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Isolation of Anaerobic Bacteria from Clinical Specimens in Veterinary Medical Teaching Hospital

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Abstract : The emergence of bacterial resistance to antibiotics during therapy is a matter of great problem in clinical medicine. This may be because many veterinarians have used inappropriate antibiotics without bacteriological culture. Therefore, the purpose of this study is to determine isolation of anaerobic bacteria as pathogens from veterinary clinical specimens as well as susceptibility pattern for choosing antibiotics. Various anaerobic bacteria were isolated from clinical specimens of dogs, cats, rabbits at Veterinary Medical Teaching Hospital of Seoul National University from May 2001 to October 2002. The total number of isolated anaerobic bacteria was 13 isolates; *Bacteroides* spp. (3 isolates), *Fusobacterium* spp. (2 isolates), *Peptostreptococcus* spp. (2 isolates), *Porphyromonas gingivalis* (2 isolates), *Prevotella* spp. (3 isolates), and *Propionibacterium acnes* (1 isolate). For evaluating the antibiotic susceptibility patterns of the isolates, disk diffusion method was used. All isolates were susceptable to all tested antibiotics except only one *Fusobacterium varium* was resistant to norfloxacin

Key words **:** antimicrobial susceptibility, anaerobic bacteria, dog, cat, rabbit

Introduction

Antibiotics are essential in the therapy of animal diseases, especially in the bacterial infections.

The presence of anaerobic bacteria as well as aerobic bacteria as pathogens is important for choosing the antimicrobial agents to be used. Anaerobic bacteria may colonize many different anatomical sites as part of the indigenous microflora of animals[28]. Many anaerobic infections in animals are caused by bacteria that may be part of the hosts normal flora[28]. Infection with aerobic bacteria can make the local tissue conditions more favorable for the growth of anaerobes. Conditions predisposing patients to anaerobes infections include the exposure of sterile sites to a high inoculum of indigenous mucous membrane flora, reduced blood supply, and tissue necrosis, which lowers the oxidation-reduction potential and favors the growth of anaerobes[6]. Conditions that lower the blood supply include trauma, foreign body, malignancy, surgery, edema, and shock. The host defenses can become impaired by anaerobic conditions and anaerobic bacteria. Many anaerobic infections in animals are caused by bacteria that may be part of the hosts normal flora[28].

The establishment of anaerobic bacteria as pathogens has occurred since Pasteur first isolated anaerobic bacteria and for over 100 years their importance in medicine has been variably recognized[10]. However, a comprehensive view of their role as normal flora, and thus potential pathogens, in the animal species encountered in veterinary medicine is lacking. This is, in part, due to their fastidious growth requirements, both of which may not be available in veterinary medicine. Anaerobic bacteria will not grow on the routine media incubated in 5-10% in room air. As in human medicine, anaerobic bacteria isolated from animals have been associated with a number of different diseases process[28].

In this study, we report anaerobic bacteria isolated from animal patients in Veterinary Medical Teaching Hospital of Seoul National University as well as susceptibility pattern. The purpose of this study is the isolation of anaerobic bacteria as pathogens.

Materials and Methods

Bacterial isolates

Anaerobic bacteria were isolated from clinical specimens of dogs, cats, and rabbits at Veterinary Medical Teaching Hospital of Seoul National University from May 2001 to October 2002. Clinical specimens in culturing anaerobic bacteria were swabs of purulent material, pus, draining tracts, lower respiratory tract, nasal discharge, peritonial aspirates, ear, eye, urine, postoperative infection site, and genital system. Tracheal washing and nasal discharge were collected from the dog with chronic respiratory tract infection.

Anaerobic bacteria were isolated from tumors or tissue damage of urinary bladder, although they rarely caused urinary tract infection[3].

Clinical specimens were collected by the swab method using Amies transport medium (BBL Culture swab plus®: BBL Microbiology System, MD, USA). Liquid specimen which was collected by the needle method was plated directly on the agar. Urine specimens were collected by cystocentesis which was sterile[9,19].

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Anaerobic culture from clinical specimens

The isolation of anaerobic bacteria requires appropriate methods of collection, transportation, and cultivation of specimens[14]. The most convenient method for culturing anaerobic bacteria in the clinical laboratory includes the use of selective and nonselective plate media and incubation of the plates in an anaerobic environment[7,25]. Media for initial processing always included blood agar containing 5% of the defibrinated sheep blood (KOMED, Korea), MacConkey agar (KOMED, Korea), and brucella blood agar (KOMED, Korea) as nonselective agars, and phenylethyl alcohol agar (PEA) (KOMED, Korea) as a selective agar. PEA was used for inhibition of facultative Gram-negative bacilli[27]. Among them, brucella blood agar and PEA were stored in the GasPack® jar (Becton Dickinson Microbiology Systems, MD, USA) with GasPack Plus®(Becton Dickinson Microbiology Systems, MD, USA) before incubation. Plates used for initial processing of specimens were maintained in a reduced environment.

Swabs were inoculated onto blood agar (KOMED, Korea), MacConkey agar (KOMED, Korea), brucella blood agar (KOMED, Korea), and PEA (KOMED, Korea). Blood agar (KOMED, Korea) and MacConkey agar (KOMED, Korea) were incubated aerobically at 37°C for 24 hrs, whereas brucella blood agar (KOMED, Korea) and PEA (KOMED, Korea) were placed in GasPack® jar (BBL Microbiology Systems, Cockeysville, MD) at 37°C for 48 hrs.

After comparison of colonies on aerobic plates with anaerobic plates, colonies appeared on only anaerobic plates were selected. Subculture was done onto chocolate agar (incubated aerobically for 24 hrs) to test for aerotolerance and onto brucella blood agar (incubated anaerobically for 48 hrs) to obtain a pure culture[7]. If colonies appeared on the chocolate agar, these were considered as facultative anaerobes[20]. After the anaerobic character of the isolate was confirmed, the Gram stain classification was determined. Identification of isolated anaerobes was based on colony morphology, Gram-staining and production of indole, and analysis with ANI card by VITEK system (bio Merieux Vitek, Hazelwood, MO, USA)[17].

Thioglycolate was used as the usual backup medium[11].

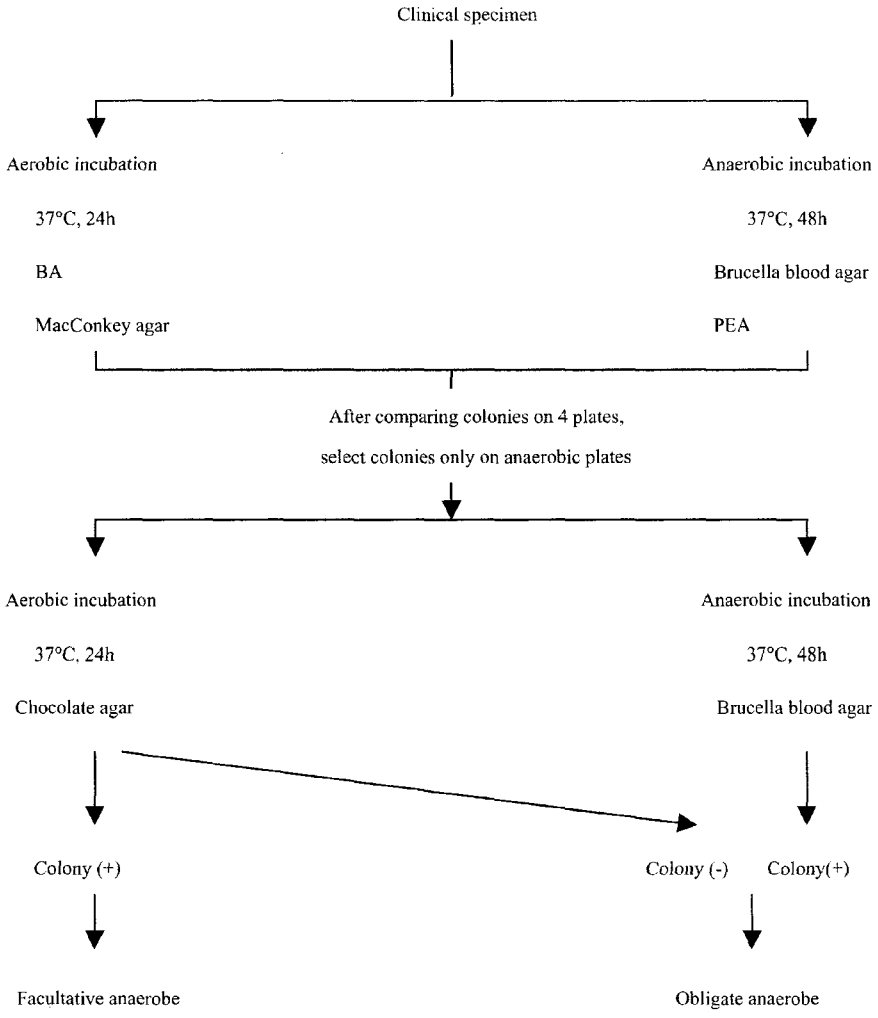

Fig 1. Schematic presentation of anaerobic culture processing. BA: blood agar containing 5% of defibrinated sheep blood agar; PEA: phenylethylalcohol agar.

Antimicrobial susceptibility testing for isolated anaerobic bacteria

The susceptibility test of isolates was determined by the disk diffusion method. Bacterial suspension of the anaerobic isolate (McFarland 0.5) was spread over the surface of a plate of reduced brucella blood agar by the direct suspension method[27,29]. Antibiotics used were penicillin G (10 unit), amoxacillin/clavulanic acid (20/10 μg), ciprofloxacin (5 μg), norfloxacin (10 μg), cephalothin (30 μg), ceftazidime (30 μg), clindamycin (2 μg) (BD Biosciences, USA). The plate was inverted and placed it in an incubator at 37°C anaerobically. After 48 hrs, the diameters of the zones of complete inhibition were measured.

Results

Isolated anaerobic bacteria from clinical specimens and antimicrobial susceptibility

This study shows the involvement of 13 strains of anaerobic bacteria. These came from 9 dogs, 3 rabbits and 1 cat. These animal patients have suffered from chronic diseases although they have been treated with antibiotics which were focused on aerobic bacteria for a long time.

Most of anaerobic isolates were isolated from abscess, necrotic tissue, and postoperative inflammation site. Anaerobic isolates were *Bacteroides* spp. (3 isolates), *Fusobacterium* spp. (2 isolates), *Peptostreptococcus* spp. (2 isolates), *Porphyromonas gingivalis* (2 isolates), *Prevotella* spp. (3 isolates), and *Propionibacterium acnes* (1 isolate) (Table 1).

Additional information, all of 12 anaerobic isolates, except *Fusobacterium varium*, was susceptible to all tested antibiotics (Table 2). Only one strain of *Fusobacterium varium* was resistant to norfloxacin.

Table 1. Anaerobic bacteria from clinical specimens

Organism	No. of Isolates	Sampling site	note (species)
Bacteroides spp.	3		
B. distasonis	1	eye	rabbit
B. eggerthii	2	nasal discharge	dog
Fusobacterium spp.	2		
F. necrophorum	1	oral cavity	dog
F. varium	1	postoperative infection	dog
Peptostreptococcus spp.	2		
P. tetradius	1	eye	rabbit
P. micros	1	postoperative infection	dog
Porphyromonas gingivalis	2	oral cavity/ tracheal washing	dog
Prevotella spp.	3		
P. disiens	1	nasal discharge	dog
P. buccae	1	postoperative infection	dog
P. melaniogenica	1	urine plug	cat
Propionibacterium acnes	1	eye	rabbit

Table 2. Antimicrobial susceptibility of the anaerobic isolates

	AMC	CAZ	CF	CIP	NOR	P	CC
Bacteroides spp.							
B. distasonis	S	S	S	S	S	S	S
B. eggerthii	S	S	S	S	S	S	S
Fusobacterium spp.							
F. necrophorum	S	S	S	S	S	S	S
F. varium	S	S	S	S	R	S	S
Peptostreptococcus spp.							
P. tetradius	S	S	S	S	S	S	S
P. micros	S	S	S	S	S	S	S
Porphyromonas gingivalis	S	S	S	S	S	S	S
Prevotella spp.							
P. disiens	S	S	S	S	S	S	S
P. buccae	S	S	S	S	S	S	S
P. melaniogenica	S	S	S	S	S	S	S
Propionibacterium acnes	S	S	S	S	S	S	S

R: resistant, S: susceptible, AMC: amoxacillin/clavulanate, CAZ: ceftazidime, CF: cephalothin, CIP: ciprofloxacin, NOR: norfloxacin, P: penicillinG, CC: clindamycin

Discussion

When choosing appropriate antibiotics to treat diseases, periodic examination of bacteriological culture (aerobic and anaerobic bacteria) should be always performed. Clinician must judge whether certain bacteria were causing the progression of the diseases and must also consider the characteristics of those bacteria.

Anaerobic bacteria are part of the normal bacterial flora of the skin and mucous membranes of animals. Therefore, it is difficult to distinguish normal flora or true pathogens in anaerobic bacteria. Anaerobic bacteria isolated in this study could not been distinguished normal flora or pathogens. However, these patients have received antibiotics to treat infectious diseases for a long time and clinical specimens came from chronic inflammation sites. Its suggested that anaerobic bacteria are found in chronic infections. It might postulate that isolated anaerobic bacteria were true pathogens.

Anaerobic bacteria become involved when sinusitis turns chronic and oxygen levels decline. And, oral infections are predominantly anaerobic[24]. *Prevotella* and *Porphyromonas* species were frequently isolated from the oral cavity of animals[4,15,16]. *Porphyromonas gingivalis* was isolated from tracheal washing of the dog with chronic respiratory disease in this study. The usual source of bacteria in anaerobic lung infections is the oral cavity-presumably the gingival crevice[13,22]. Lansing *et al.* noted that anaerobic bacteria from the upper airways may cause lung infections, based upon the canine

model[18]. In the nosocomial pneumonias, the role of anaerobic bacteria is often under appreciated. If the role of anaerobic bacteria is neglected and active therapy against anaerobic bacteria not administered then the possibility of abscess formation must be considered[2].

Clinical clues to anaerobic infection include the presence of a foul odor to a discharge or fetid wound, the presence of sulfur like granules in a discharge, the presence of gas in the tissue, infection in the proximity to a mucous membrane (oral, abdominal and pelvic areas), infection associated with necrotic or hypovascularized tissue, the presence of abscess, failure to respond an antimicrobial agent with no anaerobic activity (as aminoglycosides)[12,27].

However, there are not always specific clinical clues to distinct between mixed infection (involving a number of anaerobic, aerobic bacteria) and aerobic infection. Therefore, anaerobic bacterial culturing is recommended in suspected anaerobic infection cases, routinely[25]. However, the isolation of anaerobic bacteria is required complex methods. Plates used for initial processing of anaerobic culture were maintained in a reduced environment so that the transition by the anaerobic bacteria from reduced host tissue to synthetic media is not made more difficult by the presence of oxygen[7]. Oxygen, dissolved in culture media that were stored aerobically, may prevent the growth of anaerobic bacteria due to raising the oxidation-reduction potential of medium through the oxidation of organic substances in the medium[23]. And, use of thioglycolate for storage of anaerobic isolates may present some problem. Claros *et al.* noted that 25% of pure cultures of various anaerobes died after 2 weeks of storage in thioglycolate broth[8]. This would imply that laboratories, which do not do routine identification on anaerobic isolate, may not have the opportunity to recover the isolate if it is stored in thioglycolate[8].

Most veterinarians may choose broad-spectrum empirical antibiotics without anaerobic bacterial culturing in many cases, because of complexity and high cost. However, broad-spectrum empirical antibiotics therapy may not be effective and promotes the development of resistance to antibiotics[26]. Once the presence of anaerobic bacteria is confirmed, veterinarians are able to use this information to select antibiotics for therapy.

The veterinarians may have a dilemma on how to choose the best therapy for mixed aerobic and anaerobic infections, a situation which is far from satisfactory. Fortunately, several compounds such as clindamycin, metronidazole, the β-latamase inhibitor combinations and the carbapenems have maintained good activity against almost all anaerobes and resistance is up until now, infrequent[12]. The therapy of anaerobic infections involves both appropriate antimicrobial treatment as well as draining pus, improving circulation and debridement when necessary[12].

The susceptibility test of anaerobic isolates was executed by disk diffusion method. Only one strain of *Fusobacterium varium* was resistant to norfloxacin in this study. Barry et al. have evaluated modifications of the disk diffusion technique for use with rapidly growing anaerobes[1]. At present, however, this test is not considered appropriate for anaerobic susceptibility testing[27]. Carrasco *et al.* noted that anaerobic isolates were susceptible to tested antibiotics, even though there was heavy use of antibiotics[5]. It means that anaerobic bacteria are susceptible to most of all antibiotics until now. Additional information, routine susceptibility testing of anaerobic isolates is not recommended[21]. In this study, there was not significant for anaerobic susceptibility, because the total number of isolated anaerobic bacteria was low and the distribution of those bacteria was variable. Also, anaerobic susceptibility test is not being performed routinely in human medicine in Korea, because of low resistance.

This study emphasized the need for the isolation of anaerobic bacteria as pathogens in order to select appropriate antibiotics. To select appropriate antibiotics may be helpful to reduce antibiotic resistance and to treat animal patients.

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대학 동물병원 임상 검체로부터 분리된 혐기성 세균과 항생제 감수성 양상

박세원 · 정진영 · 황철용[1] · 윤화영 · 한홍율

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요 약 : 치료 실시중 세균의 항생제 내성 발현은 임상 분야의 중요한 문제점중 하나인데 중요한 원인중 하나는 원인 세균의 분리와 그에 따른 항생제 감수성 검사 결과에 바탕을 두지 않은 무분별하며 적절치 않은 항생제를 선택함에 있다. 따라서 본 연구에서는 대학동물 병원에 내원한 임상증례로부터 채취한 임상검체에서 혐기성 세균을 분리 동정하고 항생제 감수성 양상을 검사하고자 하였다. 이를 위해 2001년 5월부터 2002년 10월까지 서울대학교 동물병원에 내원한 개, 고양이 및 토끼로부터 채취한 임상검체에 대해 혐기성 배양을 실시하고 분리 동정된 세균에 대해서는 표준 디스크 검사법을 이용해 항생제 감수성을 평가하였다. 총 13주; *Bacteroidesspp.* (3주), *Fusobacterium spp.* (2주), *Peptostreptococcus spp.* (2주), *Porphyromonas gingivalis* (2주), *Prevotella spp.* (3주), *Propionibacterium acnes* (1주)의 혐기성 세균이 분리동정 되었으며 항생제 감수성 검사에서는 *Fusobacterium varium* 1주만이 norfloxacin 에 저항성을 나타내었으나 그 외 모든 분리주가 검사대상 항생제에 대한 감수성이 있는 것으로 나타났다.

주요어 : 항생제 감수성, 혐기성 세균, 개, 고양이, 토끼