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The bacteriology of recurrent pyogenic cholangitis and associated diseases

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SUMMARY

Ninety-five patients with recurrent pyogenic cholangitis had a range of specimens taken at laparotomy for bacterial culture. Bacteria were isolated from 68 % of cases. *Escherichia coli* was the predominant pathogen. Other aerobic and anaerobic intestinal bacteria were also isolated. Liver biopsy, bile and gallstones were the most rewarding specimens for culture. Infection was usually localized, but systemic infection occurred occasionally. The site of infection is probably in the liver parenchyma; however, the route by which intestinal bacteria invade the liver is not known. Sixty per cent of the cases of recurrent pyogenic cholangitis had gallstones and 20 % were infected with *Clonorchis sinensis*.

INTRODUCTION

The term 'Recurrent Pyogenic Cholangitis' (RPC) was first applied to a biliary tract disease by Cook *et al.* (1954). These workers believed that RPC, which has also been called Cholangio-hepatitis (Fung, 1961) and which is very common in Chinese people in Hong Kong, is a primary pyogenic infection of the biliary tract and not a sequel to the cholecystitis often found in European patients.

The pathological findings in RPC are mainly the histological reactions of the biliary tract against the invading organisms. These reactions are similar to those seen in clonorchiasis complicated by bacterial infection (Hou, 1955). *Escherichia coli* is the commonest infecting organism (Cook *et al.* 1954; Hou, 1955; Ong, 1968; Wong, 1978). However, *Salmonella typhi* has been implicated in this disease (McFadzean & Ong, 1966).

Apart from infection, two additional conditions are common in RPC patients. Gallstone formation is very frequent (Cook *et al.* 1954; Ong, 1962; Teoh, 1963; Wong, 1978), and the eggs of *Clonorchis sinensis* were found in 25 % and 21 % of specimens of bile from RPC patients by Ong (1962) and Wong (1978) respectively.

Clinically, a triad of symptoms has been described for this disease, namely pain, fever and jaundice (Cook *et al.* 1954; Ong, 1962). In 1968 Ong added vomiting to

this triad, and also claimed that RPC was one of the most common acute abdominal emergencies admitted to hospital in Hong Kong.

In this paper we describe bacteriological findings in 95 Chinese patients with a clinical diagnosis of RPC who were admitted for laparotomy to the surgical units of two general hospitals, Queen Mary Hospital and Kwong Wah Hospital, in Hong Kong.

MATERIALS AND METHODS

Collection of clinical specimens

Twenty-five patients investigated in 1975 had one or more of the following specimens taken for study: (A) portal blood, (B) lymph node, (C) liver biopsy, (D) duodenal juice or swab, (E) gall bladder bile, or swab, (F) common bile duct bile, or swab, and (G) peripheral blood. In order to avoid cross-contamination of the specimens in the theatre these specimens were always collected strictly in the order A to G detailed above.

In 1977 a further 70 patients with RPC who also had gallstones were investigated. In this group collection of specimens was reduced to the minimum needed to make a definite diagnosis, and only gall bladder bile or swab and intact gallstones were taken for study.

Processing of clinical specimens

Two different liquid media were available for direct inoculation of the surgical specimens in the operating theatre. Lymph nodes, liver biopsies and blood (for anaerobic culture) were placed in Robertson's cooked meat medium. Nutrient broth was inoculated with duodenal juice and with blood for aerobic culture. Bile was transferred directly into a sterile plain bottle for transfer to the laboratory, where a portion was also inoculated into Robertson's cooked meat medium and into nutrient broth, and a sample also examined for eggs of *Clonorchis sinensis*.

After overnight incubation at 37 °C the broth cultures were subcultured to horse blood agar and MacConkey agar plates which were incubated aerobically at 37 °C for a further 24 h and to horse blood agar with and without added neomycin for anaerobic culture at 37 °C in an atmosphere containing 5% CO_2 for 48 h. All bacteria isolated were identified by conventional methods.

Culture of gallstones

Intact gallstones were cleaned in sterile saline, soaked in 70% ethanol and flamed. Successful sterilization of the surface of the stone was confirmed by washing the stone in nutrient broth which was then incubated at 37 °C aerobically and anaerobically, and in all cases yielded no growth.

The stones were then crushed and ground in a sterile mortar. The pulverized material was shaken with nutrient broth for 30 min and the broth then streaked on blood agar and MacConkey agar plates for aerobic culture and inoculated into Robertson's cooked meat broth for anaerobic culture.

Identification of eggs of Clonorchis sinensis

A simple concentration method was applied by centrifugation of the rest of the bile specimen after culture was done. Sediment was directly examined under the

Table 1. *Results of bacterial culture of surgical specimens from* 25 *patients studied in* 1975

	Portal blood	Lymph node	Liver biopsy	Duodenal juice	Gall bladder bile	CBD bile	Peripheral blood	Overall total
	Ec (3)	Ec (3)	Ec (8)	Ec (7)	Ec (7)	Ec (10)	Ec (1)	—
	Pv (1)	Pv (1)	K (3)	K (3)	K (2)	K (3)	NG (14)	—
	C (1)	NG (3)	SN (1)	C (2)	C (1)	C (3)	—	—
	NG (10)	—	Pa (3)	Pa (1)	SN (1)	Pa (1)	—	—
			Pm (1)	Af (1)	Pa (1)	Pm (2)	—	—
			NG (4)	NG (1)	NG (3)	NG (3)	—	—
No. of cases	15	7	17	14	15	17	15	25
No. positive	5	4	13	12	12	14	1	17
Positive (%)	33	57	77	86	80	82	0 7	68

The figures in parentheses indicate the number of positive cases.

Af, *Alkaligenes faecalis*; Pm, *Proteus mirabilis*; C, *Coliform* bacilli; Pv, *Proteus vulgaris*; Ec, *Escherichia coli*; SN, Non-haemolytic streptococci; K, *Klebsiella* species; NG, No growth; Pa, *Pseudomonas aeruginosa*.

Table 2. *Results of bacterial culture from 70 patients studied in 1977*

	Bile				Gallstones				Total	
	Aerobic		Anaerobic		Aerobic		Anaerobic		Aerobic	Anaerobic
	Ec	25	Cp	3	Ec	26	Cp	9	—	—
	K	19	Bf	2	K	20	Bf	2	—	—
	Pa	7	NG	41	Pa	2	NG	35	—	—
	Sv	1		—	Sv	1		—	—	—
	Sf	6		—	Sf	6		—	—	—
	Pv	2		—	Pv	1		—	—	—
	Pm	1		—	Pm	1		—	—	—
	Ck	1		—	Ca	1		—	—	—
	NG	27		—	NG	10		—	—	—
No. studied		70		70		70		70	70	70
No. positive		43		4		35		10	45	11
Percentage positive		61		6		50		14	64	16

Bf, *Bacteroides fragilis*; Pa, *Pseudomonas aeruginosa*; Ca, *Candida albicans*; Pm, *Proteus mirabilis*; Ck, *Candida krusei*; Pv, *Proteus vulgaris*; Cp, *Clostridium perfringens*; Sf, *Streptoccus faecalis*; Ec, *Escherichia coli*; Sv, *Streptococcus viridans*; K, *Klebsiella* species; NG, No growth.

light microscope. A positive case was determined by finding the characteristic eggs of *Clonorchis sinensis*, which are flask-like in shape with an operculum at one end and bile-stained. The size of a typical egg was 16 by 20 microns.

RESULTS

In 1975, 17 of the 25 patients (68%) had bacteria isolated from one or more of the specimens examined (Table 1). Liver biopsy and common duct bile gave more positive results than the other specimens examined. Anaerobic culture was not done. Fifteen of the patients had gallstones as well as RPC; there was a close correlation between the presence of gallstones and the presence of bacteria (Table 2).

In 1977 aerobic bacteria were isolated from bile or gallstones or both from 45 (64·3%) of the 70 cases studied. Anaerobic bacteria were isolated from 11 (15·7%) of the cases. The bacteria isolated are detailed in Tables 1 and 2. Mixed infections were common, and in rather more than a quarter of the cases yielding bacteria from both bile and gallstones the bacteria isolated from bile differed from those isolated from the stones.

Eggs of *Clonorchis sinensis* were found in 20% of the patients with RPC by examination of the bile specimens. 15·6% of patients with positive bacterial cultures and 28% of uninfected patients had clonorchis eggs in the bile (Table 3).

DISCUSSION

Recurrent pyogenic cholangitis is an insidious disease. The degree of severity fluctuates from time to time and from patient to patient. In our study patients were initially classified into mild, moderate and severe acute and chronic stages by their clinical manifestations. We expected that specimens collected from

Table 3. *Incidence of gall stone formation in patients with RPC*

Bacterial culture	No. of cases studied	Stone formation	
		No. of cases	%
Positive	17	12	70·6
Negative	8	3	37·5
Total	25	15	60·0

patients with severe acute illness would yield a higher positive culture rate, since RPC is a chronic disease with acute exacerbations due to repeated infections. However, we were unable to establish a satisfactory correlation between clinical severity and positive cultures obtained at operation except that in summer acute cases were more commonly encountered and more positive cultures were obtained. The relationship between the disease and seasonal factors has been discussed by Wong (1978).

Escherichia coli, enterococci and *Clostridium perfringens* are human intestinal bacteria, and were the predominant pathogens isolated in this study. Other intestinal bacteria, including *Klebsiella* species, *Pseudomonas aeruginosa*, *Proteus* species and *Bacteroides fragilis* were also found. The route of entry of these intestinal bacteria into the hepatic system is unknown.

Aerobic bacteria were isolated more frequently than anaerobes. The low isolation rate of anaerobic bacteria may reflect the natural character of the disease or may be due to the nature of the various surgical specimens. For example, anaerobic bacteria were more commonly isolated from the cores of stones than from bile. This might be due to the adverse effects of the chemical properties and oxygen content of the bile.

In our series, 60 % of RPC patients had gallstones, and of patients with stones, 80 % had bacterial infection of the biliary system.

The bacterial species isolated from bile and from gallstones from any patient were often identical, and it is likely that the bacteria in both bile and stones originate in the same infected lesion (Wong, 1978). We suggest that the primary site of infection in patients with RPC is likely to be in the liver parenchyma, and that bacteria may be present in the hepatic cells, sinusoids or portal areas of the liver.

The high incidence of isolation of bacteria from the interior of the stones may indicate that bacterial cells can provide the primary nucleus around which the stones form. In this way, in RPC patients infection may indicate stone formation, and the stones in the biliary tract then lead to persistent infection in a vicious circle similar to that involving renal stones and urinary tract infection (Hou, 1955, 1964). Eradication of the infection is rarely possible without complete removal of the stones from the infected system.

The initial infection in RPC patients may be related to infestation with the trematode *Clonorchis sinensis*, an association which has been described as concomitant bacterial infection (Wong, 1978). Examination of bile samples revealed clonorchiasis in only 20 % of the patients in this study, but a higher incidence might have been obtained if stool samples had also been examined (Wong, 1978). It is

also possible that once bacterial infection has been established the invading bacteria produce conditions unfavourable to the infective larvae of the worms, which may be killed but which leave a persisting bacterial infection (Wong, 1978). This would explain why, in this series, we found eggs of *Clonorchis sinensis* more often in the absence of bacterial infection than in its presence.

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