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PJBS

ISSN 1028-8880

# Pakistan Journal of Biological Sciences

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

Pakistan Journal of Biological Sciences 4 (6): 696-702, 2001


# Isolation and Characterization of Bacterial Species from Surgical and Non-surgical Wounds Located on Body Surface of Buffaloes, Cattles, Sheep and Goats

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**Abstract:** Bacteriae were isolated and characterized on biomorphological variations into eleven different species, (*Streptococcus pyogenes, S. uberis, Staphylococcus aureus, S. intermedius, Corynebacterium diphtheriae C. pyogenes, Escherichia coli, Proteus vulgaris, Pseudomonas aeruginosa, Micrococcus luteus* and *Stomatococcus mucilaginosus*). They varied from cocci to rod shape and were gram-positive or gram-negative. Organisms produced a variety of colonies on different media. Some were spherical, swarming and spreading colonies on agar media while in broth, granular turbidity with powdery deposits were also seen. A few species produced hemolysis (α and β) of red blood cells. Biochemical activities of different organisms revealed that among two similar species of *Streptococcus, S. pyogenes* had not hydrolyzed aesculin but *S. uberis* had hydrolyzed aesculin. The other difference between two was that the first produced ß hemolysis while the second produced α. *Staphylococcus aureus* was coagulase positive, while *S. intermedius* was not. However, other two species *Corynebacterium diphtheriae* and *C. pyogenes* were also biochemically tested. The first had not liquefied gelatin while the other had.
**Key words:** Isolation, characterization, bacterial species, wounds, animals

## Introduction

Bio-morphological variations in the characteristics of bacteria that cause wound infections are described by Bergey's (1992) and Shaikh (1999). In addition, several workers have described the bacterial species bio-morphological characteristics throughout the world (Dinev *et al.*, 1987; Talan *et al.*, 1989). This means biochemical and morphological characteristics should make a reliable source for isolation and identification of species independent of their bio-morphological, and antigenic characteristics. The bio-morphological characteristics demonstrated here, however, are presented on the investigation of naturally infected wounds by bacterial species in animals.

## Materials and Methods

About one hundred samples were collected during 1999 from surgical and non-surgical wounds of buffaloes, cattle, sheep and goats. The samples were obtained from the above animals, brought from various places to Veterinary Hospitals for their wound treatment. All samples were processed and examined at Central Veterinary Diagnostic Laboratory, Tando Jam, Sindh, Pakistan for isolation and identification of bacterial organisms.

The surroundings of the injured, surgically operated and abscesses were cleaned with antiseptic (spirit) and then swabs were obtained by removal of extraneous contaminant organisms. While in case of abscesses, the surface of abscesses was cleaned by antiseptic and then incision was made and samples were collected into tubes and also by cotton swabs.

Before processing the samples, all preparations were made as described in Bergey's (1992). The media to be needed for proper cultivation and identification of bacterial organisms were prepared, inoculated and identification characteristics whether of physical, cultural, biochemical and morphological were recorded as adopted by Cruickshank (1968).

A biochemical tests were conducted to confirm the identification of bacterial organisms, for this purpose, oxidase, coagulase, indole, Voges Proskauer, urease, methyl red, gelatin liquefaction, Simmon's citrate, $H_2S$ production, catalase and TSI tests were carried-out (Difco, 1960) while for sugar fermenting properties, eight different sugars of 1% were prepared and used for each bacterium as demonstrated by Cruickshank (1970). The sugars were: glucose, sucrose lactose, maltose, mannitol, inositol, arabinose and raffinose.

## Results

During present investigation, morphological, cultural, staining and chemical characteristics of various bacterial species of wounds of animals were recorded and presented in different Tables and Figures.

***Streptococcus pyogenes* and *uberis***: The organisms of both the species *Streptococcus pyogenes* and *Streptococcus uberis* were in cocci form, measured from 0.5-0.75$\mu$ in diameter and were arranged in pairs or chains. In broth culture, the chains were noted of varying lengths. They were non-motile and non-spore forming, gram-positive and facultative anaerobic. On blood agar plates, both the species, produced a zone of α and ß hemolysis around colonies respectively. In broth, a moderate growth with granular turbidity and powdery deposits was noted (Tables 1, 2 and Fig.1 and 2; Fig.2, 1 and 2).

***Staphylococcus intermedius* and *aureus:*** *Staphylococcus aureus* and *Staphylococcus intermedius* were measured 0.8-1.0$\mu$ in diameter and were present singly/pairs, short chains and sometimes were observed in irregular clumps. They were found non-motile and gram positive. On agar plates, colonies were opaque to golden yellow in colour, glistening, smooth and in circular form. On blood agar, they produced beta hemolysis. Whereas on MacConkey's agar, pinkish colonies with about 0.5mm in size were noted (Tables 1, 2; Fig.1, 3 and 4; Fig.2, 3).

***Corynebacterium pyogenes* and *diphtheriae***: The morphological and cultural characteristics of *Corynebacterium pyogenes* and *diphtheriae* were recorded and presented in Tables 1, 2; Fig.1, 5 and 10; Fig. 2, 4 and 5. The organisms of the above species were rod shaped with 0.38-1.0$\mu$ in size. They always occurred singly and frequently swollen at one or both ends. They were gram-positive and usually did not stain uniformly and were non-motile. On nutrient agar, grayish white colonies of 1-5mm diameter were recorded. *Corynebacterium diphtheriae* organisms were aerobic, facultative anaerobic. On MacConkey's agar, produced disk like pin point opaque and white colonies while on blood agar, beta hemolysis was observed.

***Escherichia coli***: *Escherichia coli* were rod shaped with 0.5x1.0 to 3.0$\mu$ size. Almost present in cocci form with long rods, always occurred singly but sometimes in pairs and occasionally in short chains. They were motile and non-spore forming, gram-negative and non acid-fast (Tables 1, 2 and Fig.1, 6; Fig.2, 6). On nutrient agar, circular, smooth, white colonies and some times yellowish white colonies of 1-3mm diameter were recorded. They were non-hemolytic and on MacConkey's agar, *Escherichia coli* gave circular, smooth and convex colonies with pink colour.

***Proteus vulgaris:*** Isolates of *Proteus vulgaris* were straight or slightly curved in shape by 1.0-2.5X0.4-0.6$\mu$ in size arranged singly/pairs and short chains in fresh culture, long filamentous form was common. Stained negatively with numerous peritrichous flagella. They produced spreading colonies with slightly raised layer of growth with an ammonical odour. On blood plates, uniform growth was noted. Whereas on MacConkey's medium plates, it produced colourless discrete or partly confluent colonies (Tables 1,2 and Fig.1, 7).

***Pseudomonas aeruginosa***: Rod shaped, 0.5-0.6x1.5$\mu$ size. They were always in pairs and or in short chains. They were motile, possessed one to three polar flagella. On nutrient agar medium, 1-2mm in diameter colonies with spreading moist, greyish, dark centre in colour and translucent edges were observed. In broth, abundant growth with dense turbidity, yellowish green colonies, non-spore forming, gram-negative and non acid-fast characteristics were recorded. A similar growth pattern on agar was also noted (Tables 1 and 2; Fig.1, 9).

***Micrococcus luteus***: Mostly present in irregular clusters. They were gram-positive. On nutrient agar plates, *Micrococcus luteus* formed opaque, butyrous colonies with white-yellow pigment. Various shades of red or orange were also common. The organisms were non-spore forming and non-motile in nature (Tables 1,2; Fig.1, 8).

***Stomatococcus mucilaginosus***: The organisms of this specie were similar to that of *Streptococcus*. They were arranged in pairs or singly. In broth cultures, they were present singly and in pairs (Tables 1, 2; Fig.1, 11) and produced a powdery deposit. They were non-motile, non-spore forming, gram-positive and non acid-fast, aerobic and facultative anaerobic. Whereas on blood agar plates, colonies were non-hemolytic.

The biochemical reactions (activities) of various bacterial species from surgical and non-surgical wounds of buffaloes, cattle, sheep and goats have been studied and presented in Table 3 and sugar fermentation properties also presented in Table 4.

***Streptococcus pyogenes* and *uberis***: Both the species, *Streptococcus pyogenes* and *Streptococcus uberis* fermented glucose, maltose, sucrose, and mannitol and failed to ferment lactose, arabinose, raffinose and inositol. They did not produce catalase reaction. They were also negative for $H_2S$ production and triple sugar iron. Organisms formed chain in liquid medium. *Streptococcus pyogenes* identified during the present study, did not hydrolyze aesculin, while *Streptococcus uberis* did. (Tables 3, 4 and Fig.3, a. $a_2$; c. $c_1$, $c_2$).

***Staphylococcus aureus* and *intermedius***: *Staphylococcus aureus* and *Staphylococcus intermedius* fermented glucose, maltose, sucrose, mannitol and lactose but did not ferment arabinose, raffinose and inositol. Both the species were positive for catalase. Triple sugar iron test was A/A (Acid butt and Acid slant) and $H_2S$ production was negative. They did liquefy gelatin whereas only the species *Staphylococcus aureus* was coagulase positive and *Staphylococcus intermedius* was negative (Tables 3, 4 and Fig.3, b. $b_1$; d. $d_2$; h. $h_2$ and i).

***Corynebacterium pyogenes and diphtheriae*:** During this study biochemical tests were carried-out and their results are given in Tables 3 and 4. Glucose, maltose and sucrose were fermented while failed to react with mannitol, lactose, arabinose, raffinose and inositol. They were negative for Voges Proskauer reaction, indole production, citrate utilization, $H_2S$ production and urea decomposition. They were positive for catalase and negative for oxidase reactions. The organisms of *Corynebacterium* species were failed to react with TSI, whereas *Corynebacterium diphtheriae* did not liquefy gelatin, and positive for methyl red reaction, while *Corynebacterium pyogenes* did liquefy gelatin and negative for methyl red reaction.

***Escherichia coli:*** The species produced both acid and gas from glucose, maltose, mannitol, arabinose but they only produced acid from sucrose and lactose. Where raffinose and inositol were not fermented. Positive results were recorded for methyl red, indole and catalase reactions. They were negative for Voges Proskauer, oxidase and citrate utilization, hydrogen sulphide productions (Tables 3, 4 and Fig.3, a. $a_3$; k. $k_1$; m. $m_1$; n. $n_1$ and o) and gelatin liquefaction and TSI (triple sugar iron) test was A/A (Acid butt acid slant).

***Proteus vulgaris:*** Organisms fermented glucose, maltose, sucrose and also produced gas during fermentation. Arabinose was fermented by them but mannitol, lactose, raffinose and inositol were not. They were positive for indole production, citrate utilization and gelatin liquefaction. They were negative for Voges Proskauer, methyl red, catalase, oxidase reactions and urea hydrolysis. They did produce $H_2S$ and triple sugar iron test was A/A (Tables 3, 4 and Fig.3, b. $b_2$; g and l. $l_1$).

Table 1: Morphological and staining characteristics of bacterial species identified from wounds of domestic animals

| Bacterial species | Shape | Arrangement | Motility | Staining reaction |
|---|---|---|---|---|
| *Streptococcusp yogenes* | Spherical or circular | Pairs/pairs | Non-motile | G+ |
| *Streptococcus uberis* | Cocci | Pairs/chains | Non-motile | G+ |
| *Staphylococcus aureus* | Cocci | Pair, tetrads or clusters | Non-motile | G+ |
| *Staphylococcus intermedius* | Spherical or circular | Pairs, tetrads or clusters or irregular clusters. | Non-motile | G+ |
| *Corynebacterium diphtheriae* | Rods | Singly | Non-motile | G+ |
| *Corynebacterium pyogenes* | Rods | Club shaped or arranged in palisades. | Non-motile | G+ |
| *Escherichia coli* | Rods | Singly/pairs | Motile | G⁻ |
| *Proteus vulgaris* | Rods | Singly or in pairs or in short chains. | Motile | G+ |
| *Pseudomonas aeruginosa* | Rods | Singly/in pairs or in short chains | Motile | G⁻ |
| *Micrococcus luteus* | Cocci | Tetrads and irregular clusters. | Non-motile | G+ |
| *Stomatococcus mucilaginosus* | Spherical or circular | Singly | Non-motile | G+ |

Table 2: Cultural characteristics of various bacterial organisms isolated from wound samples of domestic animals

| Bacterial species | Colony characteristics | | |
|---|---|---|---|
| | Solid medium | | Broth medium |
| | Shape | Colour | Shape |
| *Strept.pyogenes* | Circular, discrete,transparent, dew drop like and while on blood agar produced ß hemolysis of red blood cells | - | In broth granular turbidity and powdery. deposits with short chains or moderately long chains. |
| *Strept.uberis* | Circular, discrete transparent, dew drop like colonies on N/A, but on blood agar, alpha hemolytic were recorded. | - | In broth granular turbidity and powdery deposits with short or moderately long chains. |
| *Staph.aureus* | On N/A, *Staphylococcus aureus* produced rough, shiny, circular and convex colonies. It also produced beta hemolytic colonies on B/A, whereas on M/A, it produced small colonies. | White golden and yellowish white. Pink or pinkish. | In broth, uniform turbidity was recorded. |
| *Staph.intermed.* | On N/A, it produced convex and entire smooth colonies, where on B/A plates, beta hemolytic colonies were observed. | Grey white. | Sediment and pellicle. |
| *Coryn.diphth.* | On N/A, colonies were disk like, pin point and opaque and on B/A plates, beta hemolytic colonies were seen. | Grayish white. | - |
| *Coryn.pyogenes* | On N/A, it produced dew drop like, dry opaque and while on M/A, small round colonies were produced. On B/A, ß hemolytic colonies were recorded. | White and grayish- white | - |
| *E.coli* | On N/A, colonies were large, opaque, smooth and low convex. On M/A, colonies were circular, smooth and convex. Whereas on B/A no hemolysis were seen. | Grey and yellowish- white and red on B/A. | - |
| *Prot.vulgaris* | *Proteus vulgaris* produced slightly raised layer of spreading with an ammoniacal odor colonies on N/A. Colonies were discrete or partly confluent on M/A. On B/A plates, uniform growth over whole of the surface with indistinct single colony with non -hemolytic character. | Colourless. | - |
| *Pseud.aerugin.* | *Pseud.aeruginosa* showed large, spreading, moist, translucent edges and irregular colonies on N/A. While on M/A, colonies were spreading moist , large, translucent and irregular. Hemolytic colonies were seen on B/A. | Grayish with dark centre and pale or yellowish pale on M/A. | - |
| *Micro.luteus* | On N/A, colonies were circular, entire convex and on M/A, colonies were opaque and round. Non-hemolytic colonies were also seen on B/A. | - | - |
| *Stomat.mucilag.* | On N/A, colonies were mucoid, translucent, adherent with moist surface But on M/A, colonies were round, entire convex, mucoid and translucent. Non-hemolytic colonies were observed on blood agar. | Whitish on N/A and white on M/A. | In N/B, uniform turbidity with no chain. |

N/A=Nutrient agar M/A =MacConkey's agar B/A=Blood agar N/B=Nutrient agar

Table 3: Biochemical properties of bacterial organisms recognized from wound samples of domestic animals

| Bacterial species | Simmon's citrate | Methyl red | Voges Proskauer | Indole | Hydrogen sulphide | Gelatin liquefact. | Cata-lase | Oxidase | Coagu-lase | TSI | Haemo-lysis | Aesculin | Chain in broth | Urease |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Strept pyogenes* | - | - | - | - | -ve | - | -ve | - | - | -ve | β | -ve | +ve | - |
| *Strept. uberis* | - | - | - | - | -ve | - | -ve | - | - | -ve | α | +ve | +ve | - |
| *Staph. aureus* | - | - | - | - | -ve | +ve | +ve | - | +ve | A/A | β | - | - | - |
| *Staph. intermedius* | - | - | - | - | -ve | +ve | +ve | -ve | -ve | - | β | - | - | - |
| *Coryn. diphtheriae* | -ve | +ve | -ve | -ve | -ve | -ve | +ve | -ve | - | -ve | β | - | - | -ve |
| *Coryn. pyogenes* | - | -ve | -ve | -ve | -ve | +ve | +ve | -ve | - | -ve | β | - | - | -ve |
| *Escherichia coli* | -ve | +ve | -ve | +ve | -ve | -ve | +ve | -ve | - | A/A | -ve | - | - | -ve |
| *Proteus vulgaris* | +ve | -ve | -ve | +ve | +ve | +ve | -ve | -ve | - | A/A | -ve | - | - | -ve |
| *Pseudo. aeruginosa* | -ve | -ve | -ve | -ve | -ve | +ve | +ve | +ve | - | K/K | β | - | - | -ve |
| *Microco. luteus* | - | - | - | - | -ve | +ve | +ve | +ve | - | K/A | -ve | -ve | - | - |
| *Stom. mucilagino.* | - | - | - | - | -ve | +ve | -ve | -ve | - | -ve | -ve | +ve | -ve | - |

A/A = Acid slant and acid butt K/A = Alkaline slant and acid butt K/K = Alkaline slant and alkaline butt.
TSI= Triple sugeir iron test

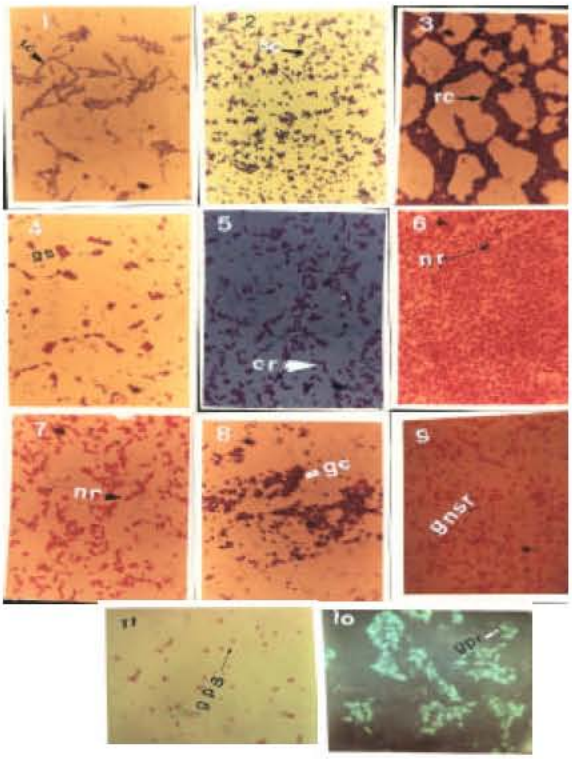


Fig. 1: The morphological and staining characteristics of bacterial species identified from wound samples of animals, stained by Gram's method X100. 1. *S. pyogenes* Gram-positive (G+ve) with long chain; lc, long chain. 2. *S. uberis* G+ve; ; Sc, short chains 3. *Staph. intermedius* G+; rc, regular cluster. 4. *Staph. aureus* G+ve; gs, grape shape. 5. *Coryneb. pyogenes* G+ve; cr, coccobacilli rods. 6. *E. coli*, Gram-negative (G-ve) negative (G-ve); nr, negative rods. 7. *Prot. vulgaris* G-ve; nr, negative rods. 8. *Microc. luteus* G+ve; gc, Gram-positive cocci. 9. *Pseud. aeruginosa* G-ve; gnsr, gram-negative single rods. 10. *C. diphtheriae* G+ve; gpr, gram-positive rods and 11. *Stomat. mucilagenosus* G+ve; gps, Gram-positive singles

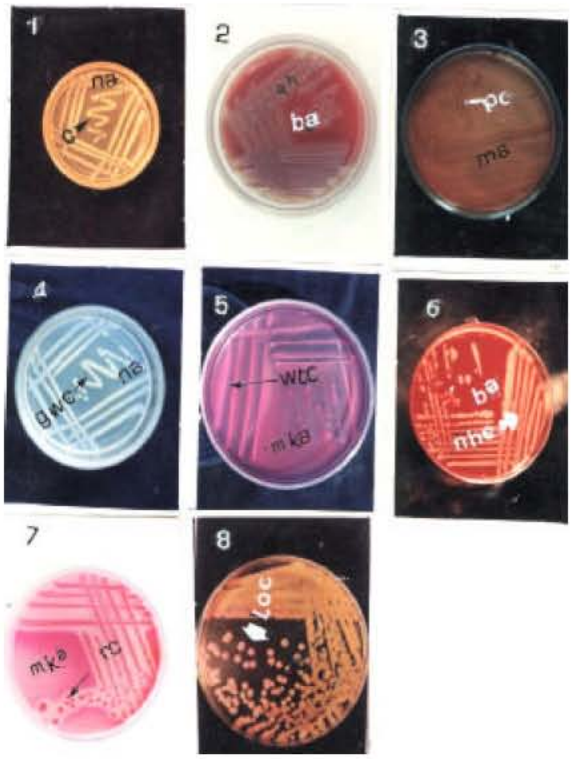


Fig. 2: Photographs show cultural characteristics of various bacterial species recognized from wound samples of animals. 1. *Strept. pyogenes* yielded white opaque colonies on nutrient agar; na, nutrient agar; c, colonies. 2. *Strept. uberis* produced αh, alpha hemolysis. 3. *Staph. aureus* produced pink colonies on MacConkey's agar; ma, MacConkey's agar; pc, pink colonies. 4. *Coryneb. pyogenes* produced grayish white colonies on nutrient agar; na, nutrient agar ; gwc, grayish white colonies. 5. *Coryneb. diphtheriae* yielded white colonies on MacConkey's agar; ma, MacConkey's agar. Wc, white colonies. 6. Non-hemolytic colonies of *E. coli* on blood agar; ba, blood agar, nhc, non-hemolytic colonies. 7. *E. coli* produced radish colonies on MacConkey's agar during 24 hours incubation; ma, MacConkey's agar; Rc, radish colonies. 8. *E. coli* produced large opaque colonies on nutrient agar; loc, large opaque colonies

***Pseudomonas aeruginosa:*** These organisms fermented glucose only but not sucrose, lactose, maltose, mannitol, inositol, arabinose and raffinose (Tables 3, 4 and Fig.3, a. $a_1$ and f. $f_2$). They were negative to indole, hydrogen sulphide production and citrate utilization. Also negative to methyl red and Voges Proskauer but was positive to catalase, oxidase and liquefied gelatin. They did not decompose urea but produced K/K (alkaline slant and alkaline butt) in TSI.

***Micrococcus luteus:*** The results regarding *Micrococcus luteus* are demonstrated in Tables 3, 4 and Fig.3, e. $e_1$). It fermented glucose, maltose and mannitol, but not arabinose sucrose, lactose, raffinose and inositol. They were negative for $H_2S$ production and aesculin but positive for gelatin liquefaction, catalase and oxidase reactions. The tripe sugar iron test was K/A (alkaline slant and acid butt).

***Stomatococcus mucilaginosus:*** It fermented glucose, sucrose, maltose and mannitol and failed to ferment arabinose, lactose, raffinose and inositol. They did not produce chain in liquid medium and were present in single form. Moreover, they were negative for oxidase, catalase reaction, $H_2S$ production, and TSI and aesculin was hydrolyzed (Tables 3 and 4).

## Discussion

The findings about morphological, cultural and staining behaviuor encountered during current study for *Streptococcus pyogenes*, are in close agreement to that of Breed *et al.* (1957) who described the size of bacteria 0.6-1$\mu$ in diameter, spherical and sometimes ovoidal in shape.

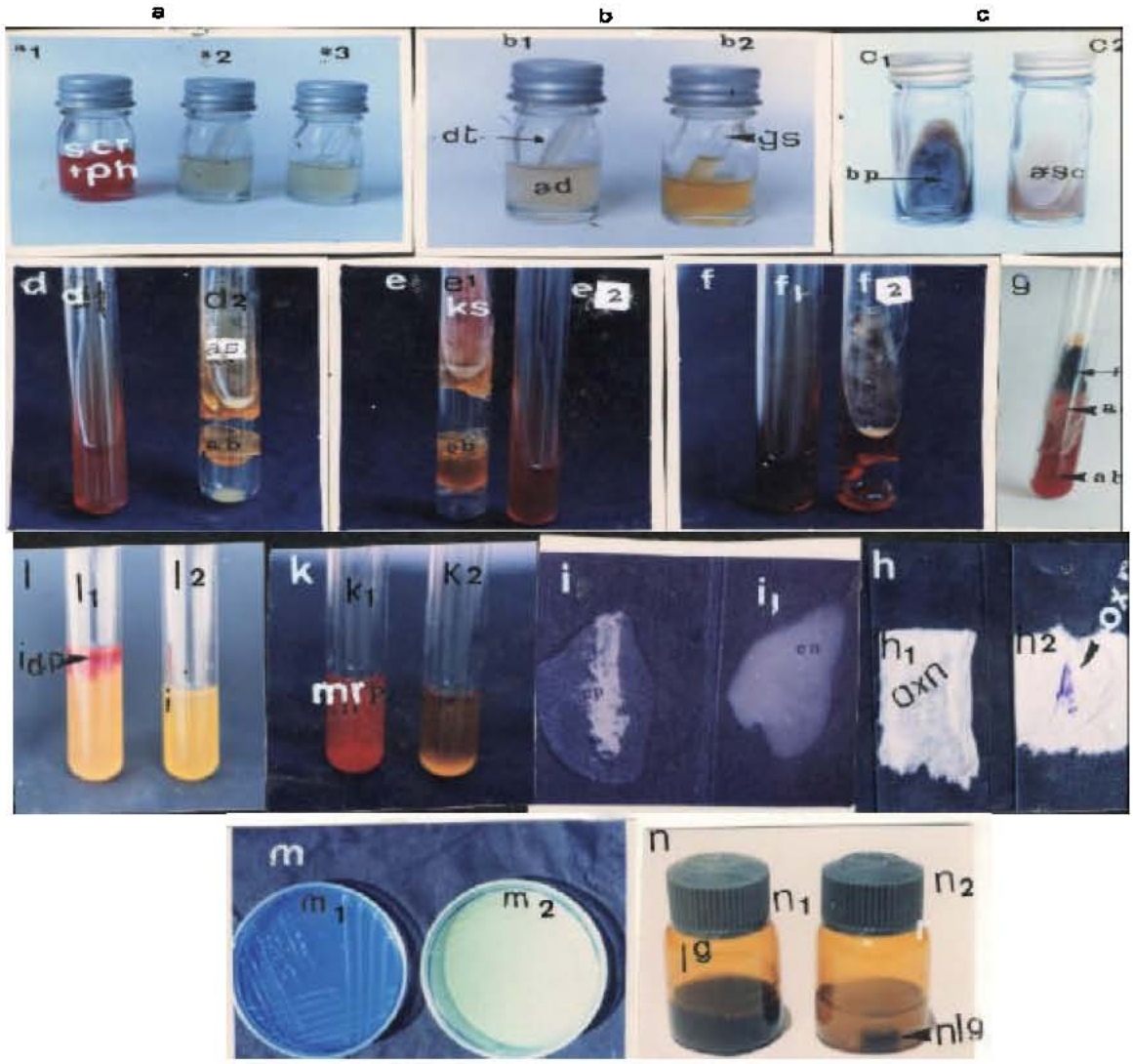


Fig. 3: Photographs show biochemical characteristics of bacterial spp. recognized from wound samples of animals. a. $a_1$ *Pseud. aeruginosa* not fermented glucose, scr+ph, sucrose+phenol red; $a_2$, *Strept. pyogenes* fermented sucrose; $a_3$, E. coli fermented sucrose. b. $b_1$, Staph. aureus fermented sucrose; dt, Durham's tube; ad, acid; $b_2$, *Prot. vulgaris* fermented glucose partially; gs, gas. c. $c_1$, *Strept. uberis* hydrolysed aesculin; bp, black preciptiin; $c_2$, Strept. pyogenes not hydrlysed aesuline; asc, aesulin. d. $d_1$, normal medium of TSI; $d_2$, *Staph. aureus* utilized TSI medium and was A/A; as, acid slant, ab, acid butt. e. $e_1$, *Microc. luteus* utilized TSI medium and was K/K; Ks, alkaline slant; ab, acid butt. f. $f_1$ normal TSI medium; $f_2$, *Pseud. aeruginosa* utilized TSI medium and was K/K. g, *Prot. vulgaris* utilized medium and produced $H_2S$, Hydrogen sulphide; as, acid slant; ab, acid butt. h. $h_1$, oxidase negative; $h_2$, *Staph. aureus* showed oxidase positive; oxn, oxidase negative; oxp, oxidase positive. i. *Staph. aureus* catalase positive; cp, catalase positive; $i_1$, cn, catalase negative. k. $k_1$, *E. coli* methyl red positive; mrp, methyl red positive; $k_2$, methyl red negative. l. $l_1$, *Prot. vulgaris* indole positive, indp, indole positive; $l_2$, indole negative. m. $m_1$, E. coli utilized Simmon's citrate medium; $m_2$, normal dish. n. $n_1$, *E. coli* liquefied gelatin disc; lg, liquefied gelatin; $n_2$ gelatin disc in intact state. o. *E. coli* sensitivity to antibiotics; sz, zone of sensitivity

In broth cultures, long chains were common and in solid medium, organisms were in chains and also in pairs. They were hemolytic in nature and produced beta zone around colonies But the characteristics about *S. uberis* demonstrated in this study are not comparable to other workers because we could not get any information in the available literature. No doubt a lot of work has been carried out on this specie throughout the world.

The morphological, cultural and staining characteristics observed for *S. aureus* in present investigation are similar to that of Smith and Conant (1962), who also reported them as gram-positive, non-motile and aerobic. On nutrient agar, colonies these were golden yellow, whereas on blood agar, produced

Table 4: The sugar fermentation properties of different bacteria identified from wounds of domestic animals

| | Bacterial species | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | *Strept. pyogenes* | *Strept. uberis* | *Staph. aureus* | *Staph. intermedius* | *Coryn. diphtheriae* | *Coryn. pyogenes* | *Escherichia coli* | *Proteus vulgaris* | *Pseud. aeruginosa* | *Microc. luteus* | *Stomat. mucilag.* |
| **Glucose** | | | | | | | | | | | |
| A | +ve | +ve | +ve | +ve | +ve | +ve | - | - | +ve | +ve | +ve |
| A/G | - | - | - | - | - | - | +ve | +ve | - | - | - |
| L | - | - | - | - | - | - | - | - | - | - | - |
| -ve | - | - | - | - | - | - | - | - | - | - | - |
| **Sucrose** | | | | | | | | | | | |
| A | +ve | +ve | +ve | +ve | +ve | +ve | - | - | - | - | +ve |
| A/G | - | - | - | - | - | - | +ve | +ve | - | - | - |
| L | - | - | - | - | - | - | - | - | - | - | - |
| -ve | - | - | - | - | - | - | - | - | -ve | -ve | - |
| **Lactose** | | | | | | | | | | | |
| A | - | - | +ve | +ve | - | - | - | - | - | - | - |
| A/G | - | - | - | - | - | - | +ve | - | - | - | - |
| L | - | - | - | - | - | - | - | - | - | - | - |
| -ve | -ve | -ve | - | - | -ve | -ve | - | -ve | -ve | -ve | -ve |
| **Maltose** | | | | | | | | | | | |
| A | +ve | +ve | +ve | +ve | - | +ve | - | - | - | +ve | +ve |
| A/G | - | - | - | - | - | - | +ve | +ve | - | - | - |
| L | - | - | - | - | - | - | - | - | - | - | - |
| -ve | - | - | - | - | -ve | - | - | - | -ve | - | - |
| **Mannitol** | | | | | | | | | | | |
| A | - | - | +ve | - | - | - | - | - | - | +ve | - |
| A/G | - | - | - | - | - | - | +ve | - | - | - | - |
| L | - | - | - | - | - | - | - | - | - | - | -ve |
| -ve | -ve | -ve | - | - | -ve | -ve | - | -ve | -ve | - | - |
| **Inositol** | | | | | | | | | | | |
| A | - | - | - | - | - | - | - | - | - | - | - |
| A/G | - | - | - | - | - | - | - | - | - | - | - |
| L | - | - | - | - | - | +ve | - | - | - | - | -ve |
| -ve | -ve | -ve | -ve | -ve | -ve | - | -ve | -ve | -ve | -ve | - |
| **Arabinose** | | | | | | | | | | | |
| A | - | - | - | - | - | - | - | - | - | - | - |
| A/G | - | - | - | - | - | - | +ve | +ve | - | - | - |
| L | - | - | - | - | - | +ve | - | - | * | - | -ve |
| -ve | -ve | -ve | -ve | -ve | -ve | - | - | - | -ve | -ve | - |
| **Raffinose** | | | | | | | | | | | |
| A | - | - | - | - | - | - | - | - | - | - | - |
| A/G | - | - | - | - | - | - | - | - | - | - | - |
| L | - | - | - | - | - | - | - | - | - | - | - |
| -ve | -ve | -ve | -ve | -ve | -ve | -ve | -ve | -ve | -ve | -ve | -ve |

A = Acid, A/G = Acid alongwith gas, L = Late fermented -ve = Negative.

ß hemolysis. But the characteristics noted for *Staphylococcus intermedius* in the current survey can not be compared to the identification marks of the other works because they placed this specie together with genus *Staphylococcus*.

The characteristics regarding the identification of *Corynebacterium pyogenes* and *Corynebacterium diphtheriae* observed during this bacteriological examination are in close agreement with the results of Merchant and Packer (1967). Breed *et al.* (1957) measured the size of *Corynebacterium diphtheriae* as 0.3 to 0.8 x 1.0 to 8.0$\mu$ in diameter. They were rod shaped and non-motile, gram-positive. They produced translucent growth on slant, while glistening and moist colonies on nutrient agar.

Salle, (1988) described rod like shape of *Escherichia coli*, measured 1-3$\mu$ in size and it always remain in pairs and in short chains. Gram-negative produced yellowish-white colonies on nutrient agars. Similar characteristics were noted during this investigation as well.

The morphological characteristics of *Proteus vulgaris* demonstrated in this study are similar as described by Wilson and Miles (1964). Furthermore, Salle (1988) also gave similar characteristics in all respects for the *Proteus vulgaris*.

*Pseudomonas aeruginosa* was observed as gram-negative, rod shaped, arranged singly/pairs with 0.5-0.6 × 1.5$\mu$ in size during this research work. Wilson and Miles (1964) described similar morphological, cultural and staining characteristics. Therefore, the findings recorded about the species are in agreement with the above authors.

The results about *Micrococcus luteus* and *Stomatococcus mucilaginosus* and their characteristics observed in this study are in line to the findings of Salle (1988) and Bergey's (1992).

The findings regarding biochemical properties of *S. pyogenes* and S. *uberis* obtained during this survey do agree with the results of Barnham and Neilson (1987). In current investigation, we found only two differences between these two species. *S. pyogenes* hydrolyzed the aesculin and produced beta hemolysis, on the other hand *S. uberis* could not hydrolyze aesculin and produced alpha hemolysis. Breed *et al.* (1957) also reported similar properties in their survey on these two species.

In present study *Staphylococcus aureus* was found coagulase positive and *Staphylococcus intermedius* was negative. The other properties tested during biochemical investigation, were the same. These results are similar as reported by Talan *et al.* (1989).

According to Breed *et al.* (1957), organisms of *Corynebacterium diphtheriae* produced acid from glucose and also fermented other sugars and reacted negatively to indole. This is in accord with the present work. The biochemical properties described for *Escherichia coli* during present work were the same as investigated by Breed *et al.* (1957).

However, the biochemical changes produced by *Proteus vulgaris* in different sugars and chemicals were also observed by Wilson and Miles (1964), who mentioned all the properties similar as noted in this study. The biochemical activities observed during present experimental work for *Pseudomonas aeruginosa* are in accordance to the activities established by Wilson and Miles (1964). However, in this study, it did not react with indole and also

negative for hydrogen sulphide production and citrate utilization.

The species *Micrococcus luteus* and *Stomatococcus mucilaginosus* demonstrated in this study were the organisms detected for the first time from the wound samples of domestic animals in the province of Sindh. *Micrococcus luteus* fermented a majority of sugars but failed to respond hydrogen sulphide production, aesculin, gelatin liquefaction and catalase and oxidase reactions. More or less similar results were recorded for *Stomatococcus mucilaginosus,* but in nature this speice was very close to *Streptococcus.* The physio-chemical nature of these two bacteria recorded here are similar to that of Bergey's (1992).

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