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Detection of *Streptococcus bovis* from hospitalised patients with inflammatory bowel disease

By

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Abstract

This final year research project is about the detection of *Streptococcus bovis* (*S. bovis*) in hospitalized patients that suffers from inflammatory bowel disease or colonic carcinoma. From 50 samples collected from patients, one have been positively identified for *S.bovis*. From Chi-square analysis, it has been determined that there is no significant association between the presence of *Streptococcus bovis* species and the age, sex and the type of disease of hospitalized patients at HUSM. There is possible association between the race of patients and presence of the bacteri . Several factors regarding patients condition, validity of the sample and collecting and processing method have been identify as reasons why such results have been achieved.

Abstrak

Projek penyelidikan tahun akhir ini adalah mengenai pengesanan bacteria *Streptococcus bovis* dalam pesakit di hospital yang mengidap penyakit radang usus besar kronik atau barah usus. Dari 50 sampel yang diambil dari pesakit, satu adalah positif untuk *S. bovis*. Kesimpulan yang diperolehi ialah tiada hubungkait diantara kehadiran bacteria *S. bovis* dengan umur, jantina dan jenis penyakit yang dihidapi oleh peakit HUSM. Terdapat kemungkinan hubungkait di antara kaum dan kehadiran bakteria tersebut. Beberapa faktor yang perlu diambil kira adalah keadaan pesakit, cara mengumpul dan memproses dan kesahihan sampel. Faktor-faktor ini mungkin memberi kesan ke atas keputusan yang diperolehi.

1. Introduction

This dissertation is prepared as a partial fulfillment for the Bachelor of Health Science in Biomedicine. This project concerns about the detection of *Streptococcus bovis (S. bovis)* in hospitalized patients with inflammatory bowel disease (IBD) and colonic carcinoma. Research on detection of *S. bovis* in hospital settings had been performed by Klein *et.al.* (1977) and the results concluded that there are significant levels of *S.bovis* in IBD or colon cancer patients fecal isolates. Jean *et.al.* (2003) indicated that *S.bovis* is present in many types of IBD and not concentrated on a specific type of bowel disease. Waisberg *et.al.* (2002) indicated that many studies have warned of the need for investigation of colonic lesions among patients, especially elderly ones, who have bacteraemia and/or endocarditis from *S. bovis*. The common knowledge is that there is an association between S. bovis and carcinoma of the colon and this could have important clinical implications for early detection of the lesion (Leport *et.al.* ,1987). In contrast, Norfleet and Mitchell (1993) and Potter *et.al.* (1998) reported that there seems to be no clear association between *S. bovis* and human colorectal carcinoma. Other studies concerning *S. bovis* have been about trying to find association between the bacteria and colonic carcinoma. To date, there have been no conclusive evidence on whether the *S.bovis* directly causes colonic carcinoma or it's presence is a marker for bowel disease (Sinave, 2001).

Currently, there is no research conducted on detection of *S. bovis* from hospitalized patients with IBD and colonic carcinoma in HUSM. This study could prove vital in understanding the scope of this problem in HUSM.

1.2 Objectives

There are two main objectives for this project. First objective is to obtain and isolate *S. bovis* from fecal culture of samples from hospitalized patients. The second, positive tests will be tabulated using statistical analysis and any relationship between the carrier state, sex, age and race will be determined. This study will be used to compare whether the presence of *S. bovis* in hospitalized patients will be as significant as studies done previously.

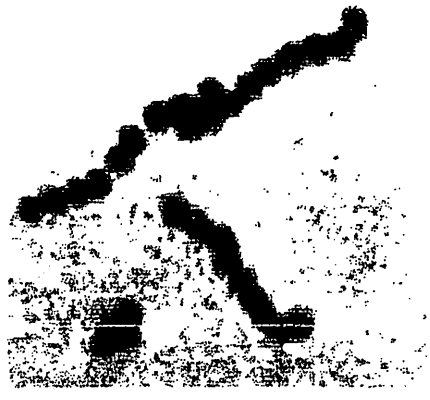
2. Literature Review

2.1 Characteristics of *Streptococcus bovis*

It is important to understand the general characteristics of *S. bovis* before any research can be performed in order to differentiate the bacteria from the rest of the species. *S. bovis* is a non beta-haemolytic gram positive-cocci in chains (figure 2.1). Previous studies indicated that *S. bovis* is a bacterium that can be found in cattle ruminaries (Skinner and Quensel, 1978). It is a normal inhabitant of cattle, but it might be transmitted to humans through contaminated milk or food. Recent studies still unable to find the proper mode of transmission of the bacteria from cows to human. What have been determined is that *S. bovis* causes 24% of all infective endocarditis cases (Songy *et.al.*, 2002).

S. bovis is a streptococcal group D bacteria (table 2.1). It have type D lipotechoic acid antigen on its outer cell wall. *S. bovis* also accounts for the majority of group D bacteria in human (Moellering *et.al.*,1974). The other types , *S equinea* and *S. alactolyticus* are only present in animal's gut system (Forbes *et.al.*,1998). There are two distinctive biotypes of *S. bovis* which are *S. bovis* I and *S. bovis* II (Farrow *et.al.*,1984). *S. bovis* II can also be further divided to subtypes II/1, which mostly affected endocardium region, and II/2 which usually isolated from bowel, urinary tract or biliary tract of patients (Collier *et.al.*,1998). This study will concentrate to the whole *S. bovis* bacteria and not trying to identify each specific biotypes. Groves (1997) indicated that *S. bovis* share other properties in common with other enterococci including their ability to grow in 40% of bile and to hydrolyze esculin. Growth in 6.5% Sodium Chloride (NaCl) or the pyrrolidonyl arylamidase (PYR) reaction can help in differentiating *S. bovis* from enterococci or viridian streptococci (Parker and Duerden, 1990).

2. 1 Figure of *S. bovis* in Blood Agar Media



(reproduced from www.qfever.com)

Table 2.1 Table showing medically important Streptococci

TABLE 13-1 Medically Important Streptococci

Type species	Lancefield serogroup	Normal habitat	Significant human disease
S pyogenes	A	Humans	Acute pharyngitis and others
S agalactiae	B	Cattle, humans	Neonatal meningitis and sepsis and infections in adults
S equisimilis	C	Wide human and animal distribution	Endocarditis, bacteremia, pneumonia, meningitis, mild upper respiratory infection
E faecalis			
S bovis (nonenterococcus)	D	Human and animal intestinal tracts; dairy products; bacteremia	Biliary or urinary tract infection, endocarditis
S anginosus	F, G[a]	Humans, animals	Subcutaneous or organ abscesses, endocarditis, mild upper respiratory infection
S sanguis[b]	H	Humans	Endocarditis, caries
S salivarius	K	Humans	Endocarditis, caries
None	O	Humans	Endocarditis
S suis	R	Swine	Meningitis
"Viridans" *S mitis*, *S mutans*[c]	None identified	Humans	Caries; endocarditis
Anaerobic or microaerophilic	None identified	Wide human and animal distribution	Brain and pulmonary abscesses, gynecologic infections
S pneumoniae	None identified	Humans	Lobar pneumonia and others

[a] Strains of the "*S milleri*" group (*S constellatus*, *S intermedius*, *S anginosus*, *minute strains*) may possess antigens of groups A, C, F, or G, or no identifiable Lancefield group antigens; a heterogeneous group, genetically related but with a wide variety of phenotypic and biochemical characteristics.
[b] Disparate grouping undergoing further definition.
[c] Other viridans streptococci (*S sanguis*, *S salivarius*, "*S milleri*," *S bovis*) have identified group antigens(a); nutritionally variant streptococci may be included in this diverse category.

(reproduced from http://gsbs.utmb.edu/microbook/ch013.htm)

2.2 Characteristics of the patients

Patients that are suitable for this project are those who are suffering from any type of inflammatory bowel disease or colonic carcinoma. Other types of patients that can be taken under considerations are those who are being treated for urinary tract infections, miscellaneous infections and bacteraemias. Patients from USM hospital will be asked to participate in this research. 50 patients will be determined and must agree to participate in the study. The patients will be picked randomly regardless of race, sex and age. This is in

order to get samples that are valid for this study and can be represented to the whole population. The patients must be able to defecate in order to obtain sample for isolation and identification. This is because the bacteria usually resides in large bowel of human. Patient's family members or guardians and HUSM staffs will informed if the sample need to be collected from patients who are incapable or debilitated.

Sinave (2001) found out that there is no significance differences in sex, race and geographical background for patients with *S. bovis* in their system. The bacteria is usually found in greater number in older patients with underlying colonic carcinoma and inflammatory bowel disease. Human factors such as patient's co-operation, difficulty in defecating and validity of collected sample needs to be addressed before any sample is taken. This will ensure that valid sample with patient's consent is taken for testing.

Streptococcus bovis is the main human pathogen among nonenterococcal group D streptococci. *S. bovis* is a well-documented cause of infective endocarditis (Ellen *et.al*,1990). The organisms were isolated more frequently from stools of patients with GI malignancies. It is unclear whether *S. bovis* plays a causative role in colon cancers or is only as a marker of the disease. There is no variation occurs among different geographical areas, race and sex. Nearly all patients with *S. bovis* are older than 50 years (Sinave, 2001).

Streptococcus bovis is a bacterium normally found in the rumen of ruminant animals such as cattle and sheep. *S. bovis* is also rarely isolated from humans but was reported to cause such disease as endocarditis and meningitis. Recent reports also suggest a correlation between increased levels of *S. bovis* and human colonic cancer. Differences in biochemical

characteristics between human and ruminal strains of *S. bovis* contribute to difficulties of identifying human isolates of the bacteria (Whitehead , 1999).

The association of Streptococcus bovis endocarditis with colonic neoplasia can be described and exceeds 50%. The prevalence of *S. bovis* in fecal cultures from patients with carcinoma of the colon is significantly increased (56%) as compared to controls (10%) and also patients with IBD (28%) (Zabiela *et.al.*, 2002)

Streptococcus bovis causes 24% of all streptococcal infective endocarditis cases from his studies of clinical isolates. The research also indicated that there are reports linking the presence of *S. bovis* bacteraemia and endocarditis with various forms of gastrointestinal disease, primarily colonic cancers. The research also found out that *S. bovis* biotype I strain is much more frequently isolated in cases of gastrointestinal disease (Songy , 2002).

It is not clear whether *S. bovis* plays an etiological role in carcinoma of the colon or is merely marker for the disease. Several studies have shown that increase in stool carriage in patients with malignant or premalignant lesion of the colon as compared with normal subjects from whom *S. bovis* is rarely isolated in stool cultures (Groves, 1997).

There is the need for investigation of colonic lesion among older patients, who have bacteraemia and/or endocarditis from *Streptococcus bovis*. Bacteraemia and infective endocarditis from endocarditis may be related to the neoplastic lesions in the large intestine. This report described patients who presented infective endocarditis from *S. bovis* associated with colonic carcinoma and tubular-villous carcinoma. The knowledge is that

there is an association between endocarditis from *S. bovis* and carcinoma of the colon has important clinical implications (Waisberg *et.al.*, 2002).

There is possible association between colonic adenocarcinoma and *S. bovis* endocarditis. Non-enterococcal Group D streptococci were isolated from fecal cultures of 11 of 105 controls, 35 of 63 patients with carcinoma of the colon, 7 of 25 with IBD, 4 of 21 with non-colonic neoplasms and 5 of 37 with GI disorders. The presence of *S. bovis* in fecal cultures from patients with carcinoma of the colon was significantly increased ($P<0.001$) as compared to those in controls, and also to all the other groups ($P<0.001$). Patients with inflammatory bowel disease were more frequently carriers. The carrier state was related to age, hospitalization status, colonic stasis, gastrointestinal bleeding or recent examination. The implications of this associations are unknown (Klein *et.al.*, 1977).

A prospective analysis of patients with *S. bovis* septicaemia had documented the presence of gastrointestinal lesion. 29 patients were studied and 15 of those undergone gastrointestinal evaluation. Two out of fifteen patients have GI neoplasms in the colon. This lesion ranged from adenomatous polyps to adenocarcinomas. From this report, it was suggested that patients with *S. bovis* septicaemia undergoes thorough investigation of the GI tract even in the absence of the symptoms (Klein *et.al.*, 1979).

3. Materials and Methods

3.1 Materials

3.1.1 Patient's sample collection

I. Questionnaires and Consent forms

II. Patient's fecal sample (50 samples)

Criteria for fecal sample:

- Patients are diagnosed with IBD or colonic carcinoma.
- Patients are not under medication prior to sample collection.
- Sample must be processed within 2 hours of collection.

III. Transport media (normal collecting bottle)

3.1.2 Patient's sample isolation

1. Blood Agar media (enrichment media)
2. MacConkey agar media (differential media)

3.1.3 Patient's sample identification

1. Optochin disks
2. Bile solubility test
3. Bile-esculin reaction
4. Growth in 6.5% NaCl
5. PYR (pyrrolidonylarylamidase reaction)
6. ESC (hydrolysis of esculin)

7. VP (Voges-Proskauer reaction)
8. Sugar broths : Mannitol , Melibiose , Sorbitol , Trehalose
9. New Strept grouping kit from Oxoid

3.1.4 Statistical analysis

SPSS software will be used to perform statistical analysis from the results of the studies .

3.2Methods

3.2.1 Samples collection

Fifty fecal samples (25 samples from male and 25 samples from female) were collected from patients suffering from inflammatory bowel disease or colonic carcinoma are identified. The patients are briefed with informed consent regarding the procedure and must agree to participate in the study. The patients are required to answer questionnaire concerning their conditions. The patients will be provided with specimen collecting bottle. The patients is asked to fill the collecting bottle with their stool with assistant from their guardians or HUSM staff. The fecal sample will be collected and sent to Microbiology Department Laboratory for identification and processing. The sample must be processed within two hours after sample collection.

3.2.2 Samples isolation

Each sample collected was streaked on Blood Agar media (BA) and MacConkey Agar media (Mac). The plates were incubated at 37°c overnight. Each plate is observed for colony appearance. The appearance of *S. bovis* is small, smooth, mucoid colony and non-haemolytic. Gram stain is performed on a slide using colony from the BA media. Microscopic appearance of Gram positive cocci in chains is an indication of presence of streptococcus species in the sample. Biochemical test needs to be performed in order to confirm the presence of *S. bovis* from the samples collected.

3.2.3 Samples identification

Several biochemical test can be use to indicate the presence of *S. bovis* in the sample . These tests helped in differentiate *S. bovis* from other pathogen.

3.2.3.1 Optochin disks

This test is used to determine the effect of optochin (ethylhydrocupreine hydrochloride) on an organism. Optochin disks is placed on the streaked area of suspected colonies on BA. Incubate the plate for 18-24 hours at 35-37°c in 5% CO2. Zone of inhibition (14mm) is measured. Optochin lyses pneumococci (positive test) but alpha-streptococci are resistant. The colony should remain intact for this test.

3.2.3.2 Bile solubility Test

Bile solubility test differentiate *S. pneumoniae* (positive) and alpha-haemolytic streptococci (negative). Bile or solution of bile salt, such as sodium desoxycholate lyses pneumococcal colonies. One or two drops of bile salts is placed on isolated colony on BA. Gently wash liquid over colony and incubate the plate at 35°c for 30 minutes. Lysis of colony is examined.

3.2.3.3 Bile Esculin Reaction

Gram positive bacteria other than group D streptococci and enterococci are inhibited by the bile in this medium. Group D streptococci can grow in the presence of 40% bile and hydrolyze esculin. They will turn the indicator, ferric ammonium citrate, a dark brown colour. One or two colonies from overnight culture is inoculated onto the surface of the slant. Incubate at 35° for 48 hours. Blackening of the agar slant will occur for positive results.

3.2.3.4 Growth in 6.5% NaCl

This test is used to determine the ability of an organism to grow in high concentration of salt. It is used to differentiate enterococci (positive) from nonenterococci (negative). The broth contain a small amount of glucose and bromcresol purple as the indicator for acid production. Colony from overnight culture is inoculated into 6.5% NaCl broth. Incubate overnight and check daily for growth. No turbidity and no colour change for *S. bovis.*

Pyrrolidonylarylamidase reaction (PYR), Hydrolysis of Esculin (ESC), Voges-Proskauer Reaction (VP), Sugar Broths and Strept grouping kit is not performed for this study. The use of Optochin disks reaction, bile esculin agar, bile solubility test and growth in 6.5% NaCl are adequate in order to identify the presence of *S. bovis* in fecal samples isolates .

3.2.4 Statistical analysis

The results collected from patients sample were tabulated. Statistical analysis were used to determine whether there is significance between the patients status and age, sex, race and hospitalization status. Chi-square (χ^2) analysis of the linkage was used to identify association between *S. bovis* and the parameters of age, sex, race and types of diseases. SPSS software was used for this analysis.

3.2.4.1 Calculation method for Chi-square (χ^2) analysis of the linkage

χ^2 analysis for the linkage between detection of *Streptococcus bovis* and the parameters

1) Hypothesis

Ho – There is an association between the parameters and detection of *S.bovis*

H_A – There is no association between the parameters and detection of *S. bovis*

2) Level of significance

Level of significance is set at $\acute{\alpha} \approx 0.05$

3) Assumption

i) Data is independent

ii) Expected frequency (EF) = 0 $\rightarrow \chi^2$

(EF) $< 5 \leq$ 20% of the cells $\rightarrow \chi^2$

(EF) $< 5 >$ 20% of the cells $\rightarrow$ Fisher's exact

4) Statistical test

Results

	+	−	
A	a	c	**a+c**
B	b	d	**b+d**
	a+b	**c+d**	**Total**

$$\chi^2 = \frac{\Sigma (O - E)^2}{E}$$

O = Observed outcome
E = Expected outcome

Degree of freedom (DoF) = (r – 1) (c – 1)

r = Total number of rows
c = Total number of columns

χ^2 calculated (P)

$$\frac{(a-E)^2}{a+b} + \frac{(c-E)^2}{c+d} + \frac{(b-E)^2}{a+b} + \frac{(d-E)^2}{c+d}$$

P =

χ^2 tabulated ($\acute{\alpha}$)

DoF = (r - 1) (c-1) =

$\acute{\alpha}$ = **(χ^2 critical values at level of significance≈ 0.05)**

5) Interpretation

χ^2 calculated , P = ____→ fall in rejection zone or acceptance zone

χ^2 tabulated , $\acute{\alpha}$ = ____

χ^2 cal > χ^2 tab → reject null hypothesis at P ≈ 0.05

χ^2 tab > χ^2 cal → accept null hypothesis at P ≈ 0.05

6) Conclusion

Δ There is **an** association between presence of S. bovis and parameters

Δ There is **no** association between presence of S. bovis and parameters

4.1 Results

The results of this study are summarized in table 4.1, which shows two cases of colonic carcinoma and twenty-three cases of IBD in male patients. Table 4.2 shows presence of colon cancer in two female patients and twenty-three cases of IBD. Table 4.3 indicates four samples that are positive for pure streptococcus species in male patients. Table 4.4 shows 3 samples that are positive for streptococcus in female patients. Seven samples in total are positive for streptococcus species. The seven samples were isolated and biochemical test performed on each sample. One sample was identified as positive for *Streptococcus bovis* from the biochemical test (Table 4.5)

Table 4.1 Male samples collected for isolation

No	Reg. no.	Sex	Age	Race	Diseases
1	B5	M	<50	M	IBD
2	B257983	M	<50	M	IBD
3	B261960	M	<50	M	IBD
4	B262691	M	<50	M	IBD
5	B062847	M	<50	M	IBD
6	B8	M	<50	M	IBD
7	4277279	M	<50	M	IBD
8	B3	M	<50	M	IBD
9	B262587	M	<50	M	IBD
10	B13	M	<50	M	Colonic carcinoma
11	B16	M	<50	M	IBD
12	B15	M	<50	M	IBD
13	B258352	M	>50	M	IBD
14	B263363	M	>50	M	IBD
15	B259385	M	>50	M	IBD
16	B17	M	>50	M	IBD
17	B14	M	>50	M	IBD
18	B254691	M	>50	M	IBD
19	B258084	M	>50	M	IBD
20	A122122	M	>50	M	IBD
21	B262330	M	>50	M	IBD
22	B262346	M	>50	M	IBD
23	B6	M	>50	M	IBD
24	B7	M	>50	C	Colonic carcinoma
25	B085956	M	>50	C	IBD

Age: <50 = under the 50 years of age
>50 = over the 50 years of age

Race: M = Malay
I = Indian
C = Chinese

Table 4.2 Female samples collected for isolation

No	Reg. no.	Sex	Age	Race	Diseases
26	A158937	F	<50	M	IBD
27	A014463	F	<50	M	IBD
28	B175620	F	<50	M	IBD
29	A158937	F	<50	M	IBD
30	B209630	F	<50	M	IBD
31	B188778	F	<50	M	IBD
32	B165603	F	<50	M	Colonic carcinoma
33	B242345	F	<50	M	IBD
34	B236189	F	<50	I	IBD
35	B259539	F	>50	M	IBD
36	B262729	F	>50	M	IBD
37	A168947	F	>50	M	IBD
38	B880144	F	>50	M	IBD
39	A837797	F	>50	M	Colonic carcinoma
40	B217259	F	>50	M	IBD
41	B259529	F	>50	M	IBD
42	A066498	F	>50	M	IBD
43	B045767	F	>50	M	IBD
44	B8259519	F	>50	M	IBD
45	A056198	F	>50	M	IBD
46	B258352	F	>50	M	IBD
47	A026285	F	>50	M	IBD
48	A885000	F	>50	M	IBD
49	A009482	F	>50	M	IBD
50	B023659	F	>50	C	IBD

Age: <50 = under the 50 years of age
>50 = over the 50 years of age

Race: M = Malay
I = Indian
C = Chinese

Table 4.3 Results of plate culture for male samples

No	Reg. no.	Plate cultures	Gram stain
1	B5	Mixed growth	G-ve bacilli
2	B257983	Small , pin-point	
3	B261960	Beta - haemolytic	
4	B262691	Beta - haemolytic	
5	B062847	Beta-haemolytic	
6	B8	Beta - haemolytic	
7	4277279	Small colony	G+ve diplococci
8	B3	Mixed growth	G-ve bacilli
9	B262587	Beta - haemolytic	
10	B13	Smooth , cream colour	**G+ve streptococci**
11	B16	Beta - haemolytic	
12	B15	Beta - haemolytic	
13	B258352	Mixed growth	G-ve bacilli
14	B263363	Smooth , cream colour	G+ve staphylococci
15	B259385	Smooth , cream colour	**G+ve streptococci**
16	B17	Smooth colony	
17	B14	Beta-haemolytic	
18	B254691	Beta-haemolytic	
19	B258084	Beta - haemolytic	
20	A122122	Smooth ,cream colour	G+ve staphylococci
21	B262330	No growth (salmonella?)	
22	B262346	Smooth , cream colony	**G+ve streptococci**
23	B6	Beta - haemolytic	
24	B7	Smooth , cream colour	**G+ve streptococci**

Table 4.3 (continued)

25	B085956	Small colony	G+ve diplococci

Table 4.4 Results of plate cultures for female samples

No	Reg. no.	Plate cultures	Gram stain
26	A158937	Mixed growth	G-ve bacilli
27	A014463	Smooth ,cream colony	G+ve staphylococci
28	B175620	Smooth , cream colony	G+ve staphylococci
29	A158937	Beta - haemolytic	
30	B209630	Beta - haemolytic	
31	B188778	Beta - haemolytic	
32	B165603	Smooth , cream colony	G+ve staphylococci
33	B242345	Mixed growth	G-ve bacilli
34	B236189	Mixed growth	G-ve bacilli
35	B259539	Beta - haemolytic	
36	B262729	Mixed growth	G-ve bacilli
37	A168947	Beta - haemolytic	
38	B880144	Smooth cream colony	**G+ve streptococci**
39	A837797	Beta - haemolytic	
40	B217259	Beta - haemolytic	
41	B259529	Smooth cream colony	**G+ve streptococci**
42	A066498	Beta - haemolytic	
43	B045767	Mixed growth	G-ve bacilli
44	B8259519	Mixed growth	G-ve bacilli
45	A056198	Smooth , cream colony	G+ve staphylococci
46	B258352	Not significant	

Table 4.4 (continued)

47	A026285	Beta-haemolytic	
48	A885000	Smooth , cream colony	**G+ve streptococci**
49	A009482	Beta-haemolytic	
50	B023659	Small-pinpoint colony	

Table 4.5 Results of biochemical testing on positive samples of *Streptococcus* species

No	**Reg. no**	**Optochin disks**	**Bile solubility**	**Bile esculin Reaction**	**6.5% NaCl**
10	B13	Resistant	Negative	Negative	
15	B259385	Resistant	Negative	Negative	
22	B262346	Resistant	Negative	Negative	
24	**B7***	**Resistant**	**Negative**	**Positive**	**Negative**
38	B880144	Resistant	Negative	Negative	
41	B259529	Resistant	Negative	Positive	Positive
48	A885000	Resistant	Negative	Negative	

* Sample positive for *S. bovis*

It had been determined in the Table 4.5 that patients with the registration number B7 was positive for *Streptococcus bovis* species. This was determined by biochemical testing that indicated the presence of group D streptococci in the patient's stool sample .

4.2 Statistical Analysis

The statistical analysis is performed in order to verify whether there was significant presence of *Streptococcus bovis* species in hospitalized patients in HUSM . Chi – square analysis will be performed on the positive sample against the age (4.6.1), sex (4.6.2), diseases (4.6.3) and races (4.6.4) of the patients . Calculations for 4.6.1 , 4.6.2 , 4.6.3 and 4.6.4 can be viewed in Appendix D

4.2.1 Chi-square (χ^2) analysis for the linkage between detection of *Streptococcus bovis* and the age of patients

Δ There is no association between the presence of *Streptococcus bovis* and the age of patients

4.2.2 χ^2 analysis for the linkage between detection of *Streptococcus bovis* and the sex of patients

Δ There is no association between the presence of *Streptococcus bovis* and the age of patients

4.6.3 χ^2 analysis for the linkage between detection of *Streptococcus bovis* and the disease of patients

Δ There is no association between the presence of *Streptococcus bovis* and the disease of patients

4.6.4 χ^2 analysis for the linkage between detection of *Streptococcus bovis* and the race of patients

Δ There is an association between the presence of *Streptococcus bovis* and the disease of patients

5. Discussion

5.1 Discussion on collection and processing of samples

All the 50 samples obtained from the patients were collected and processed in Microbiology Laboratory in Pusat Pengajian Sains Perubatan (PPSP). After culturing the samples, seven samples have been identified as *Streptococcus* species. The other samples were tested negative for *Streptococcus* species. The seven positive samples was tested using biochemical test. The results indicated that only one sample tested positive for presence of *Streptococcus bovis.* The positive sample came from a Chinese patient suffering from colonic carcinoma.

The study showed that one of four (25%) patients with colonic carcinoma and none out of forty - six samples from IBD patients are positive for *S.bovis.* This shows that 4% of all samples collected is positive for the bacteria. Further breakdown shows that none of the samples from Malay patients (0 in 46), one in three Chinese patients (33%) and none in Indian patient (0 in 1) is positive for *S. bovis.* The bacteria is present in 1 of 25 male samples (4%) and none in 25 female samples. There is no presence of this bacteria in 21 patients under 50 years old and 1 in 29 (3.5%) patients over 50 years old. Klein *et.al.* (1977) found out that 56% patients with colonic carcinoma and 28% with IBD had *S. bovis* in their system. Similar studies by Zabiela *et.al.* (2002) shows that prevalence of *S. bovis* from fecal culture in colonic carcinoma patients usually exceeds 50% and 28% positive results in patients with IBD. Several other studies also reported similar numbers in their