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Clinical study on bacteriological profile and management of surgical site infections in cattle undergoing laparo-enterectomies

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Abstract
The current investigation was carried out in cattle (n=30), that underwent laparo-enterectomies, to assess the organisms responsible for causing surgical site infection and devise measures to control the post-operative infection. Each case was followed-up for 35 days post-operatively on alternative days for checking the surgical site infection. The pus sample in positive cases was collected aseptically and sent for the identification of bacteria using culture and various biochemical tests. The antimicrobial susceptibility test was also done to know the level of resistance among various bacteria and use an appropriate antibiotic for the treatment. Surgical site infections (SSIs) occurred in 12/30 cases. The mean duration of diagnosis of SSI was found to be 8.08±0.93 days. *Staphylococcus*, *Streptococcus*, and *E.coli* were the organisms isolated. Among these, *Staphylococcus aureus* was the commonest species isolated accounting for 83.4% of the SSIs as single colonies in 6 cases and mixed colonies in 4 cases. Bacterial isolates of *Staphylococcus aureus* and *Streptococcus* spp. showed 100% sensitivity against gentamicin and enrofloxacin, followed by 66.6% each to ceftriaxone and penicillin. Isolates of *E. coli* showed maximum sensitivity (100%) against enrofloxacin and gentamicin. Consistent surgical management of the site and the specific antibiotic regimen used were effective in treating post-operative SSIs without further complications.

Keywords: Antimicrobial susceptibility, cattle, intussusception, microorganisms, surgical site infections

Introduction
Surgical site infections (SSIs) are among the most common hospital-acquired infections that occur in the postoperative period (Martone and Nochols, 2001) [12]. SSIs usually arise within 30 days after the treatment, however, in situations with additional implants, the duration of SSI can last up to a year (Richards *et al*., 2003) [17]. Surgical site infection (SSI) is a possible complication to effective recovery in veterinary patients undergoing surgery; nevertheless, there has been insufficient research on their etiology and treatment. Due to the presence of microorganisms, especially on the skin, all patients are at risk of developing SSI. (Eugster *et al*., 2004 and Vengust *et al*., 2006) [6, 20]. Break in the continuity of this physical barrier can predispose the animals to these microorganisms which can potentially cause an infection in the perioperative period (Nicholson *et al*., 2002; Eugster *et al*., 2004; Vengust *et al*., 2006) [15, 6, 20]. The contaminating pathogens in gastrointestinal surgeries are the intrinsic bowel flora, which includes Gram-negative bacilli and Gram-positive microbes, including *Enterococci* and anaerobic organisms (Schaechter *et al*., 1993) [18]. Anaerobic bacteria are a part of normal flora in the gastrointestinal tract and lower urogenital tract. The diseases caused by anaerobes occur after spillage or penetration of these organisms into normally sterile tissue. Lesions caused by anaerobes are highly specific and are often deep within the body and not exposed to air. Fewer studies have evaluated post-operative SSIs in large domestic ruminants; a comprehensive study is lacking. Therefore, the present work was done to study the bacteriological profile in cases of surgical site infections (SSIs) and their treatment in cattle undergoing laparo-enterectomies.

Materials and methods
The study was conducted in cattle (n=30) suffering from intussusceptions and on which laparo-enterectomies were performed. Each case was followed up for 35 days post-operatively and checked every alternate day. Patients were checked for systemic (fever) and local (pain, swelling, purulent drainage) signs of infections.

Surgical incision was assessed when the dressings were changed and the time of suture removal along with any wound or suture dehiscence were also recorded. Samples in positive cases (SSIs) were obtained aseptically using sterile swabs and were immediately transported and processed. Bacterial culture was done as described by Koneman, 2006 [11] in nutrient broth and solid agar (blood agar, nutrient agar, and MacConkey agar). Inoculated media were incubated at 37°C overnight in the case of solid agar and for 24-48 hours in the case of liquid broth. Growth on the culture plates was examined macroscopically for colonial morphology (size, shape, color, surface features, margins, and texture). Gram staining followed by microscopic examination was done. Depending upon the morphology and characteristics of the bacteria, they were further inoculated on the selective media. Isolation of different bacteria was done by following the methods of Crichton, 1996, WHO, 1987 and Carter *et al*, 1994 [4, 21, 3]. For isolation of *E.coli*, MacConkey's agar, Eosine Methylene Blue Agar (EMB Agar) and Hichrome E.coli specific agar were used. Similarly for *Staphylococcus*, Mannitol salt agar, Hichrome Staph selective agar and Baired Parker medium were used to confirm the presence of *Staphylococcus*. *Streptococcus* spp were identified on the basis of Gram's staining and catalase test. Biochemical tests like lactose fermentation, Indole assay, Methyl Red test, Voges Proskauer test, Citrate utilization test, Oxidase test, and catalase test were performed as given by Crichton, 1996**,** WHO, 1987 and Carter *et al*, 1994 [4, 21, 3]. Bacterial isolates were tested for antimicrobial susceptibility by the disk diffusion method using antibiotic discs (Hi Media Pvt. Ltd., Mumbai, India) by following recommendations from the Clinical and Laboratory Standards Institute (CLSI). The commonly used antimicrobial agents were tested- Enrofloxacin (Ex, 10µg) and Gentamicin (Gen, 30µg), Penicillin G (PG, 10 units), Ceftriaxone (Czx, 30µg) and Oxytetracycline (TE, 30µg) respectively. After 24 hours of incubation at 37ºC, inhibition zones were measured in millimeters on the Mueller–Hinton plates (Hi Media Pvt. Ltd., Mumbai, India) and interpreted. Isolates were designated as sensitive (S), Intermediate (I) and resistant (R) to various antibiotics as per zone diameter

The extent of SSI was determined as superficial or deep and accordingly general principles of management of SSI were followed. Wounds that were confined to the skin and subcutaneous tissue were classified as superficial. The presence of swelling, tenderness obvious oozing of pus were the main determinants for inclusion into this category. Deep/ organ SSI was determined either through clinical signs of intra-abdominal sepsis or at operation. For treatment, the infected surgical site was first washed with lukewarm water to remove all the gross debris. The surgical incision was re-opened and any necrotic skin, fat and fascia were removed. In the case of superficial SSIs, the incision was opened from the last two sutures, and the pus was drained. A counter opening was made from the first 2 sutures for flushing with potassium permanganate solution. Finally, a sterile gauge filled with betadine was packed in the area. Broad-spectrum antibiotics (enrofloxacin/gentamicin) were administered initially and then the antibiotics were administered as per the culture sensitivity results. The owners were advised to clean the area with lukewarm water daily and to keep the animal in a clean place. Regular dressing of the site was also advised to the owner.

In the case of deep SSIs, the incision was opened and the source of contamination, wound, or suture dehiscence if any, was addressed and the pus (mostly inspissated) was removed. Potassium permanganate was flushed into the site. A healthy wound bed was made and the area was either left to heal by secondary intension or partially sutured followed by packing of gauge into the area. Antibiotics were administered similarly to superficial SSIs.

Results and Discussion

Despite the development of antisepsis, contemporary surgical practices, and antibiotic regimens, surgical site infection remains an issue in patients having invasive procedures. In the current study, the overall rate of SSIs in laparo-enteroectomies was 40% (12/30). Using the CDC criteria of determining the extent of SSIs, 83.33% of these patients had superficial SSIs, whereas 16.67% had deep SSIs. The mean duration of diagnosis of SSI was 8.08±0.93 days. Gronlund and Bergstrom (2013) [8] also reported a median duration of 7 days for SSI diagnosis in horses. Danchaivijitr and Chokloikaew (1996) [5] stated that over 50% of SSIs occur within the first week after operation, and 90% within 2 weeks. The area of SSIs showed signs of warmth, swelling, and pus drainage**.** Single pathogens (*Staphylococcus aureus* and *E.coli*) were isolated from 8/12 (66.67%) cases while 4/12 (33.33%) samples after inoculation grew mixed pathogens (*Staphylococcus*, *Streptococcus* and *E.coli)*. *Staphylococcus aureus* was the commonest species isolated from the cultures accounting for 83.4% of the SSIs, as pure infection in 6 cases and as mixed infections in 4 cases. The distribution of various pathogens isolated along with their respective percentage is shown in Table 1. Previous reports have also shown *Staphylococcus* spps. to be the most common bacteria found in SSIs in dogs and cats (Kadlec *et al*., 2010, Nienhoff *et al*., 2011 and Frank *et al*., 2012) [10, 16, 7]. Isgren *et al*. (2017) [9] also reported Staphylococcus and Escherichia coli to be the pathogens responsible for causing SSIs in horses (n=287) after laparotomy (small intestinal resection). National Nosocomial Infection Surveillance (NNIS) report (1998) [13] also showed that the majority of SSIs were caused by *Staphylococcus* spp. (20%) followed by *E.coli* (8%) and then by *Streptococcus* spp. (3%). Nichols (2001) [14] reported gram-negative *E.coli* to be predominately present in abdominal surgeries.

Table 1: Distribution of pathogens isolated and their respective percentage

As pure culture	Number of cases	Percentage	Type of SSIs
Staphylococcus aureus	6	50%	Superficial SSI
E. coli	2	16.67%	Superficial SSI
As mixed culture			
Staphylococcus aureus and *Streptococcus* spp	2	16.67%	1 in superficial SSI and the other in deep SSI
Staphylococcus aureus and *E.coli*	2	16.67%	1 in superficial and other in deep SSI (contaminated)

The biochemical tests involving catalase, oxidase and IMViC (Indole assay, Methyl red test, Voges Proskauer test and Citrate utilization test) results of the isolated organisms are shown in Table 2.

Table 2: Biochemical test results of the *Staphylococcus*, *Streptococcus* and *E.coli* isolated from post-operative SSIs.

Test	*E. coli*	*Staphylococcus*	*Streptococcus*
Catalase	+	+	-
Oxidase	-	-	-
Indole	+		
Methyl red	+		
Voges Proskauer	-		
Citrate utilization	-		

Management of SSI is dependent on the selection of an efficient and suitable antibiotic which plays a significant role in both the prevention and treatment of infectious disorders (Bradford, 2001) [2]. Five anti-bacterial agents were tested against bacterial isolates and the results were recorded on dichotomous scale as resistant (R), intermediate (I), and susceptible (S). The relative response of bacterial isolates to different antibacterials were shown in Table 3.

Table 3: *In-vitro* sensitivity pattern of bacterial isolates obtained from SSIs post-operatively

Antibiotic used / Bacteria isolated	Penicillin G	Gentamicin	Ceftriaxone	Tetracycline	Enrofloxacin
Staphylococcus aureus	I	S	I	R	S
Streptococcus spp.	I	S	I	R	S
E.coli	R	S	I	R	S

Bacterial isolates of *Staphylococcus aureus* and *Streptococcus* spp. showed 100% sensitivity against enrofloxacin and gentamicin as shown by a zone of inhibition followed by 66.6% to ceftriaxone and penicillin. Isolates of *E.coli* showed maximum sensitivity (100%) against enrofloxacin and gentamicin. All the isolated organisms showed resistance to tetracycline. In the present study, both types of SSIs (superficial and deep) were treated with antimicrobial therapy and by surgical intervention. The surgical intervention included drainage, flushing of the surgical site, debridement of the wound bed, and packing of the cavity with a gauge. Anderson (2011) [1] and Stevens *et al*. (2014) [19] have also stated that if physical examination and imaging suggest a deeper infection, then suture removal, drainage, and debridement of necrotic tissue should be performed.

In conclusion, the present study provided insight into microorganisms and antimicrobial sensitivity patterns identified from post-operative surgical site infections. The study also emphasized the need for effective surveillance in lowering the risk of post-operative infections. This monitoring system should be implemented in all hospitals, and standards for antibiotic usage among surgical patients should be devised and closely adhered to, in order to provide a true estimate of the occurrence of SSIs. According to the findings of this study, microbes isolated from the surgical site infection developed resistance to more routinely used medications such as penicillin and even cost-effective tetracyclines. We are now down to a few reserve medications that must be handled with caution. Even after 160 years of discoveries by Louis Pasteur and Joseph Lister, there are still some unresolved facts about the pathophysiology and monitoring of post-operative infections. Therefore, more such studies should be encouraged so that the current situation of antimicrobial resistance and its role in the occurrence of SSIs can be assessed and acted upon timely.

Conflicts of Interest: None.

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