OTITIS MEDIA

AND THE NASOPHARYNGEAL MICROFLORA

OF THE MONGOLIAN GERBIL - *MERIONES UNGUICULATUS*

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by

Terry A. Thompson

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ABSTRACT

Terry A. Thompson. OTITIS MEDIA AND THE NASOPHARYNGEAL MICROFLORA OF THE MONGOLIAN GERBIL - MERIONES UNGUICULATUS. (Under the direction of Dr. Robert S. Fulghum). Department of Biology, November, 1979.

Otitis media, an infection of the middle ear, is the leading cause of conductive and sensorineural hearing loss in the United States. Children, especially those 10 years of age and younger, have the highest incidence of otitis media. The etiology of otitis media remains incompletely defined. The Mongolian gerbil, Meriones unguiculatus, is one laboratory animal with no reported history of otitis media and, as such, provides an excellent choice as an animal model for otitis media research. This study was undertaken to determine the specific normal microflora of the nasopharynx of the gerbil. This information is required for the evaluation of the gerbil as a model for otitis media. In this study, the nasopharyngeal microflora was determined for 6 gerbils. Each gerbil (6/6) had at least one species of Staphylococcus and 4/6 gerbils had at least one species of Corynebacterium. Most other facultatively anaerobic bacteria isolated, appear to be transitory. No anaerobic bacterium could be identified as being a consistent member of the nasopharyngeal microflora of the Mongolian gerbil.

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INTRODUCTION AND LITERATURE REVIEW

Otitis Media in Humans

Otitis media is the inflammation of the mucoperiosteal lining of the middle ear cleft, which includes the eustachian tube, tympanic cavity, attic, mastoid antrum, and mastoid air cells (Proctor, 1972). In general, otitis media is classified as acute, subacute, and chronic. Acute otitis media refers to the invasive stages of the disease, subacute refers to the intermediate stages, and chronic otitis media involves persistent infection with perforation of the tympanic membrane. A variety of terms describing these stages of otitis media are found in the literature. These include, serous otitis media, secretory otitis media, sero-purulent otitis media, catarrhal otitis, eustachiitis, glue ear, and mucoid ear. These are based on the extent of the disease and the type of fluid present. Generally, three types of fluid are recognized as being present with otitis media; serous, purulent, or mucoid fluid (Friedman, 1974). Serous otitis media effusion consists of a very pale colored, low viscosity fluid with a few neutrophils, lymphocytes, and low numbers of bacteria (Senturia, 1970). Purulent effusions are gray-white, turbid, and increased in viscosity. Cellular constituents tend to settle out giving a biphasic appearance. The effusion contains mucous strands, numerous neutrophils, and some phagocytes and lymphocytes (Senturia, 1970).

Mucoid effusions are highly viscous and contain numerous mucous strands and some cellular remnants (Senturia, 1970; Newby, 1972).

As a consequence of the fluid accumulation in the middle ear, ossicular movement is inhibited. For this reason, acute otitis media is the most common cause of conductive hearing loss in the United States (Davis and Silverman, 1970; Newby, 1972; Feigin and Chang, 1974) and the chronic form is the second most common cause of severe hearing impairment (Fraser, 1970). Permanent conductive or sensorineural hearing loss can occur if otitis media is not detected (Gacek, 1973). In a study of 404 patients with diagnosed chronic otitis media by English et al. (1973), depressed bone conduction thresholds were found both pre- and post-treatment. It was concluded by these researchers that sensorineural hearing loss can be a natural sequela of chronic otitis media. In a review of the effects of conductive hearing loss by Katz (1978), it was concluded that conductive hearing losses deprived the auditory system of normal stimulation and could lead to disruption in auditory perception, language, and learning.

Because of the sensitive location of the middle ear, within the cranium (Figures 1 and 2), other serious sequelae can occur if otitis media is undetected. These include; distension, retraction, or rupturing of the tympanic membrane; paralysis of the facial nerve; mastoiditis; meningitis; and the formation of brain abscesses.

Children, especially those 10 years of age and younger, have the highest incidence of otitis media. Fraser (1970) reported that 20% of the children entering first grade had secretory otitis media.

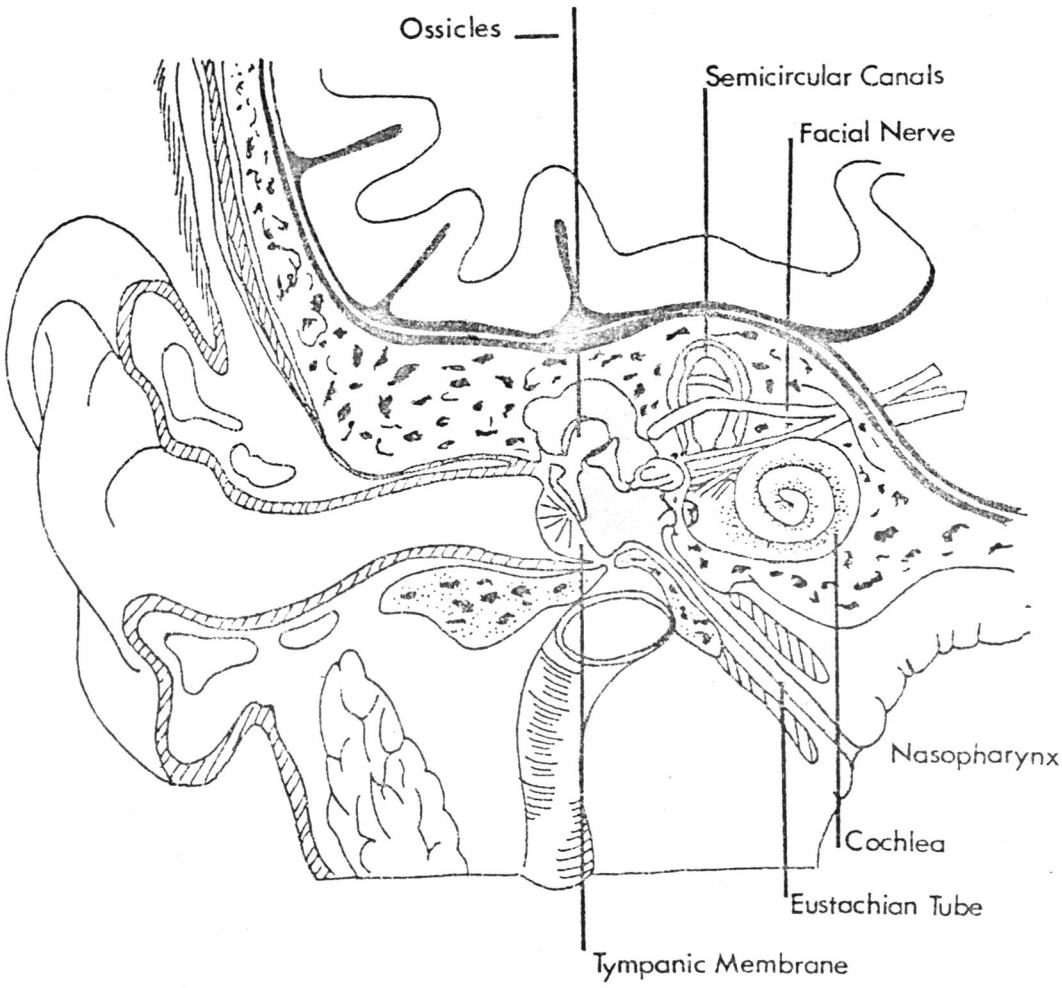


Figure 1. Overall view of the human middle ear.

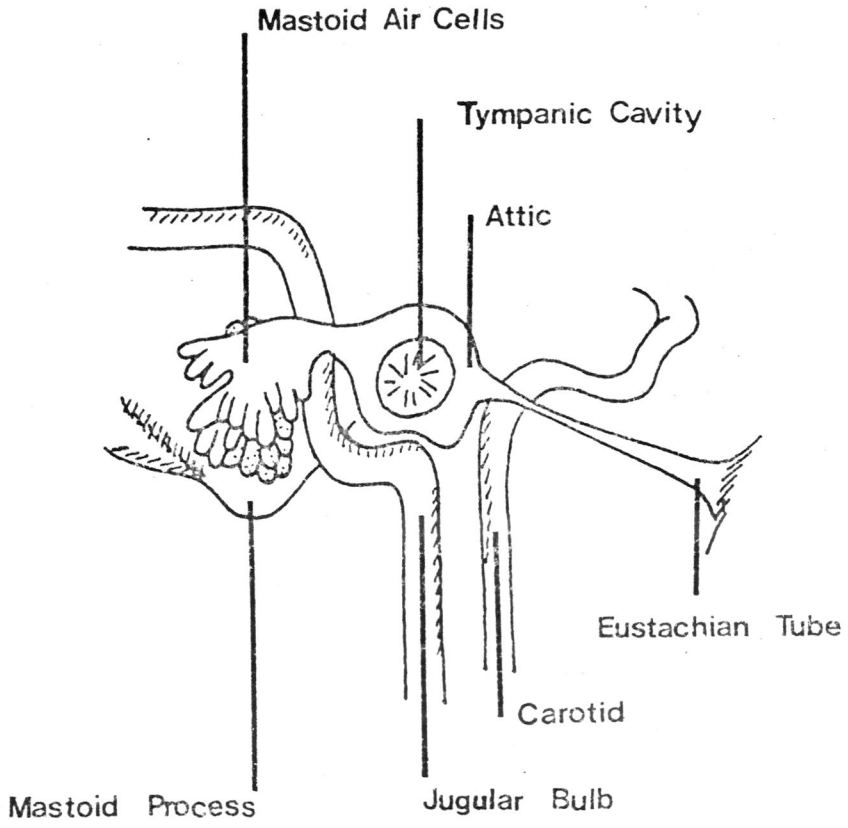


Figure 2. Schematic of the human middle ear cavity.

Feigin and Chang (1974) reported that 66% of children with acute otitis media were less than 3 years of age. In a statewide survey of primary care practices in Virginia, acute otitis media was found to be the eleventh most common diagnosis. Of over 9,000 cases in a 2-year period, 66.5% were in children 9 years of age and younger (Marsland et al., 1976).

This high incidence of otitis media in children has both anatomical and physiological bases. The function of the eustachian tube is to provide ventilation and fluid drainage of the middle ear. In children, especially those of this age group, the eustachian tube is shorter, wider, straighter, and more horizontal than in older children (Zemlin, 1968; Feigin and Chang, 1974). Also, the musculature which controls the ostium of the eustachian tube is not fully developed. Thus the ventilation and drainage of the middle ear is greatly reduced, providing an environment for the growth of potentially pathogenic bacteria. As the child matures, the eustachian tube becomes developmentally more vertical, allowing increased aeration and drainage (Zemlin, 1968). Physiologically, young children are highly susceptible to upper respiratory infections such as colds, flu, adenoiditis, tonsilitis, and allergies. Thus, young children not only have a weakened resistance, but also a reservoir of pathogenic bacteria within the nasopharynx. These pathogens may travel up the eustachian tube or be forced up the eustachian tube by coughing and nose blowing. In a study of bone chips removed during mastoid operations, it was concluded by Friedmann (1971) that invasion of the middle ear was

by an ascending infection of the eustachian tube following naso-pharyngeal or laryngeal infection. Eustachian tube infection or malfunction arising from it have often been cited as contributing factors to otitis media (Fraser, 1970; Tos and Bak-Pedersen, 1972; Friedmann, 1974; Feigin and Chang, 1974).

Bacteriology of Otitis Media

Although research regarding the bacteriology of otitis media in humans has been extensive, the findings have varied and the etiology of otitis media remains incompletely defined (Glorig and Gerwin, 1972). According to Lahikainen (1953), the most common bacteria found in acute otitis media were Streptococcus pyogenes, Streptococcus (Diplococcus) pneumoniae, Staphylococcus, Haemophilus influenzae, and Streptococcus viridans. He stated that polymicrobic infections, those involving two or more species of bacteria as a mixed culture, were rare in acute otitis media. He also found a small percentage of sterile cases. Friedmann (1971) reported Streptococcus pyogenes, Streptococcus pneumoniae, and Haemophilus influenzae as the etiological agents of acute otitis media. According to Nager (1972) the predominant pathogenic microorganisms found in otitis media were pneumococci and Group A hemolytic streptococci. He also stated that staphylococci, Haemophilus influenzae, Klebsiella pneumoniae, and Pseudomonas aeruginosa were encountered infrequently, and that, in most cases, the nasopharynx was the source of middle ear infections. Nilson et al. (1969) implicated Streptococcus pneumoniae and Haemophilus influenzae as the primary pathogens obtained by needle

aspirations. They also reported 41% of their cases were sterile. In a study of 608 private patients, Howie and Ploussard (1971) also found that the principal pathogens were pneumococcus (215 cases) and Haemophilus influenzae (146 cases). Berkman (1976) found Streptococcus pneumoniae, Haemophilus influenzae, Staphylococcus aureus, and beta-hemolytic streptococci in 45% of the nasopharyngeal cultures of children with acute otitis media. Streptococcus pneumoniae, Haemophilus influenzae, Neisseria catarrhalis, Staphylococcus aureus, beta-hemolytic streptococci, enteric bacteria, and Pseudomonas aeruginosa were reported as the agents of acute otitis media by Feigin and Chang (1974). Thirty-four percent of their cultures were sterile. Further investigation attributed these sterile cases to viruses or mycoplasmas. The view of viruses contributing to otitis media was also supported by Gwaltney (1971), but Klein (1975) found no evidence to suggest a viral etiology for serous otitis media.

Chronic otitis media has been reported as a monomicrobic infection (Dysart, 1955) and a polymicrobic infection (Meurman et al., 1951). Bacteria implicated in chronic otitis media are; Staphylococcus aureus, Staphylococcus albus, Proteus vulgaris, Corynebacterium pseudodiphtheriticum, Pseudomonas aeruginosa, Escherichia coli, Escherichia paracoli, Klebsiella pneumoniae, Haemophilus influenzae, and species of pneumococci and streptococci.

Obligately anaerobic bacteria have also been isolated from cases of otitis media. Krumwiede and Pratt (1913) isolated anaerobic fusiform bacilli from discharging ears and reported fusiforms mixed

with spirochetes. Oral Vincent's (fuso-spirochaetal) infection was found in ten cases of chronic suppurative otitis media described by Busacca (1923). Brisotto (1923) reported anaerobic bacteria from 10 of 25 cases of otorrhea. He not only found spiral organisms and fusiform bacilli, but also coryneforms, diplobacilli, and anaerobic cocci. He also reported numerous anaerobic microorganisms in 4 cases of mastoiditis. Dietrich et al. (1965) reported 3 otitis media cases yielding Bacteroides as a monoinfection, 1 case yielding anaerobic cocci as a monoinfection, 1 case with a mixed culture of several anaerobes, 1 case of chronic otitis media containing a mixed culture of anaerobic and aerobic bacteria, and 1 case of mastoiditis with Bacteroides and anaerobic cocci. Bulinska and Kalowski (1974) isolated anaerobic bacteria from 8 of 21 cases of otitis media. Most had mixed infection flora including Bacteroides species, Bacteroides melaninogenicus, Corynebacterium species, and aerobic organisms. Of 70 consecutive cases of active chronic otitis media, Jokipii et al. (1977) recovered anaerobic bacteria in 33%. Bacteroides accounted for one half of these and was always in mixed infections with facultatives. Yarborough et al. (1976) and Fulghum et al. (1977) isolated anaerobic bacteria in a mixed infection flora in 4 of 10 specimens taken at myringotomy. The isolates included Peptostreptococcus intermedius, Propionibacterium acnes, and other Gram-positive anaerobes. Dunkle et al. (1976) also reported the isolation of Propionibacterium acnes following adenoidectomy and myringotomy for chronic otitis media. In a study of 62 children (ages 4 months to 8 years) with acute otitis media, Brook et al.

(1978) isolated aerobic bacteria alone from 57% of the patients; anaerobic microorganisms alone from 15%; and mixed cultures of aerobes and anaerobes from 13%. Cultures were negative in 16% of the cases. Streptococcus pneumoniae and Haemophilus influenzae were the predominant aerobes isolated. Each was recovered from 27% of the patients. Propionibacterium (16%) and Peptococcus (11%) were the most often isolated anaerobes. The isolation of only anaerobic bacteria in 15% of the patients suggested to these researchers the possibility of a direct role in acute otitis media by these microorganisms. Brook and Finegold (1979) cultured pus from 50 patients with chronic otitis media. Only aerobes were recovered from 42% of the patients; 6% had only anaerobes; and 50% had both aerobes and anaerobes. One culture was negative. Pseudomonas aeruginosa was isolated from 72% of the patients. Proteus species and Staphylococcus aureus were other commonly isolated aerobes. Of 48 anaerobes isolated, 21 (44%) were Peptococcus species, 16 (33%) were Bacteroides species and 3 (6%) were Peptostreptococcus species. Finegold (1977) has written an extensive review of the literature on anaerobic bacteria involved in otitis media.

Otitis media with involvement by anaerobic bacteria can be the initial focus of serious infections metastasizing to other tissues. Heineman and Braude (1963) reported that chronic otitis media involving anaerobes was the probable origin of 8 of 18 cases of brain abscesses containing anaerobic bacteria. The patients involved had histories of mastoiditis, chronic otorrhea, and chronic

otitis media. Feldman (1976) reported that in 6 of 9 cases of meningitis the portal of entry of the microorganism, Bacteroides, was otitis media.

Treatment failures may also be attributed to the involvement of anaerobic bacteria in otitis media. It is now known that many anaerobes are resistant to, or are developing resistance to antibiotics. In addition, many strains of anaerobes may protect other bacteria from antibiotics. For example, many strains of Bacteroides fragilis produce potent extracellular penicillinases when growing in mixed culture lesions. Thus, microorganisms such as Bacteroides fragilis may protect other members of the infection flora from penicillin and similar drugs, resulting in treatment failures (Hackman and Wilkins, 1975).

Some anaerobes, when in mixed culture with aerobes or other anaerobes, have a synergistic ability to produce disease. Therefore mixed culture infections can be produced by bacteria which are usually non-pathogenic in pure culture. Roberts (1967) showed synergy between the anaerobe, Fusiformis necrophorus and Corynebacterium pyogenes. Fusiformis necrophorus produced a leucocidal exotoxin which protected Corynebacterium pyogenes and helped to maintain the disease process.

Otitis media, a common problem in infants and children, is a significant cause of morbidity and hearing loss. The etiology remains essentially incomplete and treatment is largely empirical (Halstead et al., 1968). Until the microorganisms contributing to the disease are identified, medical treatment of many cases will be destined to continued failure (Glorig and Gerwin, 1972).

Otitis Media in Laboratory Animals

Otitis media is quite common among laboratory animals and has frequently inhibited auditory research. The laboratory animal most often used in audiological research is the rat. Chronic respiratory disease, one of the most common pathological processes in the rat (Greselin, 1961), is often associated with otitis media in these animals (Greselin, 1961; Nelson, 1963; Olson and McCune, 1968). The symptoms of otitis media in rats, as described by McCordock and Congdon (1924), include head tilting, running in circles toward head rotation, and spinning when held by the tail. These researchers reported the incidence of otitis media as 50% in one rat colony and concluded that the infection began in the nasal passages and progressed into the middle and inner ears.

The incidence of otitis media in rats has been reported to increase as a function of age (Nelson and Gowen, 1930; Freudenberger, 1932; Retzlaff et al., 1960; Daniel et al., 1973). Incidences of 32% to 69% in colonies of young and adult rats respectively were reported by Nelson and Gowen (1930). Freudenberger (1932) reported three times the incidence in one year old rats as compared to those 12 weeks old. Retzlaff et al. (1960), using Spraque-Dawley rats, reported an incidence of 10%, while Daniel et al. (1973) found an incidence of 29%.

A variety of microorganisms have been cited as contributing to otitis media in laboratory animals. Mycoplasma pulmonis and Streptobacillus moniliformis are the two bacteria most often cited

in rats (Nelson, 1957; Lerner and Silverstein, 1958; Retzlaff et al., 1960; Nelson, 1963; Olson and McCune, 1968; Kohn and Kirk, 1969; Kohn, 1971). Mycoplasma pulmonis and Mycoplasma neurolyticum were isolated from the nasal mucosa and lungs of rats by Adler (1965). Both are thought to be agents of labyrinthitis in rats. In a study of 23 rats with chronic respiratory disease, Stewart and Buck (1975) isolated Mycoplasma arthritidis from the middle ears of 7 (30%) and from the throats of 3 (13%). Fulghum et al. (1977) isolated Propionibacterium acnes from 2 cases of bilateral otitis media in rats.

Other laboratory animals sometimes used for auditory research include mice, rabbits, guinea pigs, hamsters, and gerbils. Mycoplasma pulmonis and Pseudomonas aeruginosa are most often reported as the agents of otitis media in mice (Nelson, 1970; Ediger et al., 1971; Olson and Ediger, 1972; Halliwell et al., 1974). Intranasal introduction of Mycoplasma pulmonis (Nelson, 1970; Halliwell et al., 1974) and intravenous introduction of Pseudomonas aeruginosa (Olson and Ediger, 1972) have induced otitis media in mice. Pasteurella multocida (Vasenius and Tiainen, 1966; Fox et al., 1971; Snyder et al., 1973) and Pasteurella lepiseptica (Weiss, 1968) are most frequently isolated from middle ear exudates of rabbits. Streptococcus zooepidemicus, Streptococcus pneumoniae, and Klebsiella pneumoniae are most often associated with otitis media in guinea pigs (Kohn, 1974).

The Mongolian gerbil (Meriones unguiculatus) is one laboratory animal with no reported history of otitis media. In an attempt to

induce otitis media in the gerbil, Means et al. (1975) housed gerbils with Long-Evans laboratory rats under conditions which maximized disease susceptibility and transmission. After 6 months the gerbils were free of otitis media while the rats had an incidence of 83%.

There are several factors which may contribute to the high incidence of otitis media in rats, as compared to the apparent nonsusceptibility of the gerbil. Daniel et al. (1977) made anatomical comparisons between the rat and the gerbil. The rat's auditory bulla was found to be 6 mm in diameter while its skull measured 40 mm, giving a 0.15 ratio. In contrast, the gerbil's bulla and skull measurements were 12 mm and 28 mm respectively, giving a ratio of 0.43. The middle ear cavity of the rat is small and connected to the nasopharynx by a long (4.3 mm) and almost horizontal (15^{o}) eustachian tube. The middle ear cavity of the gerbil is hypertrophied and ovoid shaped and the eustachian tube is relatively short (1.8 mm) and nearly vertical (81^{o}). These features allow increased ventilation and drainage of the gerbil's middle ear. Also, from a behavioral aspect, gerbils spend more time than rats in upright positions which allow optimal tubal ventilation of the middle ear (Loesche et al., 1975). Sade (1974) stated that lack of ventilation was the single most important factor in the chronicity of secretory otitis media. Histological comparisons of the rat and gerbil eustachian tubes from the nasopharyngeal orifice to the posterior wall of the middle ear cavity showed additional differences. Coronal sections of the eustachian tube revealed mucous glands near

the nasopharyngeal ostium and a lack of goblet cells near the tympanic ostium in the gerbil, while in the rat there is a lack of mucous glands and an abundance of goblet cells throughout the eustachian tube. Investigations by Tos and Bak-Pedersen (1977) revealed an increase in goblet cell density in humans following tubal occlusion. Thus the goblet cell population in rats (lacking in gerbils) may also contribute to the maintenance of the disease in these animals.

Another factor which may contribute to the nonsusceptibility of the gerbil to otitis media is the specific normal flora. Since microbial invasion of the middle ear is most often by way of an ascending infection of the eustachian tube (Friedman, 1971), it is important to study the normal bacterial population in the nasopharynx of the gerbil.

As previously mentioned, anaerobic bacteria may play a role in treatment failures and sequellae of otitis media. Therefore, it is essential that any investigation of the nasopharyngeal microflora of the gerbil pay strict attention to the obligately anaerobic bacteria present. Several studies of the etiology of otitis media reported up to 41% sterile specimens (Nilson et al., 1969; Feigin and Chang, 1974). It is highly possible that the involvement of anaerobic bacteria is responsible, at least in part, for the high percentage of sterile specimens reported. In up to 40% of the cases of otitis media where strict anaerobic techniques have been used, anaerobic bacteria have been isolated (Bulinska and Kalowski, 1974; Yarborough et al., 1974; Dunkle et al., 1976; Fulghum et al., 1977; Jokipii,

1977; Brook et al., 1978; Brook and Finegold, 1979).

In view of these facts, it is possible that chronic otitis media represents an ecological progression of more than one species of bacteria. The bacteria initiating the acute stage, Streptococcus pneumoniae or Haemophilus influenzae, eventually yielding to one or more weak, opportunistic pathogens (including anaerobic bacteria) indigenous to the microflora of the patient. Once these bacteria enter the middle ear cavity of the compromised patient, they slowly extend the disease to the chronic state.

Since the gerbil is apparently not spontaneously susceptible to otitis media, it is an excellent choice as an animal model for otitis media research. Knowledge of the specific normal microflora in the nasopharynx of the gerbil is required before the gerbil can be evaluated as a model for otitis media. Also, an understanding of the microorganisms which contribute to or inhibit otitis media will aid in applying appropriate therapy for the disease. Therefore, it is important to study the normal bacterial population of the nasopharynx of an animal in which there has been no evidence of a microflora which contributes to the etiology or maintenance of otitis media - the Mongolian gerbil.

MATERIALS AND METHODS

Subjects

Six Mongolian gerbils, having no observable infections, were obtained from the East Carolina University Speech, Language, and Auditory Pathology laboratory. These gerbils were housed under conditions suggested by Schwentker (1963), in plastic cages with wire covers and were provided wood shavings for bedding. Their diet consisted of standard laboratory rat chow supplemented with sunflower seeds. The subjects were maintained at room temperature and were not used for other research.

Methods

Following sacrifice with CO_2, the head of the subject was removed. The head was skinned and the lower jaw was removed. This exposed the hard and soft palates and the posterior opening of the nasopharynx. The head was then bathed in povidone-iodine (Betadine surgical solution, Purdue Frederick Company, Norwalk, Connecticut). To obtain the sample, a sterile inoculating loop was inserted under the soft palate into the nasopharynx. Figure 3 shows the relationship of the soft palate and the ostium of the eustachian tube. Scrapings obtained in this manner were inoculated directly onto aerobic and anaerobic isolation media. Gram stains were made for morphologic analysis.

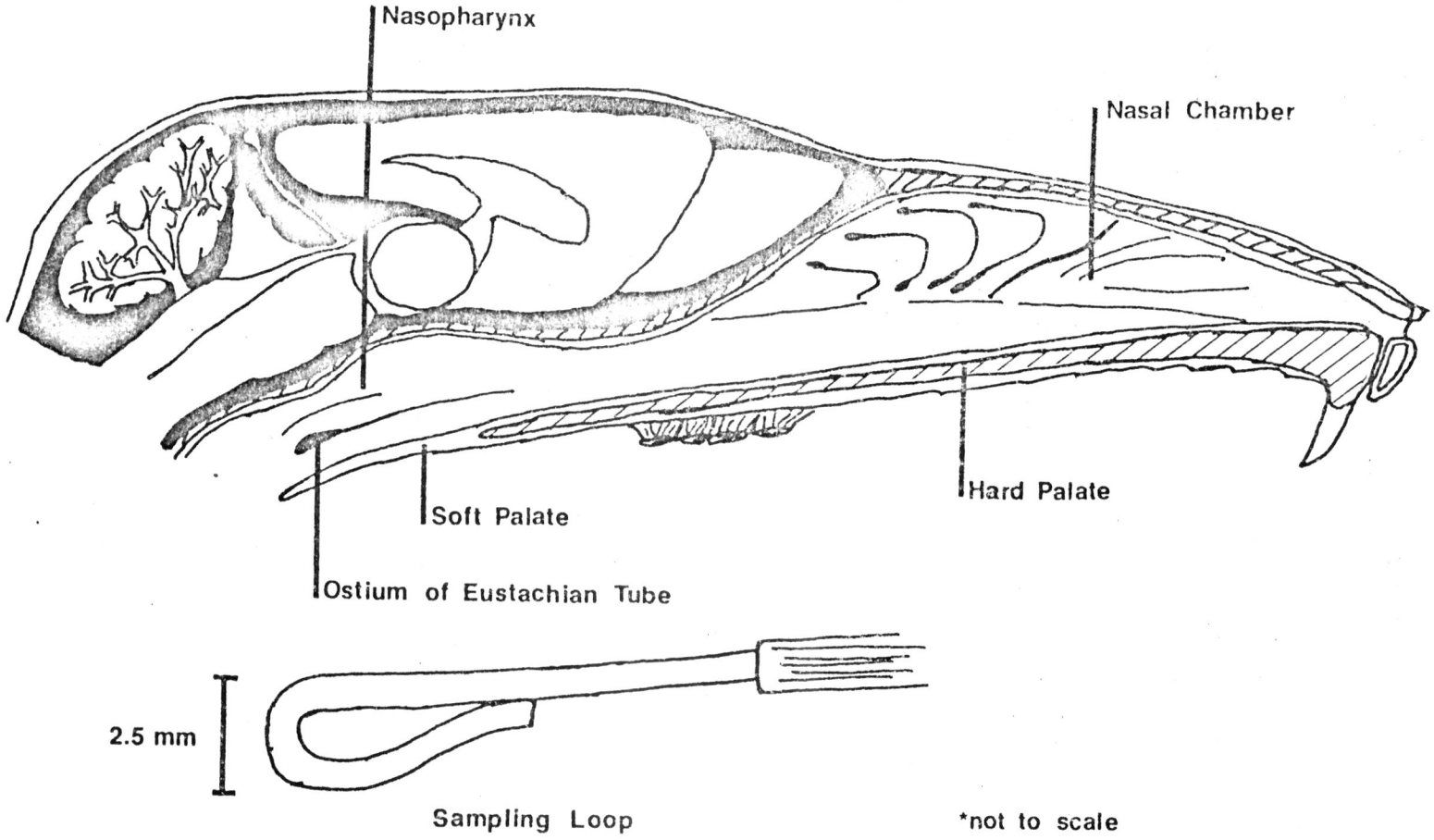


Figure 3. Midsagittal section of the gerbil head showing the location of the ostium of the eustachian tube within the nasopharnyx.

Bacteria were isolated according to respiratory function; aerobic, facultatively anaerobic, and obligately anaerobic. For initial isolation two Columbia sheep blood agar (SBA) plates were inoculated. One was incubated aerobically and the other was incubated in either a candle jar or a gas evacuation jar, which provided increased CO_2 for capnophilic bacteria.

Pre-reduced anaerobically sterilized media (PRAS media, V.P.I. Anaerobe Laboratory Manual, 1977) were used for the isolation and growth of the strict anaerobic bacteria. This technique utilizes chemically reduced media in a system of media preparation and inoculation, such that, the media and the bacteria are protected from oxygen during all manipulations. Two PRAS brain-heart infusion agar-supplemented (BHIS) roll tubes were used for the isolation of strict anaerobic bacteria. Also, one tube of PRAS chopped meat carbohydrate broth (CMC) was inoculated as a backup.

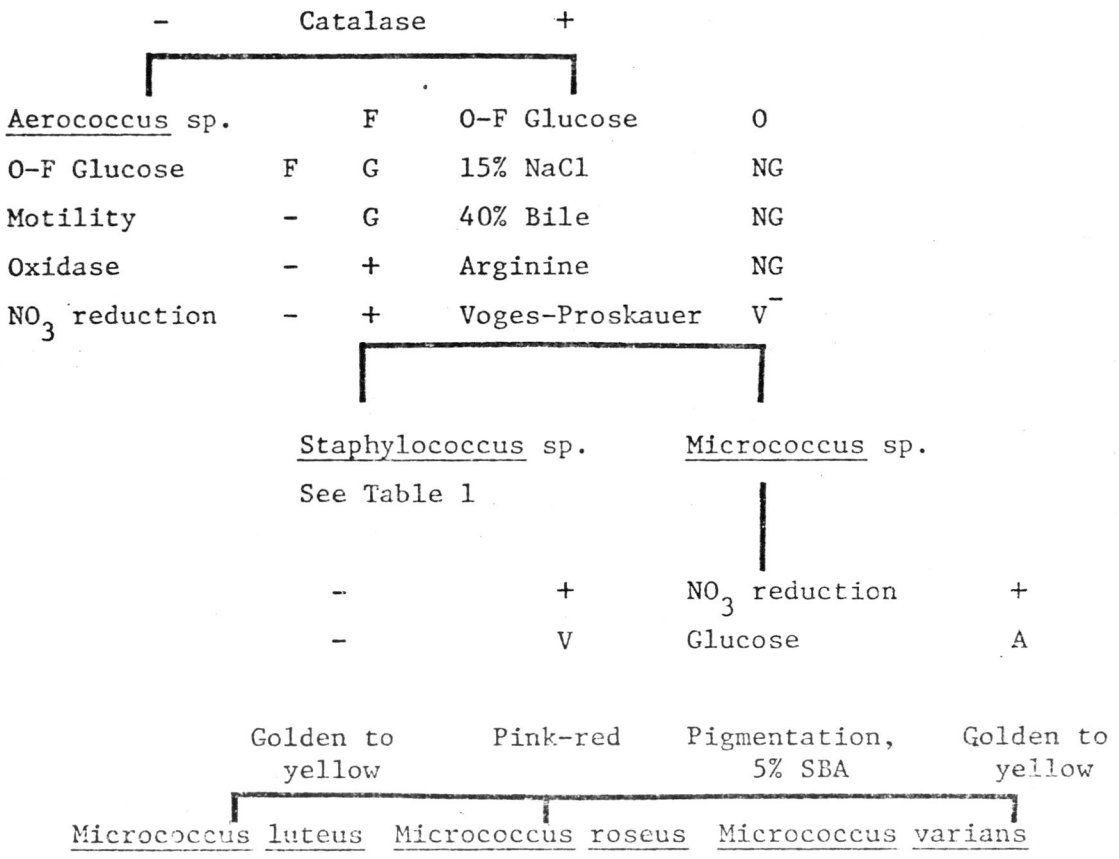
All plates and tubes were incubated at 37°C. Aerobic plates and tubed media were initially inspected after 24 hours incubation. Plates in candle jars and gas evacuation jars were initially inspected after 48 hours incubation. Thereafter, all media were inspected daily for up to 5 days.

Standard microbiological methods were used to obtain pure cultures of aerobic and facultative isolates. Identification of isolates was based on published morphological, cultural, and biochemical characteristics. Procedures followed were based on descriptions and methodologies in the Manual of Clinical Microbiology (2nd ed., 1974), Bergey's Manual of Determinative Microbiology (8th

ed., 1974), and Biochemical Tests for Identification of Medical Bacteria (MacFaddin, 1976). Figures 4 through 13 show flowcharts used in the identification of bacteria. Appendices A and B give brief descriptions of the biochemical tests used in this study. Identification of species of Staphylococcus was based on the scheme of Kloos and Schleifer (1975), depicted in Table 1. The work of Reddy and Kao (1978) was the basis for the identification of some species of Corynebacterium, Table 2. A rapid test method, the API 20E (Analytab Products Inc., Plainview, New York), was used for the identification of the Enterobacteriaceae and related Gram-negative bacteria.

Identification of strict anaerobic bacteria was based on descriptions and methodologies in the V.P.I. Anaerobe Laboratory Manual (4th ed., 1977). After an isolate was determined to be anaerobic, volatile and nonvolatile acid products were determined. Volatile acids include acetic, formic, propionic, isobutyric, butyric, isovaleric, and valeric. To detect these acid products 1 ml samples of a Peptone Yeast Glucose broth (PYG) culture were acidified with 50% aqueous H_2SO_4 and extracted with ether. The ether extracts were chromatographed. Succinic, pyruvic, and lactic acids are not volatile and cannot be detected by this procedure. Methyl derivatives of these acids are volatile and can be extracted in chloroform and the chloroform extracts chromatographed. In most cases, anaerobic isolates were identified to the genus level by the detection of these acid products. Figure 14 shows typical chromatograms of volatile and methylated acid products. Identification

Figure 4. Identification of aerobic gram-positive cocci[a]



[a]All of the following flow charts were adapted form MacFaddin (1976). Key for flow charts: +, positive; -, negative; A, acid; F, fermentative; O, oxidative; V, variable; G, growth; NG, no growth.

Figure 5. Identification of catalase-positive gram-positive aerobic bacilli

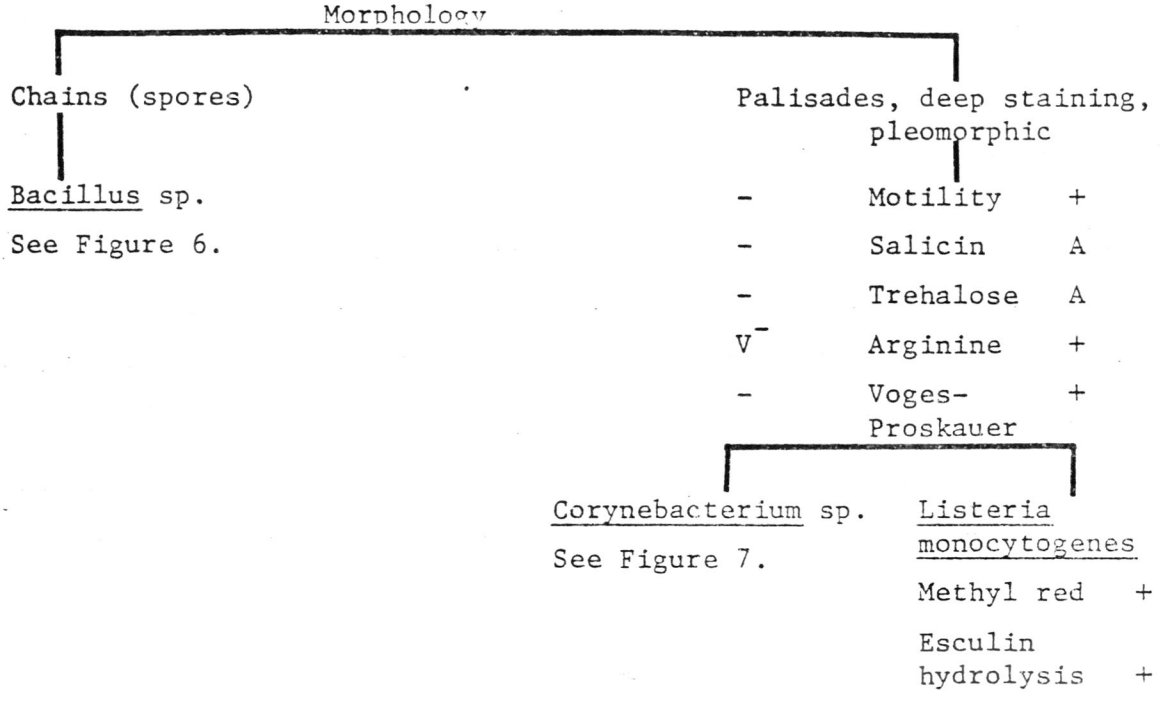


Figure 6. Identification of the three most commonly isolated *Bacillus* species

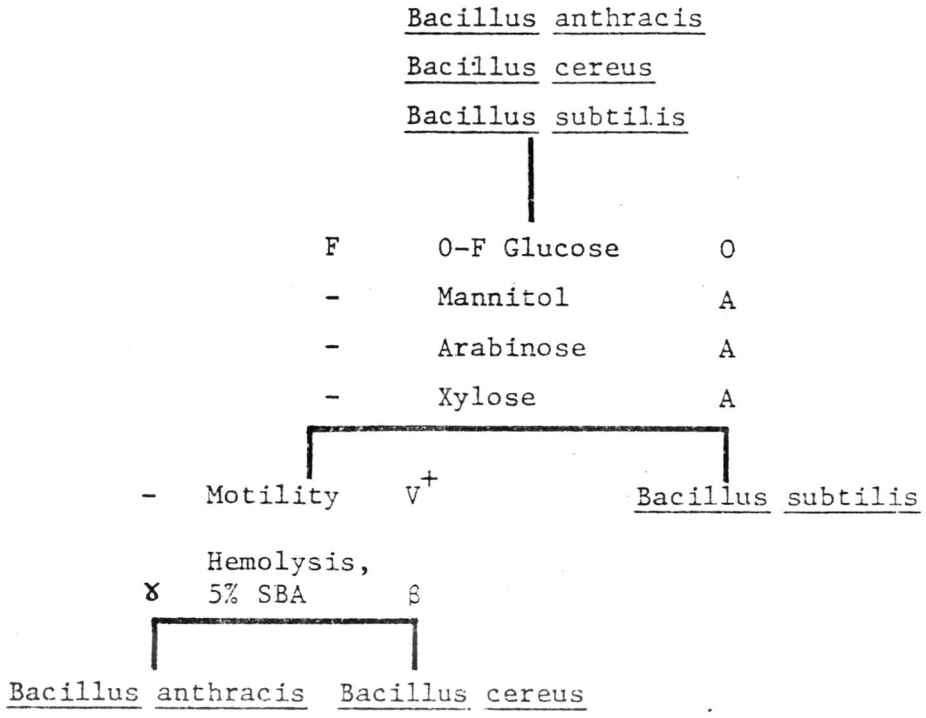


Figure 7. Identification of catalase-positive *Corynebacterium* species

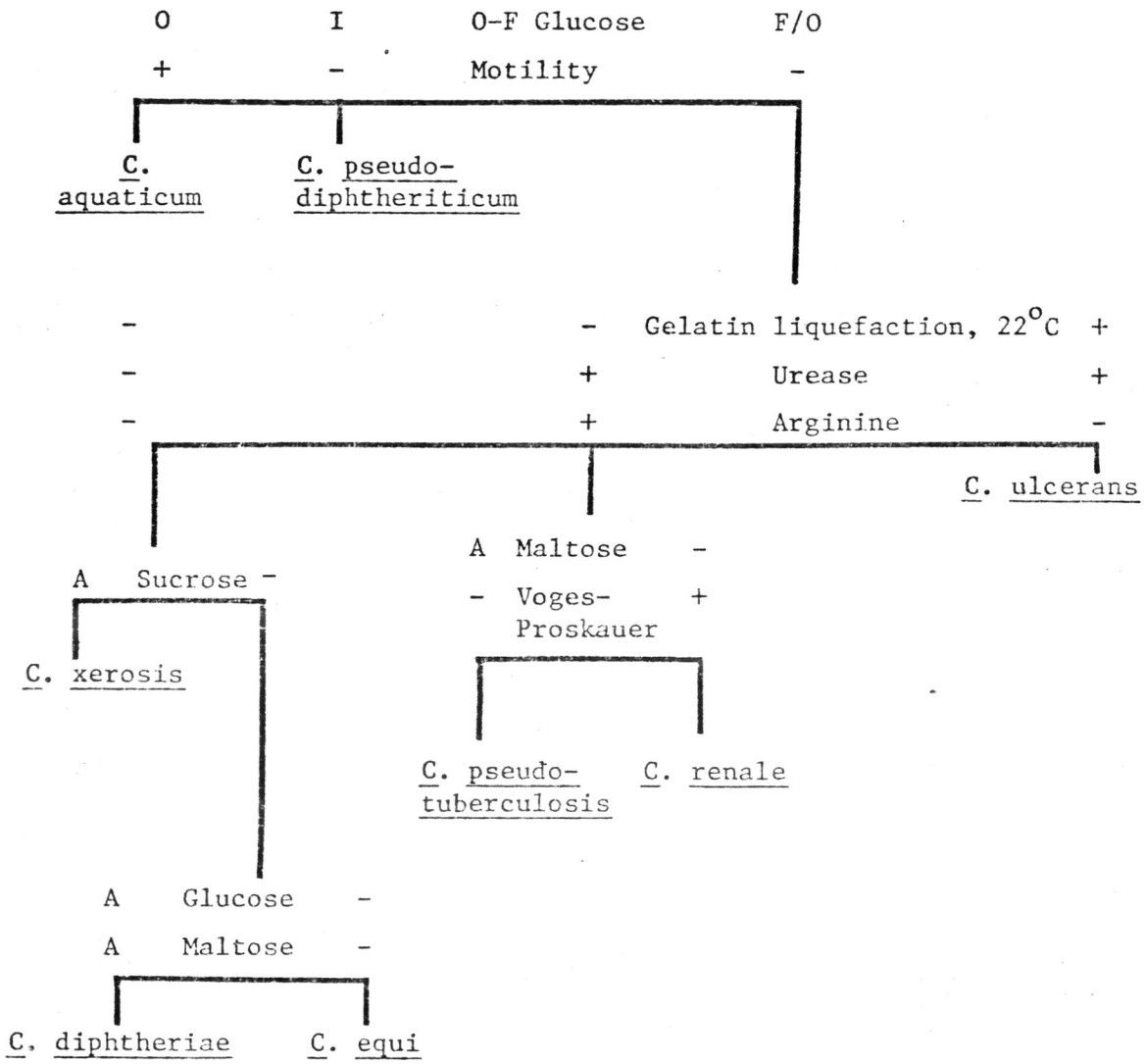


Figure 8. Identification of catalase-negative, gram-positive non-sporeforming bacilli

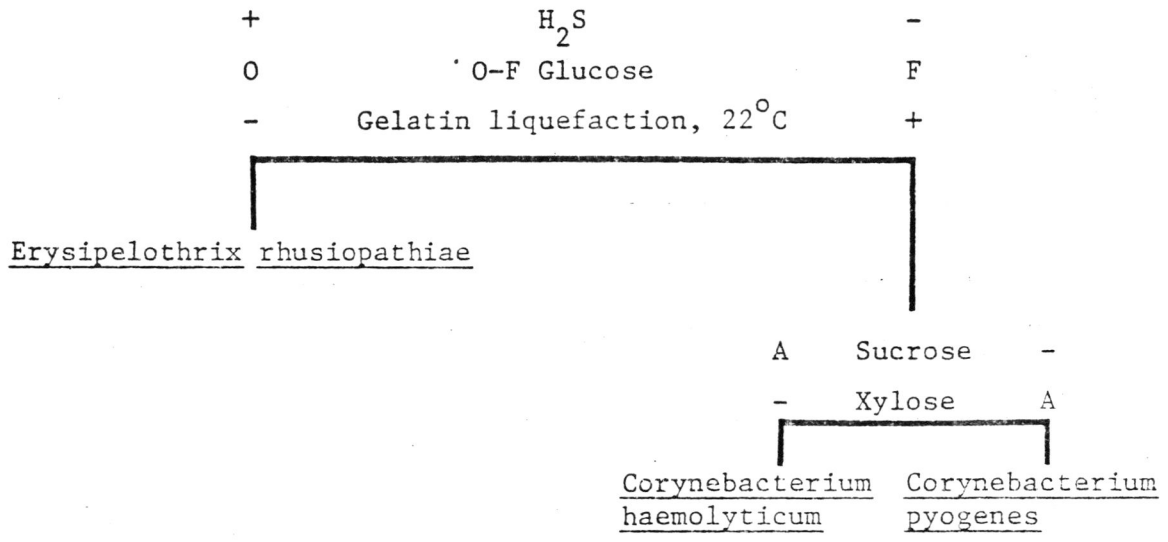


Figure 9. Identification of oxidase-positive, motile, oxidative gram-negative bacilli (coccobacilli)

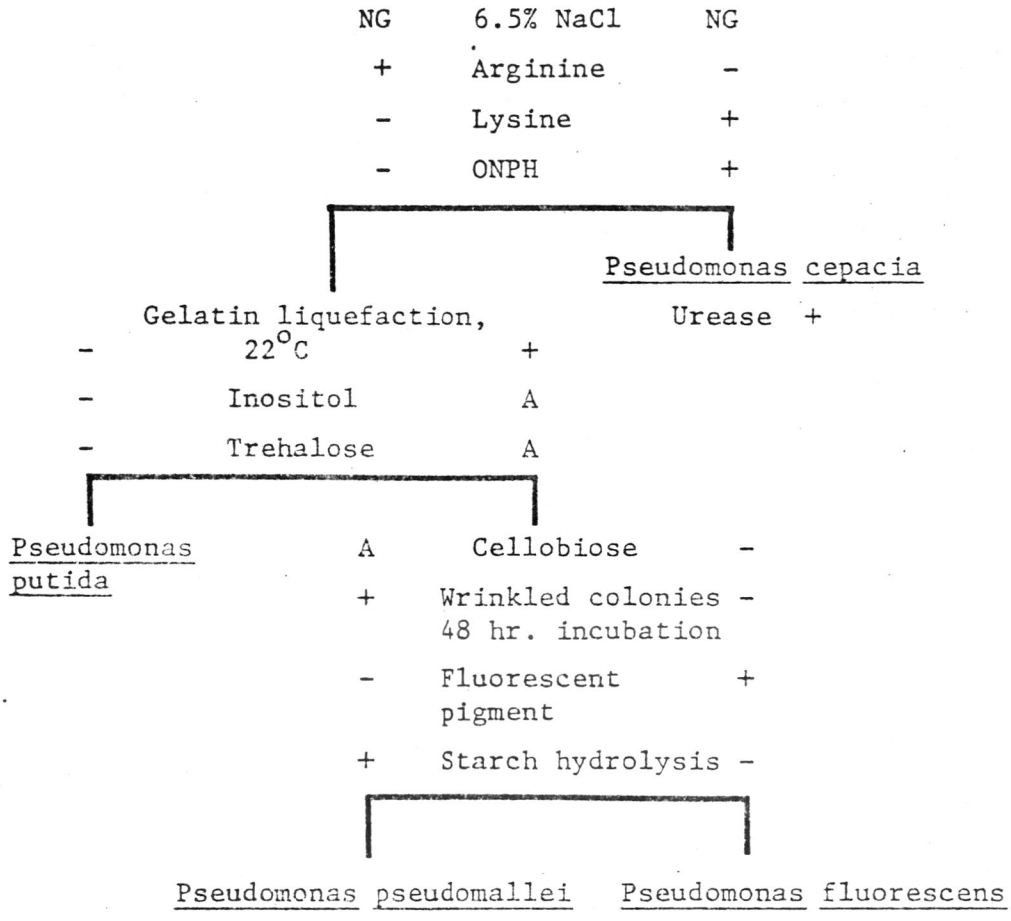


Figure 10. Identification of inert gram-negative bacilli (coccobacilli)

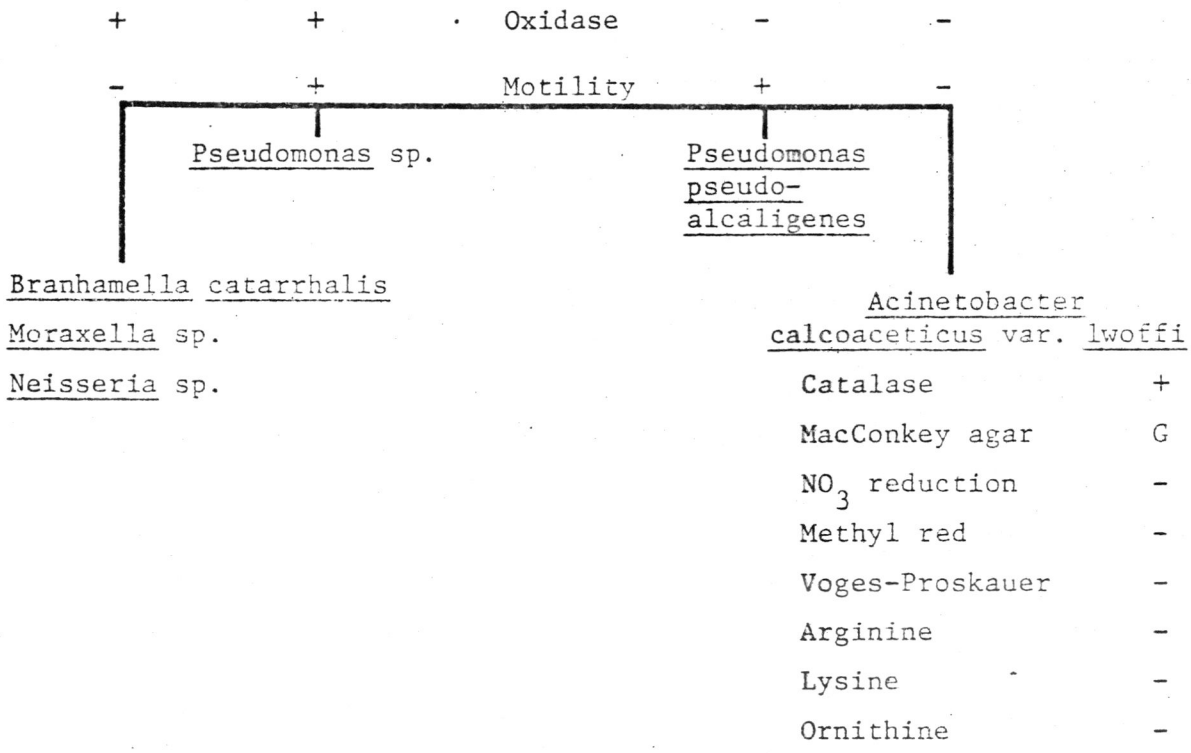


Figure 11. Identification of oxidative gram-negative bacilli (coccobacilli)

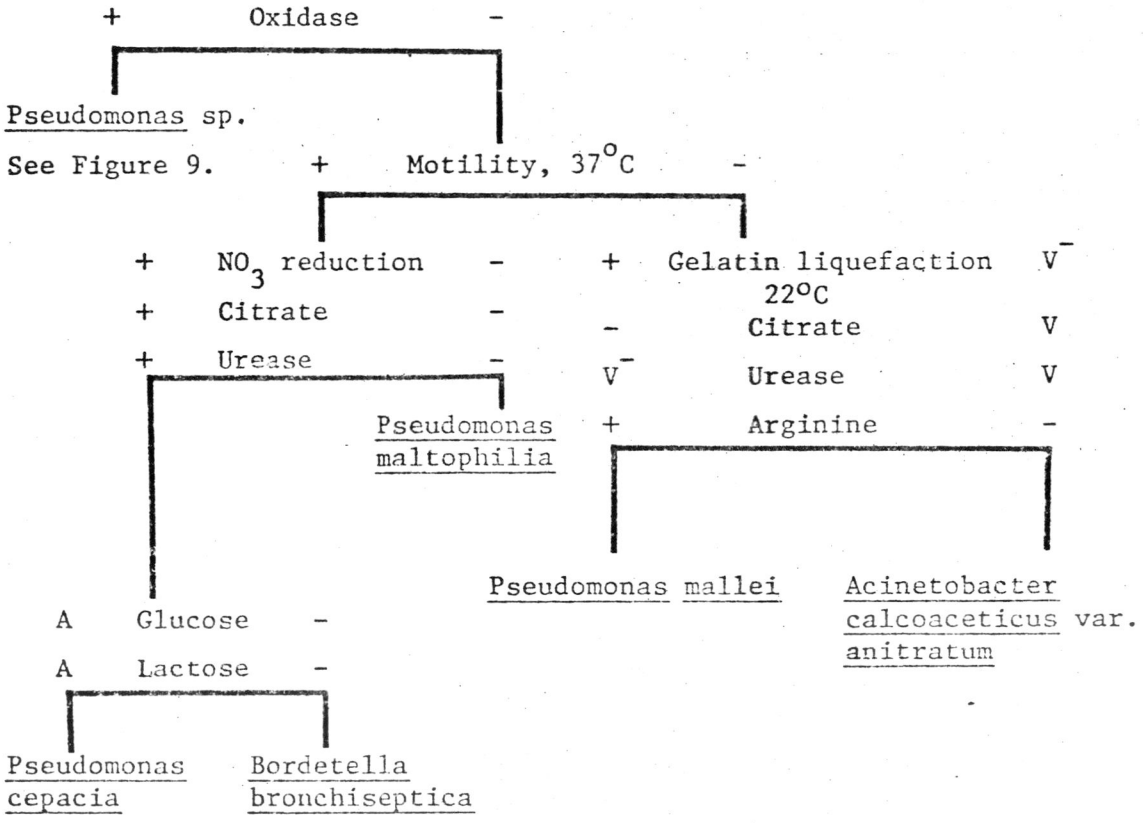


Figure 12. Identification of gram-negative fermentative bacilli (coccobacilli)

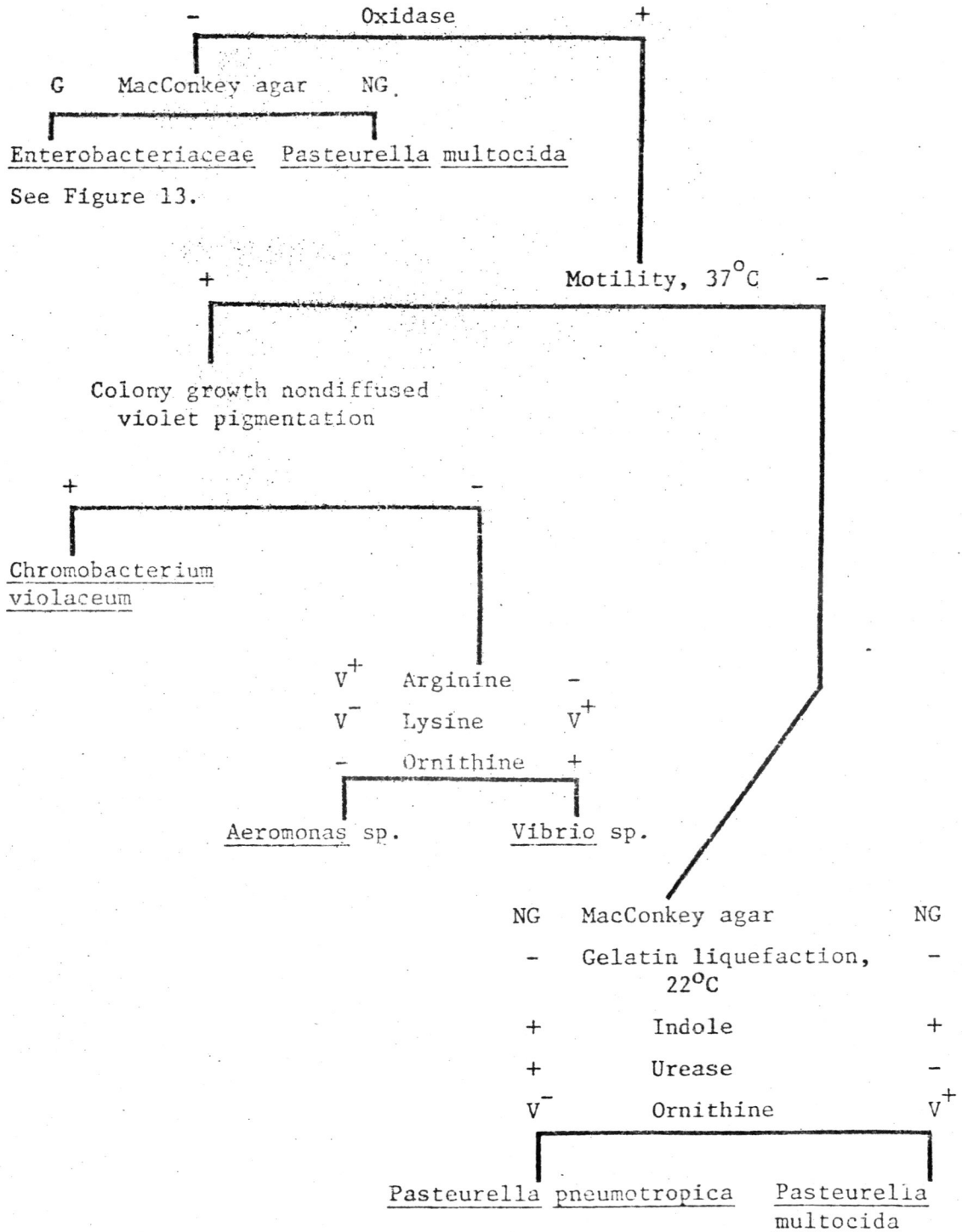


Figure 13. Identification of Enterobacteriaceae with a KIA/TSI reaction of acid/acid, with/without gas

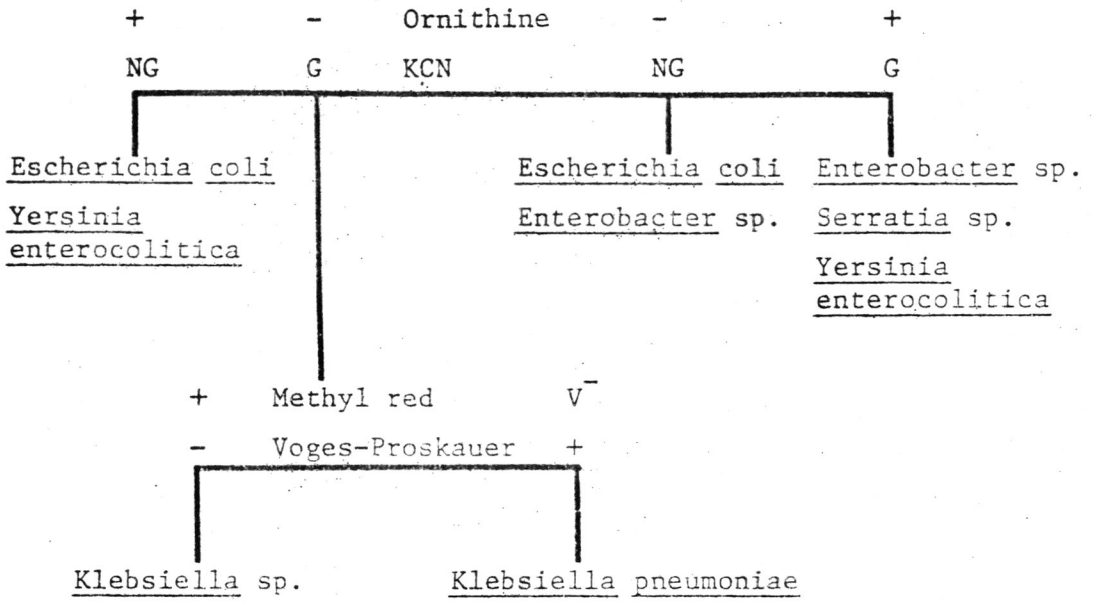


Table 1. Scheme for the identification of staphylococci[a]

Species	Coagulase	Hemolysis	Nitrate Reduction	Acid (aerobically) from Xylose	Sucrose	Trehalose	Mannitol
Staphylococcus aureus	+[b]	(+)	+	-	+	+	+
Staphylococcus epidermidis	-	-,±	(+,±)	-	+	-	-
Staphylococcus saprophyticus	-	-	-	-	+	+	+
Staphylococcus haemolyticus	-	(+)	(+)	-	+	+	+,-
Staphylococcus xylosus	-	(-)	(+)	+	+	(+)	+

[a]Table adapted from Kloos and Schleifer (1975).

[b]A single listed symbol denotes a type character frequency of 90 to 100%; parentheses around a symbol denote a frequency of 70 to 89%; two symbols are listed for a character when either type is in a frequency below 70%, but together equal 80 to 100%. Symbols for characters: +, positive; ±, weak; -, negative.

Table 2. Acid metabolic products and biochemical characteristics of two species of corynebacteria[a]

	Acid metabolic products[b]		Biochemical characteristics									
	Volatile	Nonvolatile	Catalase	Hemolysis	Glucose	Maltose	Sucrose	Lactose	NO_3 reduction	Gelatin	Urease	Anaerobic Growth
Corynebacterium equi	-	-	+	-	-	-	-	-	+	-	+	-
Corynebacterium pyogenes	a or A	Lspy	-	+	+	+	+	+	-	+	-	+

[a] Adapted from Reddy and Kao (1978).

[b] A, acetic acid; L, lactic acid; Py, pyruvic acid; S, succinic acid.
Uppercase letters indicate major products and lowercase letters represent minor products.

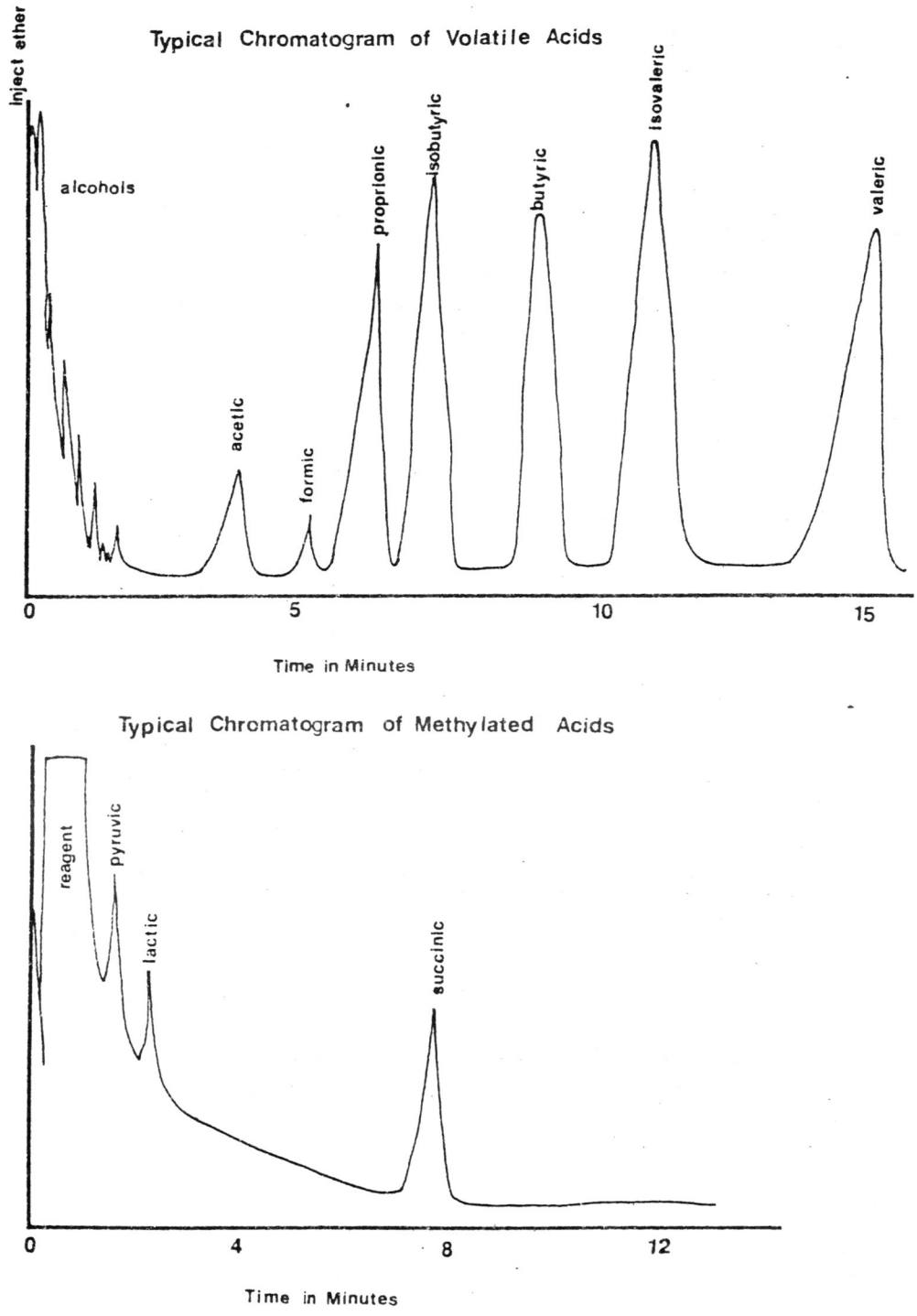


Figure 14. Typical chromatograms of volatile and methylated acids.

to the species level was accomplished with a battery of biochemical tests. Appendix B gives a brief description of these tests.

The isolation of Mycoplasma was attempted with two gerbils. Prior to scraping the nasopharynx, a small amount (microliters) of Mycoplasma broth was aseptically introduced into the nasopharynx. This was removed and added to five milliliters of Mycoplasma broth. In addition, sections of brain, liver, and intestinal tissue were surgically removed and added to tubes of Mycoplasma broth. All tubes were incubated for up to 2 weeks at 37°C. When a change in color of the medium indicated fermentation of glucose had occurred, the media was passed through a 0.22 micron filter to remove bacteria. The filtrate was then added to 5 milliliters of fresh, sterile Mycoplasma broth. These tubes were sent to Dr. Phletus P. Williams of the USDA Animal Disease Laboratory, Ames, Iowa for the isolation and identification of Mycoplasma species.

Quantitative evaluation of the bacteria within the nasopharynx was done with two gerbils. Scrapings from the nasopharynx were inoculated into one milliliter of PRAS anaerobic dilution fluid. Ten-microliter aliquots of the sample were then placed on four SBA plates and spread over the plates with sterile glass spreaders. Two SBA plates were incubated aerobically and two were placed in a candle jar. Two BHIS roll tubes and one buffered oral agar (BO) roll tube were inoculated with 10 microliters of the sample. This was done by allowing the inoculum to run down the side of the tube and then spreading with a sterile glass spreader. All plates and tubes were incubated at 37°C. The colonies on each plate and tube

were counted at 24, 48, and 72 hours. The average number of colonies was determined and this number was multiplied by the dilution factor to determine how many bacteria were present per milliliter of anaerobic dilution fluid. Methylene blue stained smears of the sample were examined for the direct microscopic clump counts (DMCC). Also, 10-microliter aliquots were examined with the Petroff-Hausser Counting Chamber (PHCC).

RESULTS AND DISCUSSION

Direct microscopic clump counts and Petroff-Hausser counting chamber results yielded 10^5-10^6 cells per milliliter of PRAS anaerobic dilution fluid. Approximately 0.1% of these counts, or 100-200 colony forming units, were recovered from each gerbil. Calculations were based on the following formulas.

Direct Microscopic Clump Count (DMCC)

(Average Count/Field) (Dilution Factor) (Reciprocal of Amount/Smears) (6.5×10^3 Fields/cm^2) = Number of Cells/Milliliter

Petroff-Hausser Counting Chamber (PHCC)

(Average Number of Bacteria/Square) (Dilution Factor) (2×10^7) = Number of Cells/Milliliter

Results of the quantitative counts are shown in the following table:

Gerbil	DMCC	PHCC	Total # of Isolates
#5	1.14×10^6	6.4×10^6	10
#6	3.0×10^5	-	119

There are several reasons why so few of the counts were actually recovered. Accurate counts with the DMCC and PHCC are difficult to obtain in such dilute samples. For Gerbil #5, 52 fields were counted, yielding 1.75 cells per DMCC field. For the PHCC there were only 8 cells in 25 squares counted. The DMCC for Gerbil #6 yielded 0.46 cells per field and the PHCC was not done due to a microscope malfunction. With both techniques it can be difficult to distinguish between cells and debris, so counts may

be higher than recovery. Also, these methods do not distinguish between living and dead cells. A break (done during sacrifice) in the left tympanic bulla of Gerbil #5 may explain the extremely low recovery. The povidone-iodine solution used to cleanse the head may have seeped into the nasopharynx, killing the majority of the microorganisms present.

The bacteria isolated from the six gerbils are listed in Tables 3 through 21. Because of the large numbers isolated, a representative number of each colony type was picked for identification. Altogether 122 isolates were identified. Of these, 71.3% were aerobic or facultatively anaerobic and 28.7% were obligately anaerobic. Staphylococcus species were most often isolated, accounting for 46.8% of all isolates. Staphylococcus aureus represented 46.3% of all staphylococci identified and was isolated from 4 of the 6 gerbils. Corynebacterium species were the next most common isolates, 35.2%. Corynebacterium pseudodiphtheriticum was found in 4 of the gerbils. Lactobacillus plantarum was isolated from 2 gerbils. It was the only obligately anaerobic bacteria isolated from more than one gerbil. No Mycoplasma species were isolated.

Of the bacteria isolated from the nasopharynx of the gerbil, only three have been implicated in otitis media of other laboratory animals; Klebsiella pneumoniae, guinea pigs (Kohn, 1974); Pasteurella multocida, rabbits (Vasenius and Tainen, 1966; Fox et al., 1977; Snyder et al., 1973), and Propionebacterium acnes, rats (Fulghum et al., 1977). Klebsiella pneumoniae and Propionebacterium acnes were isolated from one gerbil each and only one colony of each

Table 3. Characteristics of aerobic gram-positive cocci from gerbil #1

	Staphylococcus aureus	Staphylococcus epidermidis	Staphylococcus saprophyticus
Catalase	1/1[a]	2/2	1/1
O-F Glucose	1/1F[b]	2/2F	1/1F
15% NaCl	1/1	2/2	1/1
40% Bile	1/1	2/2	1/1
Arginine	1/1	2/2	1/1
Voges-Proskauer	1/1	2/2	1/1
Coagulase	1/1	0/2	0/1
β Hemolysis	1/1	1/2	0/1
Nitrate Reduction	1/1	1/2	0/1
Xylose	0/1	0/2	0/1
Sucrose	1/1	2/2	1/1
Trehalose	1/1	0/2	1/1
Mannitol	1/1	0/2	1/1

[a]Number positive/number tested

[b]F = fermentative, O = Oxidative, I = Inert

Table 4. Characteristics of aerobic gram-positive bacilli from gerbil #1

	Corynebacterium equi	*Corynebacterium pseudodiphtheriticum*	*Corynebacterium xerosis*
Catalase	2/2	1/1	1/1
O-F Glucose	2/2 F/O[a]	1/1 I	1/1 F/O
Motility	0/2	0/1	0/1
Gelatin liquefaction, 22°C	0/2	0/1	1/1
Urease	2/2 slow	1/1	0/1
Arginine	0/2	0/1	0/1
Sucrose	0/2	0/1	1/1
Glucose	0/2	0/1	1/1

[a] F = fermentation, O = oxidative, I = inert

Table 5. Characteristics of facultative gram-negative bacilli from gerbil #1

	Aeromonas species	Klebsiella pneumoniae	Pasteurella multocida
O-F Glucose	2/2 F	1/1 F	1/1 F
Oxidase	2/2	0/1	0/1
MacConkey agar	2/2	1/1	0/1
Motility, 37°C	2/2	0/1	0/1
Arginine	2/2	0/1	0/1
Lysine	0/2	1/1	0/1
Ornithine	0/2	0/1	1/1
KCN	2/2	1/1	1/1
Inositol	0/2	1/1	0/1
Sorbitol	2/2	1/1	0/1
Methyl red	2/2	0/1	0/1
Voges-Proskauer	0/2	1/1	0/1

Table 6. Characteristics[a] of anaerobic bacteria from gerbil #1

	Bifidobacterium longum var. longum	Bifidobacterium adolescentis	Peptococcus morbilloram
Volatile acids	Acetic[b]	Acetic	No major acids
Methylated acids	Lactic	Lactic	No major acids
PY	a[w]	w	-
Amygdalin	a	a	w
Arabinose	a	a	-
Cellobiose	w[-]	w[-]	w
Erythritol	w[-]	-	-
Esculin pH	-	-	-
Esculin hydrolysis	-	+	-
Fructose	a	a	w
Glucose	a	a	a
Glycogen	a	a	w
Inositol	w	-	-
Lactose	a	a	-
Maltose	a	a	w
Mannitol	w	a	-
Mannose	a	a	w
Melezitose	-	w[-]	-
Melibiose	a	a	-
Raffinose	a	a	-
Rhamnose	-	-	-
Ribose	w[-]	w[-]	-
Salicin	-	w[-]	-
Sorbitol	-	-	-
Starch pH	a	a	-
Starch hydrolysis	+	+	-
Sucrose	w	a	w
Trehalose	-	-	w
Xylose	a	w	-

Table 6. Characteristics of anaerobic bacteria from gerbil #1 (concluded).

	Bifidobacterium longum var. *longum*	*Bifidobacterium adolescentis*	*Peptococcus morbilloram*
Gelatin	-	-	-
Milk	c	c	-
Meat	+	+	-
Indole	-	-	-
Nitrate	-	-	-
Catalase	-	-	-
Pyruvate	+	+	-
Lactate	+	+	-
Threonine	+	+	-

[a] a = strong acid, w = weak acid, - = negative, + = positive, c = curd, nc = no curd

[b] Uppercase indicates major acids and lowercase indicates minor acid products.

Table 7. Characteristics of gram-positive bacteria from gerbil #2

	Staphylococcus aureus	Staphylococcus saprophyticus	Staphylococcus haemolyticus
Catalase	3/3	2/2	2/2
O-F Glucose	3/3	2/2	2/2
15% NaCl	3/3	2/2	2/2
40% Bile	3/3	2/2	2/2
Arginine	2/3	1/2	-/2
Voges-Proskauer	0/3	0/2	0/2
Coagulase	3/3	0/2	0/2
β Hemolysis	3/3	0/2	0/2
Nitrate Reduction	3/3	0/2	2/2
Xylose	0/3	0/2	0/2
Sucrose	3/3	2/2	2/2
Trehalose	3/3	2/2	2/2
Mannitol	3/3	0/2	0/2

Table 8. Characteristics of anaerobic bacteria from gerbil #2

	Lactobacillus plantarum	*Eubacterium lentum*
Volatile acids	Acetic	Acetic
Methylated acids	Lactic	Lactic
PY	-	-
Amygdalin	a	-
Arabinose	a	-
Cellobiose	a	-
Erythritol	w	-
Esculin pH	a	-
Esculin hydrolysis	+	-
Fructose	a	-
Glucose	a	-
Glycogen	w	-
Inositol	w	-
Lactose	a	-
Maltose	a	-
Mannitol	-	-
Mannose	a	-
Melezitose	-	-
Melibiose	a	-
Raffinose	a	-
Rhamnose	-	-
Ribose	a	-
Salicin	a	-
Sorbitol	-	-
Starch pH	-	-
Starch hydrolysis	-	-
Sucrose	a	-
Trehalose	a	-
Xylose	w	-

Table 8. Characteristics of anaerobic bacteria from gerbil #2 (concluded)

	Lactobacillus plantarum	*Eubacterium lentum*
Gelatin	-	-
Milk	c	nc
Meat	-	-
Indole	-	-
Nitrate	-	+
Catalase	-	-

Table 9. Characteristics of gram-positive bacteria from gerbil #3

	Staphylococcus haemolyticus	Corynebacterium pseudodiphtheriticum
Catalase	2/2	1/1
O-F Glucose	2/2 F	1/1 I
15% NaCl	2/2	ND[a]
40% Bile	2/2	ND
Arginine	2/2	0/1
Voges-Proskauer	1/2	ND
Coagulase	0/2	ND
β Hemolysis	2/2	0/1
Nitrate Reduction	2/2	ND
Xylose	0/2	ND
Sucrose	2/2	ND
Trehalose	2/2	ND
Mannitol	0/2	ND
Motility	ND	0/1

[a]ND = not done

Table 10. Characteristics of gram-negative bacilli from gerbil #3

	Pasteurella species
Oxidase	3/3
Catalase	3/3
O-F Glucose	3/3 F
β Hemolysis	0/3
MacConkey Agar	0/3
Nitrate Reduction	3/3
Motility, 37°C	0/3
H_2S	3/3
Methyl Red	0/3
Voges-Proskauer	0/3
Arginine	0/3
Lysine	0/3
Ornithine	0/3
Glucose	3/3
Lactose	0/3
Sucrose	3/3
Maltose	3/3
Mannitol	3/3
Salicin	0/3
Arabinose	0/3
Inositol	0/3
Trehalose	0/3
Xylose	0/3

Table 11. Characteristics of anaerobic bacteria from gerbil #3

	Veillonella parvula	*Bacteroides* species
Volatile acids	Acetic, Propionic	Acetic
Methylated acids	-	Succinic
PY	-	-
Amygdalin	-	-
Arabinose	w	w
Cellobiose	-	-
Erythritol	-	-
Esculin pH	-	-
Esculin hydrolysis	-	-
Fructose	w	w
Glucose	-	-
Glycogen	-	-
Inositol	-	-
Lactose	-	-
Maltose	-	-
Mannitol	ND	ND
Mannose	w	w
Melezitose	-	-
Melibiose	-	-
Raffinose	-	-
Rhamnose	-	-
Ribose	-	-
Salicin	-	-
Sorbitol	-	-
Starch pH	-	-
Starch hydrolysis	-	-
Sucrose	-	w
Trehalose	-	-
Xylose	-	-
Gelatin	-	-
Milk	nc	nc

Table 11. Characteristics of anaerobic bacteria from gerbil #3 (concluded)

	Veillonella parvula	*Bacteroides* species
Meat	ND	ND
Indole	-	-
Nitrate	+	+
Catalase	+	+
Pyruvate	+	+
Lactate	-	+
Threonine	+	+

Table 12. Characteristics of aerobic gram-positive cocci from gerbil #4

	Staphylococcus aureus	Staphylococcus epidermidis	Micrococcus varians
Catalase	8/8	1/1	6/6
O-F Glucose	8/8 F	1/1 F	6/6 O
15% NaCl	8/8	1/1	0/6
40% Bile	8/8	1/1	0/6
Arginine	8/8	1/1	0/6
Voges-Proskauer	8/8	1/1	0/6
Coagulase	8/8	0/1	ND
β Hemolysis	8/8	0/1	0/6
Nitrate Reduction	7/8	0/1	6/6
Xylose	0/8	0/1	ND
Sucrose	8/8	1/1	ND
Trehalose	8/8	0/1	ND
Mannitol	5/8	0/1	ND
Pigmentation	Yellow	White	Yellow
Glucose	ND	ND	6/6

Table 13. Characteristics of aerobic gram-positive bacilli from gerbil #4

	Corynebacterium pseudodiphtheriticum	Corynebacterium aquaticam	Corynebacterium pyogenes	Bacillus species
Catalase	1/1	1/1	0/10	1/1
Morphology	Pleomorphic	Pleomorphic	Pleomorphic	Chains
Spores	0/1	0/1	0/10	1/1
O-F Glucose	1/1 I	1/1 I	11/10 F/O	1/1 F
Motility	0/1	1/1	0/10	0/1
Salicin	0/1	0/1	0/10	ND
Trehalose	0/1	0/1	0/10	ND
H_2S	ND	ND	0/10	ND
Gelatin, 22°C	0/1	0/1	10/10	1/1
Sucrose	0/1	1/1	0/10	ND
Xylose	0/1	0/1	10/10	1/1
Glucose	0/1	1/1	10/10	1/1
Mannitol	ND	ND	ND	1/1
Arabinose	ND	ND	ND	1/1
Starch	ND	ND	ND	1/1
7.5% NaCl	ND	ND	ND	1/1
Nitrate Reduction	1/1	0/1	0/10	0/1

Table 14. Characteristics of facultative gram-negative bacilli from gerbil #4

	Acinetobacter calcoaceticus var. *lwoffi*	*Pasteurella multocida*	*Pseudomonas pseudomallei*
Catalase	1/1	2/2	1/1
Oxidase	0/1	2/2	1/1
O-F Glucose	1/1 I	2/2 F	1/1 O
Motility, 37°C	0/1	0/2	1/1
MacConkey Agar	1/1	0/2	1/1
Indole	1/1	2/2	0/1
Urease	0/1	0/2	0/1
Ornithine	0/1	2/2	0/1
Lysine	0/1	0/2	0/1
Arginine	0/1	0/2	1/1
Gelatin, 22°C	0/1	0/2	1/1
Inositol	ND	0/2	1/1
Trehalose	ND	2/2	1/1
Cellobiose	ND	ND	1/1
Starch hydrolysis	ND	ND	1/1
Fluorescent pigment	ND	ND	0/1

Table 15. Characteristics of anaerobic bacterium from gerbil #4

	Lactobacillus plantarum
Volatile acids	Acetic
Methylated acids	Lactic, Succinic
PY	a
Amygdalin	a
Arabinose	a
Cellobiose	a
Erythritol	-
Esculin pH	w
Esculin hydrolysis	+
Fructose	a
Glucose	a
Glycogen	-
Inositol	a
Lactose	w
Maltose	a
Mannitol	a
Mannose	a
Melezitose	a
Melibose	a
Raffinose	a
Rhamnose	w
Ribose	w
Salicin	w
Sorbitol	a
Starch pH	w
Starch hydrolysis	-
Sucrose	a
Trehalose	a
Xylose	a
Gelatin	-
Milk	c

Table 15. Characteristics of anaerobic bacterium from gerbil #4 (concluded)

	Lactobacillus plantarum
Meat	ND
Indole	-
Nitrate	-
Catalase	-

Table 16. Characteristics of aerobic gram-positive cocci from gerbil #5

	Staphylococcus aureus	Staphylococcus xylosus	Micrococcus luteus
Catalase	7/7	1/1	1/1
O-F Glucose	7/7 F	1/1 F	1/1 O
15% NaCl	7/7	1/1	0/1
40% Bile	6/7	1/1	0/1
Arginine	7/7	1/1	0/1
Voges-Proskauer	5/7	1/1	0/1
Coagulase	7/7	0/1	ND
β Hemolysis	6/7	0/1	0/1
Nitrate Reduction	7/7	1/1	0/1
Xylose	0/7	1/1	ND
Sucrose	7/7	1/1	ND
Trehalose	7/7	1/1	ND
Mannitol	7/7	0/1	ND
Pigmentation	Yellow	White	Yellow
Glucose	ND	ND	0/1

Table 17. Characteristics of facultative gram-negative bacillus from gerbil #5

	Pseudomonas cepacia[a]
Catalase	1/1
Oxidase	1/1
O-F Glucose	1/1 O
Nitrate Reduction	1/1
Motility	1/1
Gelatin, 22°C	0/1
6.5% NaCl	0/1
Arginine	0/1
Lysine	1/1
Citrate	1/1
Urease	0/1

[a]Also identified with the API 20E

Table 18. Characteristics of aerobic gram-positive bacteria from gerbil #6

	Staphylococcus xylosus	Aerococcus species	Corynebacterium pseudodiphtheriticum
Catalase	11/11	0/2	2/2
O-F Glucose	11/11 F/O	2/2 F	2/2 I
15% NaCl	11/11	0/2	ND
40% Bile	11/11	0/2	ND
Arginine	11/11	0/2	0/2
Voges-Proskauer	8/11	ND	0/2
Coagulase	0/11	ND	ND
β Hemolysis	0/11	0/2	0/2
Nitrate Reduction	11/11	0/2	2/2
Xylose	11/11	ND	0/2
Sucrose	11/11	2/2	0/2
Trehalose	11/11	ND	0/2
Mannitol	11/11	ND	0/2
Lactose	ND	2/2	0/2
Motility, 37°C	ND	0/2	0/2

Table 19. Characteristics of facultative gram-negative bacilli from gerbil #6

	Acinetobacter calcoaceticus var. *lwoffi*	*Pseudomonas pseudomallei*	*Pseudomonas putida*
Catalase	1/1	1/1	3/3
Oxidase	0/1	1/1	3/3
O-F Glucose	1/1 I	1/1 O	3/3 O
MacConkey Agar	1/1	1/1	3/3
Motility, 37°C	0/1	1/1	3/3
Nitrate Reduction	0/1	1/1	0/3
Arginine	0/1	1/1	3/3
Lysine	0/1	0/1	0/3
Ornithine	0/1	0/1	0/3
Gelatin, 22°C	ND	1/1	0/3
Fluorescent pigment	ND	0/1	ND
Inositol	ND	1/1	0/3
Trehalose	ND	1/1	0/3
Starch hydrolysis	ND	1/1	0/3

Table 20. Characteristics of anaerobic bacteria from gerbil #6

	Streptococcus intermedius	*Propionibacterium acnes*
Volatile acids	Acetic	Acetic, Propionic
Methylated acids	Lactic	Lactic, Succinic
PY	w	-
Amygdalin	w	-
Arabinose	w	w
Cellobiose	a	-
Erythritol	a	-
Esculin pH	w	-
Esculin hydrolysis	+	-
Fructose	a	w
Glucose	a	-
Glycogen	-	-
Inositol	w	-
Lactose	a	-
Maltose	a	-
Mannitol	w	-
Mannose	w	-
Melezitose	w	-
Melibiose	w	-
Raffinose	-	-
Rhamnose	a	-
Ribose	a	w
Salicin	a	-
Sorbitol	a	-
Starch pH	w	-
Starch hydrolysis	-	-
Sucrose	a	-
Trehalose	a	-
Xylose	w	w

Table 20. Characteristics of anaerobic bacteria from gerbil #6 (concluded)

	Streptococcus intermedius	*Propionibacterium acnes*
Gelatin	-	+
Milk	c	nc
Meat	-	-
Indole	-	-
Nitrate	-	+
Catalase	-	+

Table 21. Summary of bacteria isolated from the nasopharynx of six Mongolian gerbils

Aerobic Bacteria	Gerbils #1	#2	#3	#4	#5	#6
Staphylococcus aureus	+	+		+	+	
Corynebacterium pseudo-diphtheriticum	+		+	+		+
Staphylococcus epidermidis	+			+		
Staphylococcus saprophyticus	+	+				
Staphylococcus haemolyticus		+	+			
Staphylococcus xylosus					+	+
Acinetobacter calcoaceticus var. lwoffi				+		+
Pasteurella multocida	+			+		
Pseudomonas pseudomallei				+		+
Micrococcus luteus					+	
Micrococcus varians				+		
Aerococcus species						+
Corynebacterium equi	+					
Corynebacterium aquaticum				+		
Corynebacterium xerosis	+					
Corynebacterium pyogenes				+		
Bacillus species				+		
Aeromonas species	+					
Klebsiella pneumoniae	+					
Pasteurella species			+			
Pseudomonas cepacia					+	
Pseudomonas putida						+
Anaerobic Bacteria						
Lactobacillus plantarum		+		+		
Bifidobacterium longum var. longum	+					
Bifidobacterium adolescentis	+					
Peptococcus morbillorum	+					
Eubacterium lentum		+				
Streptococcus intermedius						+
Propionibacterium acnes						+
Veillonella parvula			+			
Bacteroides species			+			
Gram-negative bacilli				+		

was recovered. It is possible that these were contaminants. Pasteurella multocida was found in two gerbils. Again it was in two numbers, with only four isolates recovered.

The low organisms most often cited as contributing to otitis media in rats, Mycoplasma pulmonis and Streptobacillus moniliformis (Nelson, 1957; Lerner and Silverstein, 1958; Retzlaff et al., 1960; Nelson, 1963; Olson and McCune, 1968; Kohn and Kirk, 1969; Kohn, 1971), were not isolated from any of the gerbils. Neither has there been any report in the literature of isolating these two micro-organisms from laboratory gerbils.

As stated in the literature review, several factors including; lack of ventilation, size of auditory bullae, angle and length of the eustachian tube, amount of time in optimal tubal ventilation positions, and goblet cell population, contribute to the high incidence of otitis media in rats as compared to gerbils. This study and that of Gardner (1977) support these conclusions.

Gardner (1977) isolated Corynebacterium species, Staphylococcus epidermidis, Staphylococcus saprophyticus, and Gram-positive rods from the tympanic bullae of the Mongolian gerbil. Staphylococcus epidermidis was the most common isolate. These bacteria are considered to be generally non-pathogenic and were not present in numbers sufficient to initiate otitis media. This suggests that the tympanic bulla provides a poor environment for the promotion of an indigenous microbial flora. It also suggests, since the major route of invasion of the middle ear is by way of an ascending infection of the eustachian tube (Friedman, 1971), an immunological antimicrobial

defense mechanism working in conjunction with the mechanisms of ventilation and drainage stated above. Therefore, further investigations into the physiological and biochemical conditions within the middle ear and eustachian tube of the Mongolian gerbil are required.

In conclusion, each gerbil (6/6) had at least one species of the Genus, Staphylococcus, 4/6 had at least one species of the Genus, Corynebacterium, and most other facultative anaerobes appeared to be transitory. No anaerobic bacterium could be identified as being a consistant member of the nasopharyngeal microflora of the Mongolian gerbil.

APPENDIX A

Biochemical tests for aerobic and facultatively anaerobic bacteria[a]

Aerobic acid production from carbohydrates. Agar plate method of Kloos and Scheifer (1974) to detect acid production from carbohydrates under aerobic conditions. Medium employed: Purple agar base plus 1% desired carbohydrate.

API 20E. Microtechnique by Analytab Products Inc., Plainview, New York for the identification of Enterobacteriaceae and related bacteria after 24 to 48 hours of incubation.

Bile esculin test. To determine the ability of an organism to hydrolyze the glucoside esculin to esculetin and glucose in the presence of bile (10 to 40%). Medium employed: Bile Esculin Medium (pH 7.0).

Carbohydrate fermentation tests. To determine the ability of an organism to ferment a specific carbohydrate incorporated in a basal medium producing acid or acid with visible gas. Medium employed: Phenol red broth base (pH 7.4) with Durham fermentation tubes for gas production plus 1% desired carbohydrate except salicin, 0.5%.

Catalase test. To determine the presence of the enzyme catalase. Reagent employed: Hydrogen peroxide, 30% or 3%.

Citrate test. To determine the ability of an organism to use citrate as the sole source of carbon for metabolism with resulting alkalinity. Medium employed: Simmons citrate medium (pH 6.9).

Coagulase test. To determine the ability of an organism to clot plasma by the action of the enzyme coagulase. Reagent employed: Plasma, human or rabbit.

Decarboxylase tests (lysine, ornithine, arginine). To determine the enzymatic ability of an organism to decarboxylate an amino acid to form an amine with resulting alkalinity. Lysine and ornithine are decarboxylated but arginine may be catabolized by a decarboxylase or a dehydrolase pathway. Medium employed: Møller decarboxylase base (pH 6.0) plus 1% desired amino acid.

Gelatin liquefaction test. To determine the ability of an organism to produce proteolytic enzymes (gelatinases) which liquefy gelatin. Medium employed: Nutrient gelatin stab medium (pH 6.8).

Hemolysis. To determine the presence or absence of enzymes (hemolysins) which lyse red blood cells. Three classifications of hemolysis occur: β hemolysis, complete lysis of red blood cells (clear-transparent zone around colony); α hemolysis, incomplete lysis (greenish zone around colony); and γ hemolysis, absence of lysis. Medium employed: 5% sheep blood agar.

Hydrogen sulfide test. To determine if hydrogen sulfide (H_2S) has been liberated, by enzymatic action, from sulfur bearing amino acids producing a visible black color reaction. Media employed: Kligler iron agar (KIA), Triple sugar iron agar (TSI), or Sulfide-indole-motility agar (SIM).

Indole test. To determine the ability of an organism to split indole from the tryptophan molecule. Media employed: Tryptophan or peptone broth. Reagents employed: Ehrlich's or Kovac's indole test reagents.

Kligler's iron agar (KIA) and Triple sugar iron agar. To determine the ability of an organism to attack specific carbohydrates incorporated in a basal medium, with or without the production of gas, and the production of hydrogen sulfide. KIA contains 1.0% lactose and 0.1% glucose. TSI contains 1.0% lactose, 0.1% glucose, and 1.0% sucrose.

MacConkey agar. Selective and differential medium for Gram-negative bacteria. Inhibitory to most Gram-positive bacteria and allows differentiation of lactose fermenting and non-lactose fermenting Gram-negative bacteria. Bailey and Scott (1974).

Mannitol salt agar (MSA). Selective and differential medium for staphylococci. High salt concentration inhibits most other bacteria. Fermentation of mannitol is indicated by a yellow halo surrounding colonies. Bailey and Scott (1974).

Methyl red test. To determine the ability of an organism to produce and maintain stable acid end products from glucose fermentation over a long period of time (2 to 5 days). Medium employed: Clark and Lubs medium, MR/VP Broth (pH 6.9).

Motility test. To determine if an organism is motile or nonmotile. Media employed: Commercial broths or SIM (see indole test).

Nitrate reduction test. To determine the ability of an organism to reduce nitrate to nitrites or free nitrogen gas. Medium employed: Potassium Nitrate Medium (pH 7.0).

ONPG test. To determine the presence of the enzyme β-galactosidase by using the compound o-nitrophenyl-β D-galactopyranoside (ONPG). Medium employed: ONPG broth (pH 7.3).

Oxidase test. To determine the presence of the oxidase enzymes in a cytochrome oxidase system which activates the oxidation of reduced cytochrome by molecular oxygen. Oxidase enzymes have an important role in aerobic respiration. Reagents employed: Kovac's reagent, 1.0% tetramethyl-p-phenylenediamine dihydrochloride or BBL: Taxo N discs.

Oxidation-fermentation test. To determine the oxidative or fermentative metabolism of a carbohydrate. Medium employed: Hugh and Leifson's OF Basal Medium (pH 7.1) plus 1.0% desired carbohydrate.

Potassium cyanide tests (KCN). To determine the ability of an organism to live and reproduce in a medium containing potassium cyanide. Medium employed: KCN broth (pH 7.6).

Sodium chloride (NaCl), 6.5% and 15%. To determine the ability of an organism to live and reproduce in a medium with a high salt concentration. Medium employed: Nutrient broth plus 6.5% or 15% NaCl.

Starch hydrolysis test. To determine the presence or absence of hydrolytic enzymes which break down starch. Medium employed: Starch agar. Reagent employed: Gram's iodine (turns black in the presence of undegraded starch). Manual of Clinical Microbiology, 2nd Ed. (1974).

Urease test. To determine the ability of an organism to split urea to two molecules of ammonia by the action of the enzyme urease. Medium employed: Rustigian and Stuart's urea broth (pH 6.8).

Voges-Proskauer test. To determine the ability of an organism to produce a neutral end-product, acetylmethylcarbinol, from glucose fermentation. Medium employed: Clark and Lubs Medium, MR/VP Broth (pH 6.9).

[a]Except where noted descriptions of biochemical tests are adapted from MacFaddin (1976).

APPENDIX B

Biochemical tests for anaerobic bacteria[a]

Most biochemical tests are basically the same for aerobic and anaerobic bacteria. Only tests for anaerobic bacteria are performed under anaerobic conditions. Those tests which are unique to the anaerobic bacteria will be described here.

Lactate utilization. To determine lactate utilization, chromatographs of a Peptone Yeast-lactate culture and an uninoculated PY-lactate medium are compared. The disappearance of lactate (peak height of lactate in the PY-lactate culture vs. peak height of lactate in an uninoculated PY-lactate medium) indicates lactate utilization.

Meat digestion. To determine the presence or absence of enzymes which degrade meat. Indicated by disintergration of meat particles in a Chopped Meat broth culture. Digestion may require 14 to 21 days.

Milk. To determine the presence or absence of enzymes which attack milk components. Curd formation with or without digestion indicates the presence of these enzymes.

pH. The pH of carbohydrate media is recorded to determine the amount of acid produced from specific carbohydrates. In general, pH 5.5 to 6.0 = weak acid; pH below 5.5 = strong acid; and pH above 6.0 = negative.

Pyruvate utilization. To determine pyruvate utilization, chromatographs of a PY-pyruvate culture and an uninoculated PY-pyruvate are compared. Disappearance of pyruvate indicates pyruvate utilization.

Spore test. To determine the presence or absence of spores. May demonstrate spores by staining or performing a heat test. Inoculate starch broth with growth from Chopped Meat agar slant. Stoppered tubes are then clamped to retain stoppers and placed in 80 C water bath for 10 minutes. The tubes are then cooled to and incubated at 37 C. Growth is positive for sporulation.

Threonine. To determine the conversion of threonine to propionate. Chromatograph the threonine culture and the PY culture. Presence of more propionic acid in the threonine culture than in the PY culture indicates conversion of threonine to propionate.

[a]Description of biochemical tests are adapted from the V.P.I. Anaerobe Laboratory Manual (4th Ed., 1977).

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