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Qualitative assessment and antibiotic sensitivity test of the bacterial microbes involved in the surgical site infections (SSIS) and chronic wounds in animals

Ayushi Sawhney, DK Dwivedi, HR Bhardwaj, Ashok Kumar and Maninder Singh

Abstract
The present study was conducted at VCC, SKUAST-J, R.S. Pura, Jammu in the client owned 7 dogs and 1 cattle suffering from surgical site infection and chronic wounds unresponsive to the routine antibiotic therapy regimen. The objectives of this study were to determine the qualitative identification of the bacterial microorganisms in the chronic wound and surgical site infections and identification of the antibiotics effective against identified organisms, by using Antibiotic Sensitivity Test (AST). The samples were collected and cultured in the MacConkey agar and BHI to get the bacterial cultures. The organisms were identified by using catalase and oxidase tests. Pure colonies were again streaked on MHA agar and AST was done. Isolated organisms were colonies of *Staphylococcus, E. coli*, *Pseudomonas* and *Klebsiella.* AST revealed resistance to various higher generation antibiotics and intermediate to sensitive trait to amoxi-clav combination /gentamicin antibiotic.

Keywords: antibiotic, bacterial microbes, SSIS, chronic

Introduction
In 1992, the United States Centres for Disease Control and Prevention (CDC) introduced the term surgical site infection (SSI). Prior to it, term surgical wound infection was used (Kolasiński, 2019) [6]. The CDC has developed a definition for SSI as an "infection related to an operative procedure that occurs at or near the surgical incision within 30 days of the procedure or within 90 days if prosthetic material is implanted at surgery" (Owens and Stoessel, 2008) [7]. This CDC definition thus describes three levels of SSI:

- Superficial incisional, affecting the skin and subcutaneous tissue.
- Deep incisional, affecting the fascial and muscle layers.
- Organ or space infection, which involves any part other than the incision that is opened or manipulated during the surgical procedure.

When evaluated by wound classification, the National Research Council summarized the reported incidence of SSI in the veterinary literature; clean and clean-contaminated procedures have a 2-5 percent risk of infection whereas contaminated and dirty surgeries are reported at 4-12 percent and 6-18 percent, respectively. Stetter *et al*., 2021 [9] reported that SSI was detected in 5.5% (85/1550) dogs undergoing various surgical procedures. SSIs being one of the most common surgical complications are responsible for an increase in morbidity, mortality, prolonged hospital stay, increased costs and a negative impact on the emotional state of the owner (Espinel-Rupérez *et al*., 2019) [3].

Wounds can be broadly categorized as having either an acute or a chronic aetiology. Wound contaminants are most likely to come from three different places: (i) the environment (exogenous microorganisms in the air or those introduced by traumatic injury), (ii) the surrounding skin (involving members of the normal skin microflora like Staphylococcus epidermidis, micrococci), and (iii) endogenous sources involving mucous membranes (primarily the gastrointestinal, oropharyngeal, and genitourinary mucosa (Prakash *et al.*, 2022) [8].

For prompt wound healing and return to normal function, early wound infection detection and surveillance are essential. Microbiological testing and subjective clinical symptoms are the key components of infection evaluation techniques (Johnson *et al*., 2022) [5]. Emergence of resistant bacteria has reinforced the requirement for antimicrobial stewardship.

In general, sampling either wound tissue or wound fluid can be used to examine the quantitative and qualitative microbiology of wounds. To sample superficial wound fluid and tissue debris, a cotton-tipped swab is most often used, and this allows for a semiquantitative and qualitative examination of the wound microbiota (Bowler *et al.*, 2001) [2]. The procedure is simple, inexpensive, non-invasive and convenient for the majority of wounds.

Antibiotic resistance happens when standard antibiotics become less effective or ineffective against certain bacteria. An antibiotic sensitivity test (AST) can help find out which antibiotic will be most effective in treating the persistent infection. Microbiological data are important in confirming the microorganisms involved and subsequently facilitates building up a regimen most appropriate for the given disease condition.

Materials and Methods

Sample of the animals visiting V.C.C, SKUAST-J, R.S. Pura with a history of chronic wound or infected surgical wounds were collected from the affected site using sterile swabs. The swab was further dipped in sterile Normal saline solution and culturing of the swab material was done on suitable medias. The samples were swabbed on two agars *viz*. MacConkey agar and Brain heart Infusion agar, followed by incubation at 37 °C for 24-48 hours. Growth was assessed for the identification of the microbes present. The MacConkey agar plate were screened for lactose fermenting and non- lactose fermenting colonies. Further, lactose fermenting non mucoid colonies were picked and streaked on EMB agar and the plates were again incubated at 37 °C for 24 hours. For the further evaluation of the organisms isolated, catalase oxidase tests were also performed. The isolates were subjected to AST by disc diffusion method. AST was done using plates of Mueller Hinton Agar that were prepared using same sterile plates, Hi-Media powdered media and solution autoclaved and then, plates poured. The plates were then swabbed with the growth present in MH broth. For this, the pure bacterial colony was inoculated in MH broth followed by incubation at 37 °C for 16 hours. After incubation, the broth was swabbed on MHA plates and antibiotic discs were placed on the agar plate, followed by incubation at 37 °C for 24 hours. The antibiotics used in AST were Gentamicin (GEN), ceftriaxone (CTR), cefotaxime (CTX), ciprofloxacin (CIP), amoxycillin/clavulanic Acid (AMC), penicillin (P), chloramphenicol (C) and co- trimoxazole (COT). Post incubation period, the zones for each antibiotic were measured with zone measuring scale and the result was interpreted by using Hi-Media antibiotic sensitivity table.

Results and Discussion

A total of 8 samples were processed that were procured from animals having surgical site infections and wounds. The animals were selected on the basis of their chronicity and non-responsiveness to the medication prescribed**.** Details of the species, breed and sample collection site is given in Table 1.

Post incubation of the Petri plates, both MacConkey agar and Brain Heart Infusion agar plates were assessed for the colonization and the results are mentioned in table 02.

Table 1: Signalment and site of sample collection in the animals affected with SSIs/chronic wounds.

Case No.	Species	Breed	Site
1	Canine	Non-descript	Hind limb (thigh)
2	Canine	Pug	Abdomen
3	Canine	Labrador	Ear
4	Canine	GSD	Paw (nail)
5	Canine	Spitz	Skin patches
6	Canine	Pug	Elbow
7	Canine	Labrador	Hind limb (stifle area)
8	Bovine	Non-descript	Eye

Table 2: Colonization trend of the samples after streaking the MacConkey agar and Brain Heart Infusion agar

Case No.	Species	MacConkey agar	Brain Heart Infusion agar
1	Canine	Colonization present	Colonization present
2	Canine	Colonization absent	Colonization absent
3	Canine	Colonization present discoloration of media	Colonization absent
4	Canine	Colonization present	Colonization absent
5	Canine	Colonization absent	Colonization present
6	Canine	Colonization absent	Colonization absent
7	Canine	Colonization absent	Colonization absent
8	Bovine	Colonization present	Colonization absent

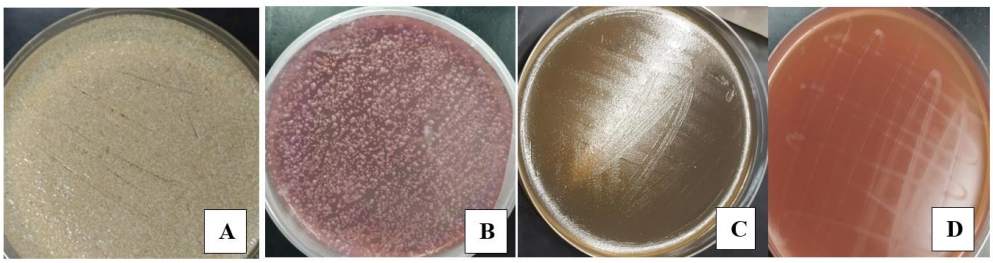

Fig 1: Growth seen on both BHI agar (A) and MacConkey agar (B) in case of case no. 1 and no growth observed on BHI agar (C) and MacConkey agar (D) in case of case no. 2

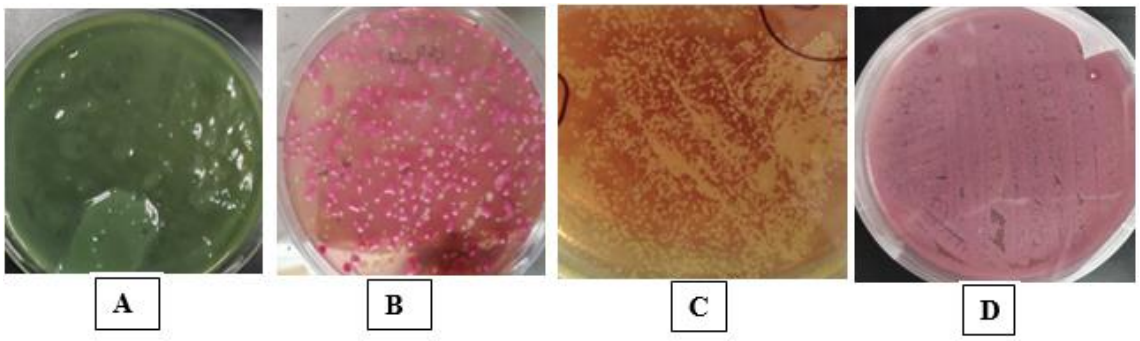

Fig 2: A) Discoloration of media and growth seen on MacConkey agar in case of case no. 3, B) Growth seen on McConkey agar case no. 4, C) Growth seen on BHI again case of sample no. 5 and D) Pink colonies growth seen on MacConkey agar in case no. 8

After the characterisation of the above growth on plates, serial streaking was done on media plates for getting pure colonies. MacConkey agar and EMB agar was used to get pure colonies serially.

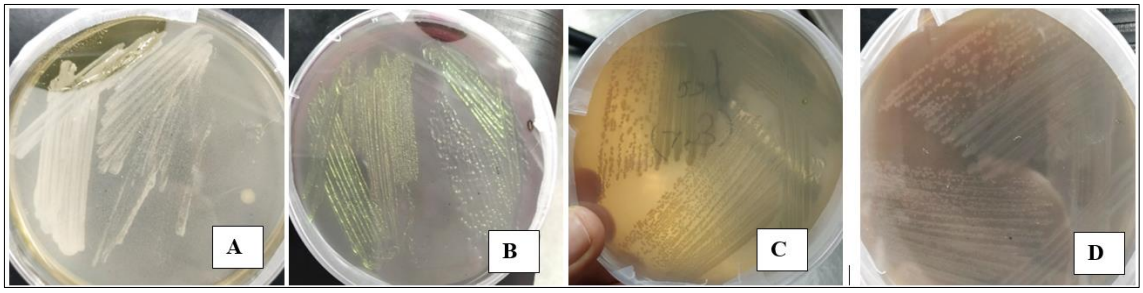

Fig 3: Isolation of pure colonies by serial streaking. (Colonies of *Staphylococcus*, *E. coli*, *Pseudomonas* and *Klebsiella* isolated from sample no. 5, 8, 4 and 1

The colonial characterization of the Lactose fermenting colonies from MacConkey agar showing greenish metallic sheen on EMB agar were deduced to be *E. coli*. Non- lactose fermenting rough serrated colonies on MacConkey agar with blue-green discoloration were known to be *Pseudomonas aeruginosa*. Pink mucoid colonies MacConkey agar were investigated to be *Klebsiella pneumoniae*. Gold-coloured colonies were formed on brain heart infusion (BHI) agar in case of *Staphyloccocus aureus.*

Andrade *et al.*, 2014 reported that *Staphylococcus spp*. was the most commonly recovered bacteria from the surgical gloves, hands, and dogs' skin, suggesting that the human and canine microbiomes are predominant contributors to the intraoperative bacterial load. In the clinical study conducted by Hagen *et al*., 2020 [4] the follow-up findings reported that signified/ indicated SSI were either a focal area of spontaneous dehiscence that was erythematous and swollen 14 days postoperatively or/and a swollen, erythematous incision with serosanguinous discharge 10 days postoperatively. In bacterial culture tests, bacteria were recovered in 92.2% cases whereas Methicillin-resistant *S pseudintermedius* was the most commonly found cultured bacterium, However, *Staphylococcus* spp. were detected in 85.7% of SSIs that were presented beyond 90 days post procedure.

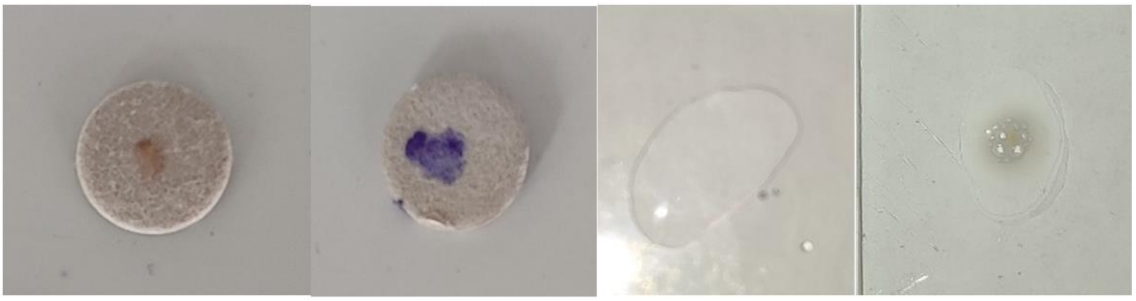

Fig 4: showing oxidase and catalase test (both negative and positive)

Antibiotic Sensitivity Test

The pure colonies were inoculated in Mueller- Hinton broth and incubated for 24 hours at 37 ºC. The plates were then swabbed with the MH broth inoculum and the 8 antibiotic discs were placed to check their sensitivity. The plates were kept for incubation and the zones were measured using Hi-media antibiotic zone scale. The index of the sensitivity was obtained by using Hi-media charts.

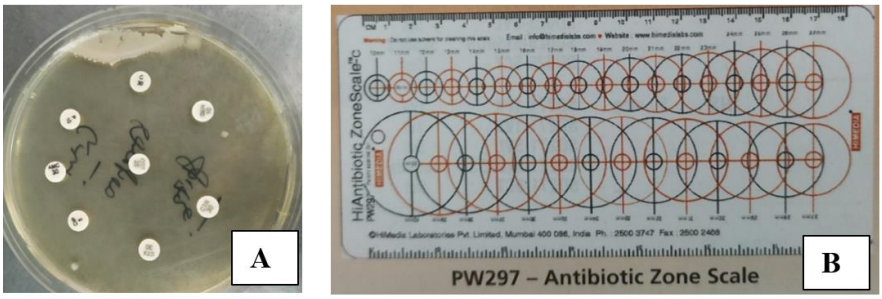

Fig 5: A) MHA plate showing antibiotics disc placement and B) Antibiotic zone scale pattern of antibiotic disc sensitivity

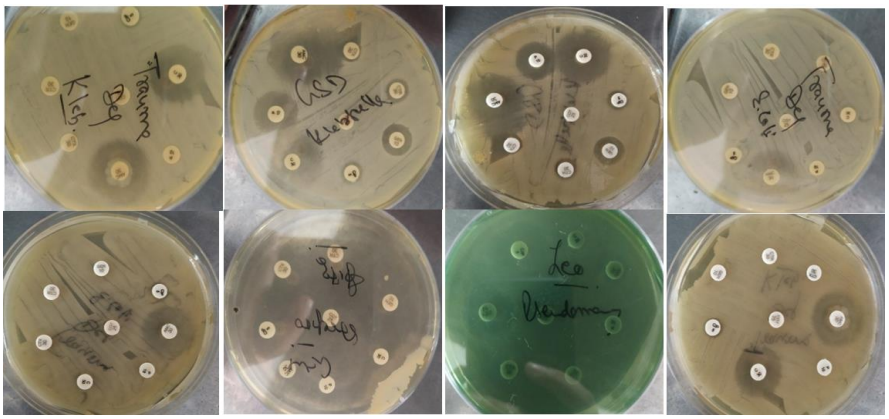

Fig 6: Plates of MH agar showing various antibiotic sensitive and resistant zones

Table 3: Measurement of zones of various antibiotic discs and their sensitivity

A)	***Klebsiella***	**Zone measured (mm)**	**Index**
	GEN	0	Resistant
	CTR	0	Resistant
	CTX	0	Resistant
	P	0	Resistant
	COT	0	Resistant
	AMC	16	Intermediate
	CIP	0	Resistant
	C	16	Intermediate
B)	***E. coli***	**Zone measured (mm)**	**Index**
	GEN	0	Resistant
	CTR	0	Resistant
	CTX	0	Resistant
	P	0	Resistant
	COT	0	Resistant
	AMC	20	Sensitive
	CIP	0	Resistant
	C	0	Resistant
C)	***Klebsiella***	**Zone measured (mm)**	**Index**
	GEN	12	Resistant
	CTR	10	Resistant
	CTX	0	Resistant
	P	16	Resistant
	COT	0	Resistant
	AMC	23	Intermediate
	CIP	10	Resistant
	C	22	Intermediate
D)	***Pseudomonas***	**Zone measured (mm)**	**Index**
	GEN	20	Sensitive
	CTR	19	Resistant
	CTX	15	Intermediate
	P	15	-
	COT	0	Resistant
	AMC	10	-
	CIP	26	Sensitive
	C	17	-

Conclusion

In the current study it was found that the chronic wounds were mostly resistant to the routine antibiotics used. It was also deduced that the organisms such as *Pseudomonas* were resistant to higher antibiotics such as ceftriaxone (CTR) and cefotaxime (CTX). However, they were sensitive to Gentamicin and Ciprofloxacin. This clearly points out to the fact that indiscriminate use of the higher antibiotics has led to the resistant microorganisms. The zones of the antibiotics show resistance towards the common antibiotics used and hence microbial characterisation and AST is the need of the hour to provide information about the microbes present in a particular wound or site and its particular remedial management. This practice will prevent the indiscriminate use of the antibiotics and hence, drug resistance.

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