro. luteus* (8) and *Stomat. mucilaginosus* (1) and the prevalence of each species recorded during the study was 37.9, 6.8, 24.1, 13.7, 37.9, 6.8, 31, 17.2, 27.5 and 3.4% respectively.

In goats, 58 bacterial isolates were isolated and mentioned in Table 2. The species recognized from samples were *Strept. pyogenes* (8), *Strept. uberis* (5), *Staph. aureus* (6), *Coryn. diphtheriae* (6), *Coryn. pyogenes* (5), *E. coli* (10), *Prot. vulgaris* (3), *Pseud. aeruginosa* (5), *Micro. luteus* (8) and *Stomat. mucilaginosus* (2). The percentage prevalence of the above organisms in wounds of goats was 30.7, 19.2, 23.0, 23, 19.2, 38.4, 11.5, 19.2, 30.7 and 7.6 respectively.

It is concluded from present investigation that the wounds of all animal species are commonly infected with *Micro. luteus*, *Strept. pyogenes*, *E. coli* and *Staph. aureus*. It is further concluded that wounds of small animals (sheep and goat) get high level of contamination of a large numbers of bacterial organisms.

The species identified and their percentage prevalence in wounds and abscesses of different animal species demonstrated during the current study (Table 2) were also recognized from wound samples of animals by various workers throughout world (Tadayon *et al.*, 1980; Dinev *et al.*, 1987; Awad-Masalmeh *et al.*, 1988; Gezon *et al.*, 1991; Saini *et al.*, 1992; Kelly *et al.*, 1992). Khan and Barya (1970) who isolated/identified more or less same bacterial species from the wound samples of cattle and buffaloes. The species recognized were *Staph. aureus*, *Strept. pyogenes*, *E. coli*, *Pseud. aeruginosa*, *Prot. vulgaris* and *Coryn. diphtheriae*. Similar bacterial species were also identified from lamb and sheep. They detected *Staph. aureus*, *Staph. intermedius*, *E. coli*, *Coryn. pyogenes* and *Streptococci* species are also recognized during this study from various species of domestic animals. On the other hand, Kelly *et al.* (1992) investigated 87 wounds from humans due to dog bites in Zimbabwe and identified *Staph. intermedius* and *E. coli* and their prevalence in samples were 23 and 18% respectively were also similar noted in the present study from cattle, buffaloes, sheep and goats. Nevertheless, it was very difficult to compare these results to other workers because they did not mention the percentage prevalence of species in different animals which were recorded in the present survey. However, Love (1989) examined samples from canine lesions and observed 97.4% *Staphylococci* species from which *Staph. intermedius* was 85.8% and *Staph. aureus* 11.5%.

The incidence of opened and un-opened wounds contaminated with bacterial species are demonstrated in Table 3. Of the 15 samples, 14 opened and 1 un-opened wound samples from buffaloes were collected and examined. Among 14 samples of opened wounds, 13 were found positive and while 1 was negative. Only 1 sample of un-opened wound was studied and found positive. The percentage incidence in both the cases, opened and un-opened wounds was 92.85 and 100 respectively. Only one sample of opened wound was examined and recorded as 7.14%.

In cattle, 22 opened wound samples were examined, of which 19 were found positive and recorded as 86.36% whereas 3 samples of opened wound were found negative and observed

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Table 1: The number and percentage of wounds infected with bacterial organisms during study period

Animal samples	Total No. of wound samples	No. of positive samples	%age of positive samples	No. of negative samples	%age of negative
Buffaloes	15	14	93.3	1	6.6
Cattle	30	27	90	3	10
Sheep	29	29	100	0	0
Goats	26	26	100	0	0

Table 2: The percentage incidence of individual bacterial species in wound samples of domestic animals

Bacterial species		Buffaloes	Cattle	Sheep	Goats
<i>Strap.</i>	No.	4	20	11	8
<i>Pyogenes</i>	%	26.6	66.6	37.9	30.7
<i>Strap.</i>	No.	3	6	2	5
<i>Uberis</i>	%	20	20	6.8	19.2
<i>Staph.</i>	No.	3	1	7	6
<i>Aureus</i>	96	20.0	3.3	24.1	23.0
<i>Staph</i>	No.	4	2	4	-
<i>Intermedius</i>	96	26.6	6.6	13.7	-
<i>Coryn.</i>	No.	-	-	11	6
<i>Diphtheriae</i>	%	-	-	37.9	23
<i>Coryn</i>	No.	-	6	2	6
<i>Pyogenes</i>	%	-	20	6.8	19.2
<i>E. coli</i>	No.	3	8	9	10
	%	20.0	26.6	31.0	38.4
<i>Proteus.</i>	No.	-	-	5	3
<i>Vulgaris</i>	%	-	-	17.2	11.5
<i>Pseud.</i>	No.	2	-	8	5
<i>Aeruginosa</i>	%	13.3	-	27.5	19.2
<i>Micro.</i>	No.	8	16	8	8
<i>Luteus</i>	%	53.3	53.3	27.5	30.7
<i>Stomat</i>	No.	2	1	1	2
<i>Mucilag</i>	96	13.3	3.3	3.4	7.6

Table 3: The number and percentage of opened and un-opened (abscess) wound samples contaminated with different species of bacterial organisms

	Animal species			
	Buffaloes	Cattle	Sheep	Goats
Opened wounds				
Total No. of wound samples examined	14	22	15	16
No. of positive samples	13	19	15	16
% of positive samples	92.85	86.36	100	100
No. of negative samples	1	3	0	0
% of negative samples	7.14	13.63	0	0
Un-opened wounds (Abscesses)				
Total No. of wounds examined	1	8	14	10
No. of positive samples	1	8	14	10
96 of positive samples	100	100	100	100
No. of negative samples	0	0	0	0
96 of negative samples	0	0	0	0

as 13.63%. In the case of un-opened wounds, a total of 8 samples were examined and all were positive with incidence of 100%.

A similar kind of investigation was carried-out on wound samples of sheep and goats (Table 3). A total of 15 and 16 opened wound samples from sheep and goats respectively were collected and investigated. All the samples of both the species were positive and incidence recorded as 100%. While in the case of un-opened wounds, 14 and 10 samples were collected from sheep and goats respectively and incidence was recorded as 100%.

Again it is concluded from the present study that un-opened wounds are caused mostly by bacterial flora whereas opened wounds also got infected by microorganisms. During this investigation, it was found that all un-opened wounds of all the

animals were infected with bacterial species.

During this study, wounds were categorized into two, one opened and the other un-opened. The un-opened wounds were considered as abscesses or subcut lesions. In scientific literature the term un-opened has not been used before for abscess. Therefore, we considered here un-opened wounds as abscesses. The reason was to make easy comparison between the present results and the findings of other authors.

A total of 33 un-opened wound samples were collected from all four classes of domestic animals and examined. All un-opened wounds were detected as positive (Table 3) and while from 67 opened wounds, 63 were found infected with different species of bacteria. The species and the incidence of infection in abscesses noted during this investigation are very close to Tadayon *et al.* (1980) who reported 100% incidence in 86 samples collected from

Table 4: The number and percentage of pure and mixed infections in wound samples of different animals

	Animal species			
	Buffaloes	Cattle	Sheep	Goats
Total No of wounds samples examined	15	30	29	26
% of positive samples	93.3	90	100	100
No. of pure samples	3	3	4	5
96 of pure samples	20	10	13.79	19.2
No. of mixed samples	12	27	25	21
96 of mixed samples	80	90	86.2	80.76

Table 5: The percentage incidence of individual bacterial species in wound samples of domestic animals (100, positive samples 96)

Bacterial species	Individual bacterial Species occurring	% of individual bacterium
<i>Streptococcus pyogenes</i>	43	44.79
<i>Streptococcus uberis</i>	16	16.66
<i>Staphylococcus aureus</i>	17	17.70
<i>Staphylococcus Intermedius</i>	10	10.40
<i>Corynebacterium diphtheriae</i>	17	17.70
<i>Corynebacterium pyogenes</i>	13	13.54
<i>Escherichia coli</i>	30	31.25
<i>Proteus vulgaris</i>	8	8.33
<i>Pseudomonas aeruginosa</i>	15	15.62
<i>Micrococcus luteus</i>	40	41.66
<i>Stomatococcus mucilaginosus</i>	6	6.25

Table 6: Number of species of bacteria occurring in individual wound samples of domestic animals

	0	1	2	3	4
No. of species present in samples					
No. of samples occurring in	4	15	49	31	1
96 of total samples	4.0	15.0	49.0	31.0	1.0

45 sheep and 41 goats. They further mentioned that no abscess was free from bacterial contamination. From these findings, it is obvious that all abscesses are being caused by

bacteria. Therefore, the results obtained in this study about un-opened wounds are in agreement with the findings reported by above workers. However, our data about percentage incidence of opened wounds infected with microorganisms are in line with the reports of other authors (Zeiller, 1966; Gezon *et al.*, 1991; Goldstein, 1992; Cruickshank, 1940) for small and large animals. The bacterial organisms recognized during this survey are more or less same as detected by the above workers.

The percentage incidence of mixed infections in wound samples of different animal species are summarized in Table 4. All 15 wound samples were examined from buffaloes, among these samples, 3 were pure infection and 12 were mixed and incidence was 20 and 80% respectively.

Thirty samples were collected from cattle and examined, here 3 were pure and 27 were mixed and recorded as 10 and 90% respectively. In case of sheep, 29 samples were processed and only 4 were found pure and 25 were of mixed infections and the percentage incidence in the samples was 13.79 and 86.20 respectively. At the same time, twenty six samples were collected from goats, 5 were with pure infection and 21 were mixed infections. The percentage incidence observed during this survey of pure and mixed organisms was 19.2 and 80.76 respectively.

It is again concluded that mixed infections are always present in the wound samples. The incidence of mixed infections in wound samples of animals observed during this survey which ranged from 80-90% in different animals and the number of species present in individual samples from 2-4 (Table 4) are in agreement to that of Talan *et al.* (1989) and Tadayon *et al.* (1980), they recorded about 60% wounds with mixed infections in small and large animals. The number of species encountered in a single sample in this study is also counted by the above workers. They counted 2-4 different species in a wound sample of sheep, goats and dogs. The information recorded about the pure infection in the current survey similar to the results of Tadayon *et al.* (1980) who determined a similar percentage of incidence in their study.

The number and percentage incidence of individual bacterial species isolated and recognized from various wound samples of animals during present investigation are given in Table 5. Eleven different species of bacterial organisms were recorded from wound samples of buffaloes, cattle, sheep and goats. The incidence of individual species in different wound samples was recorded in this survey.

The species were *Strept. pyogenes*, *Strept. uberis*, *Staph. aureus*, *Staph. intermedius*, *Coryn. diphtheriae*, *Coryn. pyogenes*, *E. coli*, *Prat. vulgaris*, *Pseud. aeruginosa*, *Micro. luteus* and *Stomat. mucilaginosus* and occurred in 43, 16, 17, 10, 17, 13, 30, 8, 15, 40 and 6 samples respectively. The percentage incidence of the above species was 44.19, 16.66, 17.70, 10.4, 17.7, 13.45, 31.25, 8.3, 15.6, 41.66 and 6.25 respectively. The high incidence of bacterial species in the wound samples of animals were found *Micro. luteus* and *Strept. pyogenes*.

All eleven species encountered from wound samples of buffaloes, cattle, sheep and goats during present investigation were recognized by Khan and Barya (1970) and Cruickshank (1940) who recorded six different species from wounds of large and small animals. Moreover, we found high incidence of *Strept. pyogenes* infection in samples compared to other species (Table 5). Awad-Masalmeh *et al.* (1988) reported 92% *Staphylococci* species infection in skin lesions of dogs and while *Streptococci* species in 22% samples. Therefore, the results about incidence of individual organism in samples agree with the reports of Awad-Masalmeh *et al.* (1988), but the findings regarding the incidence of single species in samples of the present study do not agree with the results of Talan *et al.*

(1989) who observed somewhat high incidence of bacterial species in their investigation. Furthermore, it was very difficult to compare the findings of the present investigation to the other workers because we could not find any information in the available literature.

The number and percentage incidence of above species present in individual wound samples are presented in Table 6. Of the 100 samples examined, 4 were found to be organisms free while pure infections were found in only 15. However, mixed infections 2-4 species were recorded in individual samples. While in a single sample 4 different species were detected. The present investigation reveals that mixed infections were common in wound samples of domestic animals. The number of species detected in any single wound sample ranged from 2-4 in the current investigation which agrees to that of Tadayon *et al.* (1980), Talan *et al.* (1989) who found 2-4 different species in a single sample. The wound samples examined in our study contained 1 (15%), 2 (49%), 3 (31%) and 4 (1%) species. A similar trend was encountered by Tadayon *et al.* (1980) who recorded 29.06% mixed infections that contained 2-4 different species. They further stated that mixed infections are common in wound samples.

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