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INVESTIGATIONS OF THE RELATIONSHIP BETWEEN BOWEL DERIVED ORGANISMS AND ANORECTAL SEPSIS PRE-OPERATIVE AND POST-OPERATIVE ANTIBIOTIC THERAPY OF THE COLON AND RECTUM

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ABSTRACT

The bacteriological study of anorectal sepsis showed that most cultures produced mixed organisms which were either aerobic or anaerobic or both. The most common organisms of perianal abscessess and low fistula were Staph.aureus and B. fragilis while in cases of ischiarectal abscesses and high fistulae, the most common organisms were E.coli and diphtheroids. There was no difference in the bowel derived organism and bacterial species isolated from anorectal sepsis. The organisms causing anorectal sepsis were very sensitive to neomycin sulphate followed by rifampicin. Emulsifying wax ointment base was found to be the best, among the different bases tested, for preparing antiseptic anorectal neomycin sulphate ointment. The bacteriological study of the specimen obtained from 30 patients , before and after preparation showed that the administration of oral and topical neomycin sulphate alone followed by colonic wash was the

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best while use of oral antibiotics only proved to be of low value. Also, it was found that neomycin sulphate 0.5% w/w in emulsifying wax ointment base and early treatment of anorectal sepsis gave good results.

INTRODUCTION

Anorectal sepsis is one of the humanity's most old and most common disease (1-3). The causative organisms as an aetiology for anoretal sepsis are many types according to different workers (4-6). Tyson and Spaulding (7) showed that mechanical preperation of the colon decreased faecal population. Unquestionably there are great advantages in operating on an empty colon, and preoperative mechanical bowel preparation is designed to secure that aim as far as possible (2).

The antibiotics have different disadvantages as noted by Page and Freeman (5). Therefore, the antibiotics can be used in combination with other methods as described by David and Wilson (4). The preoperative adminstration of some antibiotics to reduce the possibility of infectious complications following colorectal surgery is discussed in some reports (8,9). Also, topical antibiotics preparations containing neomycin sulphate for prevention of cutaneous infections following minor cuts, scraps and burns were discussed (10,11), Also, it is adminstered topically in treatment of infections of skin eye and nose due to susceptible Staphylococci. It's topical preparations is considered safe and effective (12). Neomycin solphate is poorly absorbed from GIT, wounds and inflammed skin. Hence, it has been given by mouth for the suppression of bacterial growth in the intestine before abdominal surgery (13).

The operative procedures of treating the abscess is to deal with the bowel contents to minimize the bacterial count (2). Also the diagnosis of anorectal sepsis depends upon the bacteriological examination of stool as well as pus or tissue from abscesses (2).

The aim of this work is to discuss the following :

1. Bacteriological study of different types of anorectal sepsis.

2. Bacteriological study of faecal specimens and correlation between bacteria present and causative organisms of anorectal sepsis.
3. The effect of different bowel preparation as preoperation procedure for colonic operation and anorectal sepsis.
4. Best line of treatment after operation.
5. The object of this study also included the investigation of neomycin sulphate antiseptic activity in various ointment bases for prophylaxis and treatment of anorectal sepsis.

EXPERIMENTAL

Materials :

1. All drugs, chemicals and materials were of analytical grade.
2. **Formulation** :

(A) Emulsifying ointment base (14) :

- White soft paraffin	500 gm
- Emulsifying wax	300 gm
- Liquid paraffin	200 gm

(B) Water absorption base (14) :

- Wool fat	10 gm
- Yellow soft paraffin	90 gm

(C) Oleaginous base (15) :

- Liquid paraffin	10 gm
- White soft paraffin	90 gm

Preparation of ointment bases in each case by melting the ingredients together and stir until cold.

Methods :

1. Samples from 50 patients with anorectal sepsis admitted in the surgical department of Assiut University Hospital and the General Hospital of Assiut were collected.

The collected pus samples were cultured on two sets of media, each composed of nutrient agar, blood agar, Mac-Conkey's media, one set was incubated at 37°C aerobically and the 2nd set anaerobically

on the preceeding media in addition to thioglycollate and cooked meat media for 48 hrs . Identification of the colonies was conducted according to Buchanan and Gibbon's (16),Bailey and Scott (17) and Cruickshank et al. (18).

Fourty out of the previous patients were chosen and were divided into 3 groups; 20 patients were subjected to mechanical wash, 10 patients took neomycin sulphate tablets (dose : 500 mg every 8 hrs , orally) for 48 hrs and 10 patients were subjected to both mechanical wash and administration of oral neomycin sulphate in the same dose. The additive effect of neomycin sulphate ointment baside oral adminstration and mechanical wash was studied. Faecal sample from each patient for bacteriological culture was taken before colonic preparation. A further specimen of faeces was also taken 24 hrs after the start of preparation and another one after 72 hrs at the end of preparation. One ml of intestinal fluid or one gram of stools was thoroughly suspended in 9 ml of Tryptic soya broth and serial dilutions made to 10^{-9}/ml. An aliquot containing 0.1 ml of the appropriate dilution was spread over the surface of the culture media such as Blood agar (18); cetrimide agar,Mac-Conkey's agar (18); Tryptone agar (18) and Mannitol salt agar. The plates were incubated at 37°C for 48 hrs for aerobic cultivation. Blood agar plates were incubated anaerobically for 72 hrs at 37°C. The isolated organisms from anaerobic cultivation were subcultured aerobically. These strains were considered anaerobic only after failing to grow aerobically. The concentration of organisms is expressed as the mean counted organisms/ml or gm of faeces. Suspicious-looking colonies from different media were examined morphologically and biochemically according to the methods described by Bailey and Scott (17) and Buchanan and Gibbon's(16).

The antibiotic sensitivity of the isolated strains from recto-anal fistula was determined by using disc diffusion method (19, 20) to detect the target antibiotics.

2. Preparation of neomycin sulphate ointments : Under aseptic conditions the base was melted on a water bath in an evaporating dish at the possible low temperature. The calculated amount of the pure

micronized drug was incorporated in the melted base. The mixture was allowed to cool to room temperature while contineously stirring to achieve homogenity of the drug in the base. The concentration of the drug in all the ointment bases was 0.5% w/w.

3. In *vitro* evaluation of antiseptic effect of neomycin sulphate ointments : According to the results of antibiotic sensitivity of the isolated strains from anorectal fistula neomycin sulphate was the most effective antibiotic. Hence, in *vitro* study of neomycin sulphate in topical ointments on Staph. aureus, which was the most common organisms of the anal fistula and bowel derived organisms , are important. A 0.1 ml of 24 hrs broth culture of Staph. aureus was inoculated into 15 ml of sterile molten nutrient agar. The inoculated medium was well mixed and poured into sterile petri dish. Six cups, each 11 mm in diameter were cut into each of inoculated plates using a sterile cork borer. The cups then aseptically filled with the respective ointment under test and then incubated for 24 hrs at 37^{0}. The diameters of the resulting inhibition zones were accurately measured.

RESULTS

Table 1, gives the types and frequencies of fistula while table 2 shows the frequency of mixed and single organisms in cultures. Table 3, shows the relationship between Bowel derived organisms and bacteriology of Anorectal sepsis. Table 4, shows the sensitivity and resistance of organisms causing anorectal sepsis to different antibiotics. Table 5, shows the antiseptic effect of different neo-neomycin sulphate ointment formulations. Table 6, gives the mean counts of different groups of bacteria in stool and after bowel preparation. Figures 1, 2 and 3, show the bacterial counts of 5 species before and after each bowel preparation.

DISCUSSION

The anorectal abscess develops in the intersphincteric spaces as an intersphiacteric abscess. The extension of this abscess results in perianal or ischiorectal abscess (Table 1). The bacter-

TABLE 1: Frequency of different types of fistula.

Type of lesion	Frequency	%
Peri and anorectal abscesses	17	21.25
Ischiorectal anorectal abscesses	3	3.75
Submucous anorectal abscesses	1	1.25
Low fistula	18	22.50
High fistula	7	8.75
Subentureous	2	2.50
Blind external fistula	1	1.25
Blind internal fistula	1	1.25
Total	80	

TABLE 2: The frequency of mixed and single organisms in cultures.

Culture	Mixed organisms	Single organisms	No. growth
Aerobic only	12	10	1
Anaerobic only	0	10	14
Both aerobic and anaerobic	30	-	3
Total of cultures	42	20	18

TABLE 3: Relationship between Bowel derived organisms and Bacteriology of Anorectal sepsis

Bacterial	Anorectal sepsis								Bowel derived
	Abscesses			Fistulae					
	Peri	Ischio rectal	Subcut-aneous	Low	High	Subcut-aneous	Ext.	Blind Inter	
Aerobic									
Ps.aeruginosa	-	-	-	-	-	-	-	-	9
Staph.aureus	5	3	-	3	3	1	-	-	6
Diphtheroid	4	-	-	6	4	-	-	-	-
Klebsiella	2	1	-	4	-	-	-	-	4
Proteus vulgaris	4	1	-	4	1	-	-	-	14
Strept.faecalis	2	-	-	3	1	-	-	-	9
Staph.epidermidis	2	1	1	1	-	1	1	-	2
E.coli	2	3	-	1	2	-	-	-	23
Micrococcus	-	-	-	-	-	-	1	-	2
Anaerobic:									
Peptococcus	3	3	-	2	1	-	-	-	2
B.fragilis	3	1	1	5	2	-	1	-	3
Cl.perfringens	3	-	-	4	1	-	-	-	-
Pepto streptococcus	-	-	-	2	1	-	1	-	5
Diphtheroid	4	-	-	1	2	-	-	-	-
B.melaninqenis	-	-	-	1	-	-	-	-	1
Total	34	13	2	37	18	2	4	-	80

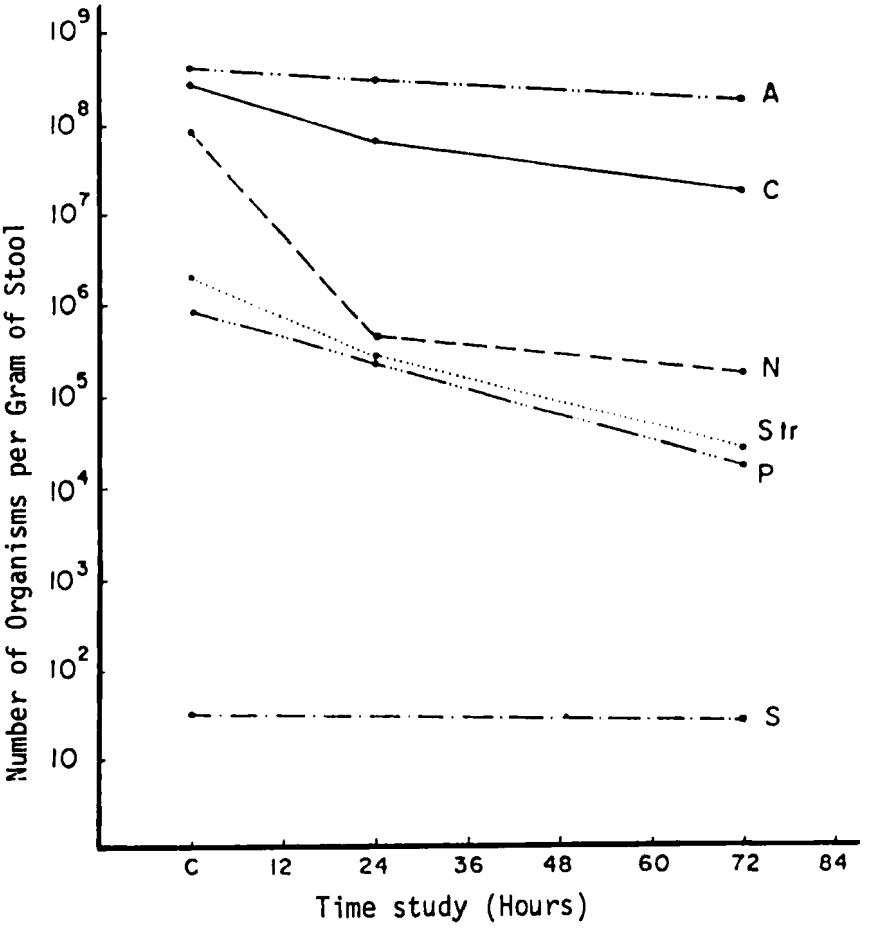

FIGURE 1. Mechanical Preparation

C : Coliform N : Non lactose fermenter A : Anaerobes
S.tr: Streptococcus P : Pseudomonus species
S : Staphylococcus

iological examination of anorectal lesions in this study showed that most cultures produced mixed organisms aerobically or anaerobically either skin derived organisms or gut derived organisms (Table 2). This concided with the work of Page and Freeman (5),and Ghoneim et al. (6). The most common aerobic organisms were Staph. aureus, diphtheroid, E. coli,klebsiella and Proteus vulgaris while the anaerobic ones were bacteroids especially B. fragilis and B.melaninogeni-

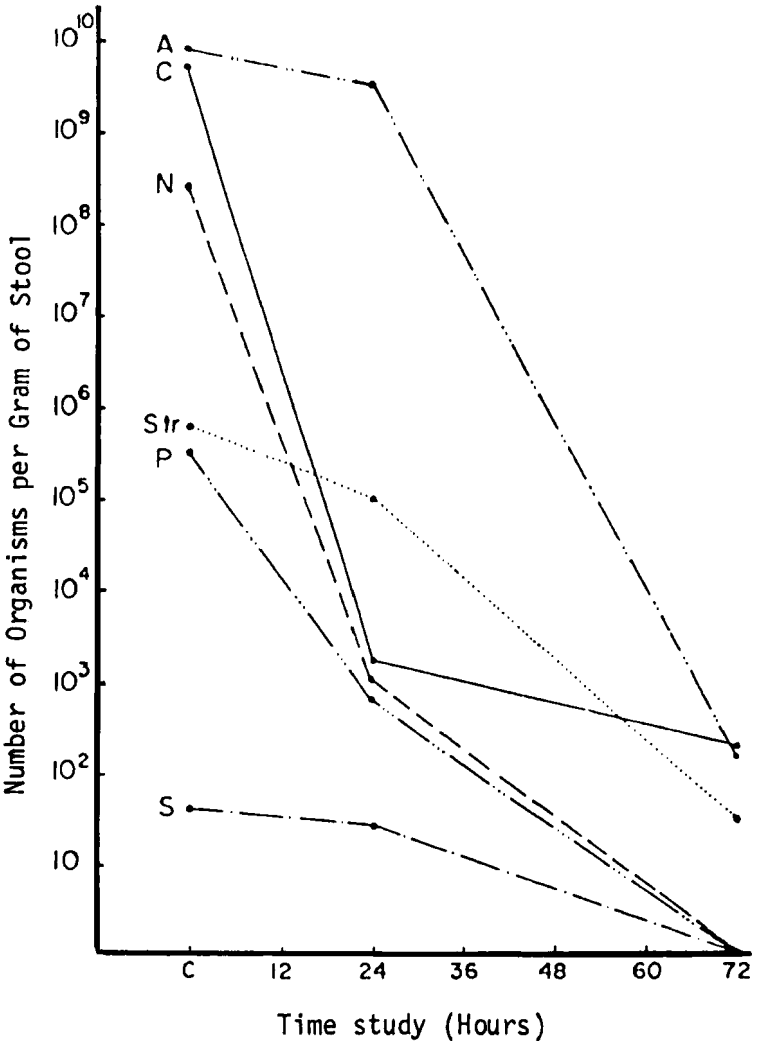

FIGURE 2. Neomycin Sulphate Preparation. Key:as in Fig.1

ous which constituted the majority of the causative organisms then came the peptococcus and clostridium perfringens (Table 3).

It was important to investigate the sensitivity and resistance of organisms causing the anorectal sepsis to different antibiotics. Table 4, obviously showsthat neomycin sulphate has the highest effect on the organisms causing the anorectal sepsis followed by rifam-

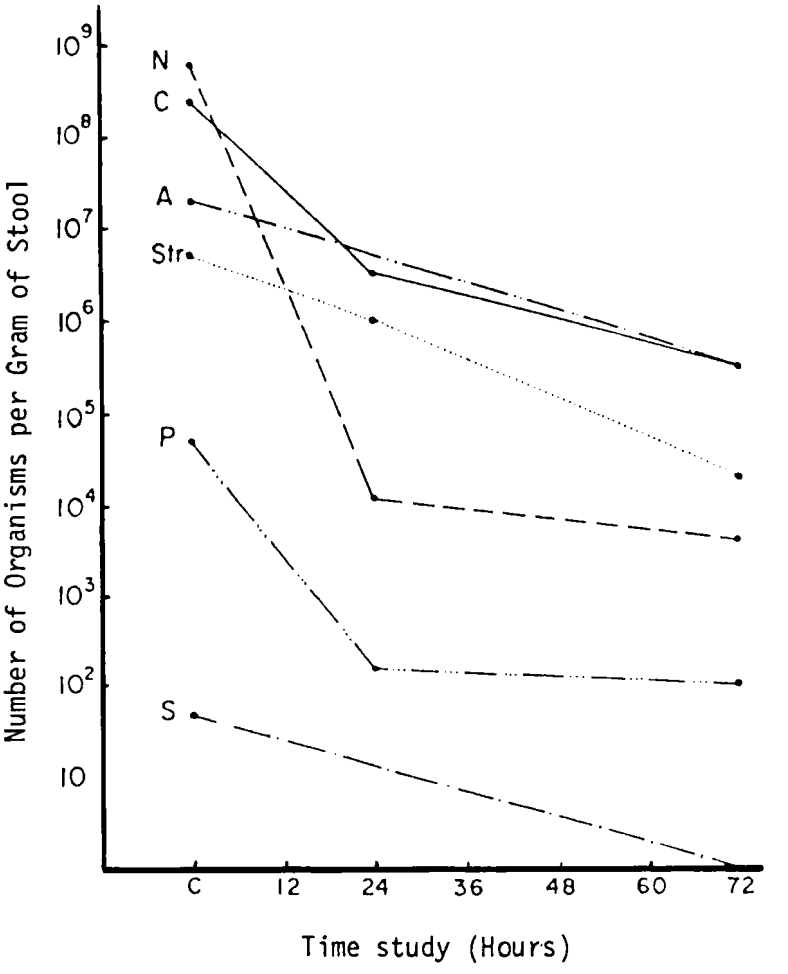

FIGURE 3. Mechanical and Neomycin Sulphate Preparation. Key: as in Fig. 1.

picin . The literature reported the favourable effect of rifampicin on the eradication of resistant Staph. aureus on a surgical unit (21) and in treatment of peritonities due to mycobacterium gastri (22). Hence, neomycin sulphate was the selected antibiotic for oral and topical administration in the in *vivo* and in *vitro* tests.

Since, the compositional variation effect the antimicrobial activity of the topical preparation (23 - 25) neomycin sulphate was

TABLE 4 : The sensitivity and resistance of organisms causing anorectal sepsis to different antibiotics.

	Staph. aureus		Diphtheroids		*E.Coli*		*Kelebsiella* sp.		*Proteus vulgaris*		*Streptococcus faecalis*		Peptococcus		*B. fragilis*		*C. perfringens*		Diphtheroids (anaerobic)	
No. of strains tested	15		14		8		7		10		6		8		13		8		7	
Antibiotics	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R
Rifampcin	11	4	13	1	5	3	5	2	10	0	6	0	4	4	10	3	4	4	5	2
Cefatrexium	7	8	7	7	4	4	2	5	4	6	1	5	4	4	2	11	2	6	2	5
Cefatrexyl	6	9	7	7	2	6	2	5	4	6	1	5	3	5	1	12	3	5	3	4
Cephaloridine	6	9	8	6	3	5	3	4	4	6	1	5	3	5	2	11	4	4	2	5
Cephaloxine	4	11	7	7	0	8	2	5	5	5	2	4	2	6	2	11	2	6	2	5
Pyopen	2	13	4	10	0	8	0	7	2	8	1	5	2	6	1	12	2	6	2	5
Thiophenicol	1	14	5	9	2	6	2	5	1	9	1	5	3	5	8	5	3	5	3	4
Clindamycin	0	15	5	9	0	8	0	7	0	10	0	6	4	4	10	3	4	4	4	3
Carbinicillin	1	14	3	11	1	7	0	7	2	8	0	6	4	4	2	11	4	4	3	4
Neomycin sulphate	**15**	**0**	**13**	**1**	**8**	**0**	**5**	**2**	**10**	**0**	**5**	**1**	**7**	**1**	**12**	**1**	**1**	**7**	**5**	**2**

S= Sensitive & R= Resistant

TABLE 5. In *vitro* Antimicrobial Effect of (0.5 w/w) Neomycin Sulphate in Three Different Ointment Bases.

Ointment Formulation	Minimum inhibition zones diameter, (mm)
1. Neomycin sulphate in emulsifying ointment base	26
2. Neomycin sulphate in water absorption base.	21
3. Neomycin sulphate in oleaginous base.	16

formulated in three different anorectal ointments. Neomycin sulphate are stable in the hydrophilic ointment formulations. Degradation was limited to 4-7% when the ointmnets were stored for one year(26). The diffusion rate of neomycin sulphate and accordingly the therapeutic effect were influenced by the bases ingredients. Quantitative informations appeared in the literature correlating drug release data with variation in the formulation composition (27, 28). Table 5 , shows relatively low drug diffusion occured from the oleaginous base.

TABLE 6: The mean counts of different groups of bacteria in stool before and after bowel preparation.

Bacterial species	Methods applied					
	Mechanical		Neomycin		Mechanical and Neomycin	
	B	A	B	A	B	A
Coliforms	$4x10^8$	$2x10^7$	$7x10^9$	$3x10^2$	$4x10^8$	$5x10^5$
Non lactose fermenter	$9x10^7$	$2x10^5$	$4x10^8$	-	$7x10^8$	$6x10^3$
Streptococci	$3x10^6$	$4x10^4$	$8x10^5$	5x10	$7x10^6$	$3x10^4$
Staphylococci	$5x10^6$	$4x10^4$	$6x10^5$	-	$7x10^6$	-
Anaerobes (Bacteroids)	$6x10^8$	$2x10^8$	$9x10^9$	$2x10^2$	$3x10^7$	$3x10^5$
Pseudomonas	$9x10^5$	$2x10^4$	$5x10^4$	-	$7x10^4$	$1x10^2$

B= Before preparation.
A= After preparation.

The fast neomycin sulphate release was obtained from the emulsifying ointment base followed by absorption base. This may be attributed to the miscibility of the emulsifying ointment base with the diffusion agar medium. This facilitate the drug transfer from this base while in case of oleaginous and absorption bases, the external phase is non polar and immiscible with the polar diffusion agar medium and hence a retarded effect drug release was shown. The effect of the type of the ointment base on the antimicrobial activity of neomycin sulphate was measured by the minimum inhibition zones diameter (mm). It is clear that neomcyin sulphate in emulsifying ointment base is most effective antiseptic formulation.

There was no correlation between the organisms grown and the clinical severity of the infection and this remark is in agreement with the work of Buchan and Grace (1) and Goligher (2). Infection remains the principal complication after colinic surgery, so suppression of the microflora of the colon before surgery would seem logical and desirable especially operation of anorectal fistula. Some workers showed that there was progressive reduction of total bacterial counts (29). Others showed that there was insignificant decrease in bacterial population consequent upon the diminution in the faecal mass, but the bacterial count of faeces remained the same (30, 31). Our results of mechanical preparation were more or less similar to that of Tyson and Spaulding (7). Bornside and Cohn (32) reported

that antimicrobials alone have little effect on the colonic content, unless the bulk of solid stool would be removed mechanically before their administration. Using neomycin sulphate alone followed by mechical preparation gave better results than after its usage without mechanical preparation (Table 6, Figures 1-3). Accordingly it is better to use oral neomycin sulphate followed by mechanical preparation. This method would suppress the posibility of introducing staphylococci and other pathogenic organisms into the colon, rectum and anus to avoid new anorectal sepsis. This was more ascertained by topical application of neomycin sulphate 0.5% w/w in emulsifying ointment base on the anorectal region.

According to the effect of neomycin sulphate on the microflora of the colon, it is observed that oral neomycine sulphate of great value in patients which are prepared for elective operative of colon or anorected fistula. A high in *vitro* antiseptic effect of neomycin sulphate ointments was also obtained on bacteria isolated from, anorectal sepsis. This study clearly demonstrated the effectiveness of neomycin sulphate followed by mechanical wash in reducing the possibility of infectious complications following colorectal surgery. A favourable clinical efficacy on the treatment of anorectal sepsis was obtained by topical application of neomycin sulphate 0.5 w/w in emulsifying wax ointment base.

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