CHAPTER 1

INTRODUCTION

Civilized man makes use of plants to satisfy his aesthetic sense and plants are bound up with civilization in a deeper sense. Many drugs that help man fight diseases are derived from plants. The crude extract of the bark of *Alstonia scholaris* R. Br. has helped the frontiersman fight malaria. Quinine a much improved antimalarial, is also a plant product. New technology has shown that inert substances are physiologically indispensable in making some drug actions effective. Plants contain, aside from active principles, trace elements of utmost importance because of their enzyme action which is valuable in therapy. It is observed that many vegetable drugs defy laboratory analyses, and therefore their therapeutic constituents could not be isolated. As a result, there is unavoidable use of the plants or their parts in herbal medicine.

The superiority of natural products can be illustrated in a case of iodine deficiency. When given iodine treatment a patient generally develops rash, but when given seaweeds rich in iodine the recovery is normal without any side effect. There must be something in the seaweeds in addition to iodine that gives a better therapeutic result. Scientists have been trying to identify that substances. In like manner, perhaps, many of our plants may not give any active prin-

ciple on analysis. The synthesizing power of plants has not been duplicated by chemistry. Analysis of the chemical constituents is never complete, for some of these products are destroyed by heat or fermentation.

For centuries vegetable drugs have been used in all parts of the world as folklore remedies even before the discovery of micro-organisms. Claims have been made that certain plants have the power of healing wounds, removing inflammation, and that others are effective in the treatment of such diseases as Cholera, and Dysentery. It might be assumed that if these observations were true the cures claimed may have been influenced in some cases by the presence of antibacterial substances in the plants.

Although modern technology has made possible the synthetic chemical production of many important drugs, plants continue to be a major source of medicines. Many of our present day drugs from plant sources were known and used, at least in raw form, by early man. Indeed some modern medicines have been discovered through study of their usage in primitive societies. Among the recent drug discoveries from plant sources are the steroidal compounds from Dioscorea which are used in the treatment of arthritis (24). The antibiotics such as penicillin and streptomycin are the most outstanding example of newly developed drugs from plant sources. In 1928, the Scottish scientist Sir Alexander Fleming started the interest in antibiotics when he accidentally discovered the inhibitory activities of a common green mold, Penicillium notatum Westing. against the pathogenic bacterium

Staphylococcus aureus Rosenbach. Since then, many antibiotics have been developed, some of which do not have any practical application at present. Antibiotics include aspergillic acid from Aspergillus flavus Link ex Fr., clavalin from Aspergillus clavatus Desmazieres, chaetomin from Chaetomium cochlioides Palliser, an Ascomycete, and gliotoxin from Trichoderma sp., a Deuteromycete.

Researchers are continually attempting to isolate more soil microorganisms which synthesize metabolic products that are of possible use in the control of bacterial infections. Since there is the possibility that penicillin, aureomycin or streptomycin may have less effective value in the control of various human diseases, the search for new antibiotics has to continue.

Survey of Literatures

Over the last 3 decades, an intensive effort has been made to search for new, clinically useful, antibiotics. A number of reviews discussing antibiotics from plants have been published. These compilations are evidence of considerable screening, but relatively few studies have been published in which the promising leads were followed up with attempts to isolate and identify the active principle(s).

Biological antagonism in aquatic microalgae was first observed in Chlorella vulgaris Beijerinck. by Pratt and Fong (1940)(66). Fatty acids of this green alga are photooxidized on standing in the light to form the antibacterial chlorellin (44, 84).

Studies on the inhibition of Haematococcus by fatty substances from Chlamydomonas were carried out by Proctor (1957), who demonstrated that long chain fatty acids, such as palmitic and linolenic acids, were toxic to sensitive species of green algae (68).

Accorinti (1963 a, 1963 b) showed that the cultures of Scenedesmus obliguus and Coelastrum microporum produce fatty acids inhibitory for Staphylococcus aureus Rosenbach (2, 3).

The green alga Pythophora has been shown to inhibit Mycobacterium tuberculosis (Schroeter) Lehmann and Neumann., in vitro experiment: where the bacterias were exposed to algal extracts (42).

Levina (1961) and Telitchenko et al. (1962) claimed that active growing of Scenedesmus and Chlorella can inhibit coliforms and Salmonella (46, 87).

Studies on Phaeocystic pouchetii blooms in Antarctic waters by Sieburth (1959, 1960, 1961) indicated that acrylic acid produced by the algae inhibits Gram-positive bacteria, yeasts and Aspergillus fumigatus Fresenius (75, 76, 77).

Paper chromatograms of ethereal and ethanolic extracts of cells of three unicellular algal species, Chlorella vulgaris Beijerinck., Scenedesmus quadricanda (Turp) Breb. and Chlamydomonas reinhardi Dang were tested on agar plates with the bacteria Bacillus subtilis (Ehrenberg) Cohn by Jørgensen (1962). The result showed that Chlorophyllides might possibly play a part as antibacterial substance in natural waters (39).

It was observed by Burkholder and Sharma (1969) that blooms of Cochlodinium and Goniodoma in Puerto Rican waters inhibit growth of bacteria and yeasts (14).

Antimicrobial properties of seaweed extracts were observed by Pratt et al. (1951) and since then numerous reports have been published on this subject (67).

It was suggested by Mautner et al. (1953) that the active substances in Rhodomela larix (Turn.) J. Ag. and Symphocladia gracilic may be brominated phenols (54).

Chesters and Stott (1956) found that ethereal extracts of certain British seaweeds, e.g., Halidrys siliquosa, Pelvetia canaliculata, Laminaria digitata (L.) Edmonson and Polysiphonia fastigiata, have marked antagonistic properties against pathogenic bacteria.

Methanolic extracts of algae from Japanese waters, including the species Laminaria angustata, Undaria pinnatifida and Rhodomela larix (Turn.) J. Ag., have been shown to inhibit several kinds of bacteria (71, 72).

In tests of 150 species of tropical marine algae growing near La Parquera, Puerto Rico, 66 species exhibited antimicrobial activity against laboratory strains of bacteria and yeasts and several kinds of marine bacteria. Among the more active species of algae were the followings: Chondria littoralis, Falkenbergia hildebrandii, Murrayella periclados, Wrangelia argus, Wrangelia bicuspidata, Laurencia obtusa and Dictyopteris justii (13).

Benthic algae collected along the west coast of Mexico and Central America by Allen and Dawson (1960) were frozen, extracted with 80% methanol, and tested against Gram-positive and acid-fast bacteria. Diverse patterns of inhibition suggested the presence of at least four different antibiotic agents in the seaweeds studied (4).

Solvent extracts from 9 species of algae were tested by Olesen, Maretzki and Almodovar (1946) against bacteria and Candida, they reported some of the chemical properties of active spots separated by thin-layer chromatography (62).

The chemical nature of the substances causing diverse effects of algae against microorganisms has received some attention. Thus, it appeared that one of the most common antibacterial components of seaweeds is acrylic acid. This compound occurs in many red, brown and green algae, as well as in various species of phytoplankton (21, 40, 76).

Challenger (1953) and Simpson (1956) demonstrated the occurence of β-dimethyl propiothetin in the red alga Polysiphonia fastigiata. Hydrolysis of propiothetin by enzymatic cleavage yields dimethyl sulfide and acrylic acid (CH_2 = CH - COOH), in red and green algae (16, 17).

Apparently substances present in Sargassum have an influence upon the fouling organisms which attach to this floating seaweed (25). Tannins of brown algae have been shown by Sieburth and Conover (1965) to retard fouling fauna and growth of the bacteria Pseudomonas and Vibrio (78).

Among the earlier investigations of antibiosis in marine bacteria is the study of 58 strains of isolated by Rosenfeld and Zobell (1947) who found 9 active against laboratory cultures (70).

Grein and Meyers (1958) isolated and tested 166 cultures of Actinomycetes from the marine littoral zone and recorded 70 active against various Gram positive and Gram negative bacteria (33).

The production of antibacterial substances by marine bacteria has been studied by Burkholder (1963) who found pigmented antagonistic bacteria in beds of tropical sea weeds, some of these liberate antibacterial compounds into the medium parallel with the growth curve (12).

Many sponges show antimicrobial inhibition when cut portions of tissue are placed upon seeded agar plates and incubated for a few hours. Both aqueous and organic solvent extractable substances from sponges have been demonstrated to have broadspectrum antibiotic effect against many kinds of test organisms (38, 59).

From red beard sponge, Microciona the antibiotic substance ectyonin was prepared from a fraction of ethyl ether extract. This broad spectrum antibiotic shows in vitro inhibition of many kinds of bacteria and Candida (60).

Substances with varying degrees of antimicrobial activity have been obtained from two temperate zone sponges, Halichondria (bread sponge), Cliona (sulfur sponge), Tedania (fire sponge), Haliclona (green sponge) Dysidea (heavenly sponge), and Oligocera (bleeding sponge)(38).

Burkholder and Sharma (1969) had collected numerous sponges from the Atlantic and Pacific Ocean and from the Mediterranean sea for antibiotic studies. The results showed that, there are many species of sponges which inhibit the growth of Escherichia coli, Pseudomonas aeruginosa, Proteus vulgaris Hauser, and many marine bacteria (14).

Uyenco (1971) pointed out that there is considerable information exists on the antibiotic activities of lichen substances. They are active against bacteria, free-living fungi and viruses. Usnic acid, a common lichen substance derived from Usnea spp., has received the most attention. It has a broad antibiotic spectrum and is the basis of some commercial drugs. Usnic acid inhibits oxidative phosphorylations, relaxes smooth muscle tissue, accelerates the rate of respiration in mammals, and has an adrenergic effect on the crop and gizzard of earth worms (92).

Very little attention is paid to the lower vascular plants, the Pteridophytes. Maruzzella's (1961) screening of 34 ferns for antimicrobial substances is the one of the works on lower vascular plants. He reported extraction of antibacterial substances from 33 of the 34 specimens tested (53).

Kshirsagar and Mehta (1972) have examined 13 species of ferns collected from different parts of Gujarat State (India) for antibacterial contents. Methanolic extracts were made of plants at different stages of growth and also of plants of different ages. Antibacterial activity was observed in few of the ferns screened. The activity was

more pronounced in old plants than in young ones. The fertile plants showed greater activity than vegetative ones. A preliminary study for the presence of alkaliods in fern species was negative (45).

The systematic investigation of higher plants for antibiotic substances was revived when Osborn (1943) reported his now classical studies on antimicrobial substances from green plants. He made the most thorough and systematic search of all for antibacterial substances in 2,300 species of higher plants belonging to 166 different families. Of these 63 genera in the Ranunculaceae, Cruciferae, Compositae and Liliaceae were found on presumptive test to contain substances which inhibit the growth of Staphylococcus aureus and Escherichia coli. The results of his study indicate that plants containing antibacterial substances are widely distributed in nature and offer a fertile field for investigation (63).

The paper of Huddleson et al. (1944) was a brief preliminary report of the results obtained from an investigation of the presence of antibacterial substances in 23 genera belonging to 15 families of fresh and stored plants. The various parts of a plant, such as root, stem, petiole, leaf, and fruit were tested separately. In examining the juices and partially purified extracts for inhibition of bacterial growth, they used serial dilution method. Each specimen was tested against Escherichia coli and Staphylococcus aureus and certain ones againt Brucella abortus (36).

Little and Grubaugh (1946) summarized the works of many investigators who pointed out that the resistance of some plant varieties

to phytopathogens might conceivably be related to the presence of more or less specific antibiotic substances in the juices of these plants. The works of these investigators were undertaken with a view to testing the validity of this postulate (47).

Carlson et al. (1948) separated 2,115 antibacterial substances from 550 species of plants collected from Ohio and Oregon states and determined their inhibitory properties in vitro. One or more antibacterial substances were separated from 115 species of the plants tested. The saline, acidic, ethereal, weak acidic (pH 4.0 buffered), and weak alkaline (pH 9.0 buffered) extracts of these plants were tested for their antibiotic effectiveness against Staphylococcus aureus and Escherichia coli (19).

Burlage et al. (1954) screened extracts of various toxic plants indigenous to Central Texas for antabonistic action upon bacteria, virus and tumors. In addition, the toxicity to experimental animals of many of these extracts has also been investigated (15).

Farnsworth et al. (1966) made a biological and phytochemical evaluation of a random-selected native plants by which 200 species and 4 varieties representing 137 genera from 47 families are included. A defatted ethanolic extract from plants was prepared for biological evaluation in vitro against 16 bacterial animal-pathogens and saprophytes, 15 fungal pathogens and material-degrading organisms, 3 fungal animal pathogens and saprophytes, 4 bacterial plant pathogens, 4 protozoa, 2 algae and 3 viruses. Also a general mouse behavior evaluation of each extract and a primary insecticide screen, utilizing

mosquito larvae were included (30).

Khanna et al. (1968) have examined the extracts of 24 plant tissue cultures grown as either static (callus) or suspension cultures for antimicrobial activity. Extracts of the callus tissues were more active against Staphylococcus aureus (14 strains) than Escherichia coli (9 strains)(41).

Mitscher et al. (1972) showed that extracts of Thalictrum rugosum Ait. were repeatedly active in a screening program designed to detect antimicrobial activity in higher plants. Fractionation led to the isolation of berberine, obamegine, thalidasine, thalrugosine, thalrugosidine and alkaloid D as active antibacterial agents. The structures and absolute stereochemistry of the new alkaloids, thalrugosine and thalrugosidine, were determined by a combination of spectroscopic and degradative experiments. A number of micro-biologically inert substances, including magnoflorine, β-sitosterol, β-sitosterol-β-D-glucoside, sucrose and an unidentified cyanogenetic glycoside, were encountered (58).

Mitscher et al. (1972) also showed that extracts of Zanthoxylum elephantiasis Macf. were consistently active against Stahylococcus aureus, Smith strain (ATCC 13709), Klebsiella pneumoniae AD (ATCC 10031), Mycobacterium smegmatis 607 B (ATCC 607) and Candida albicans (ATCC 10231) when tested in vitro using an agar dilution-streak method. Accordingly fractionation was undertaken to isolate the responsible agent(s) in pure form. Canthin-6-one, a well known alkaloid, previously isolated from this species but not hitherto known to be an

antibiotic, was shown to be the active constituent as detailed in his report (57).

It was observed by Lucas et al. (1946) that seed extracts of several species of Brassica exerted an antabonistic effect on the growth of a number of bacteria and fungi. The tests for the presence of antibiotic substances in plant materials were made according to a modification of the procedure developed by Vincent and Vincent (1946). A comparison of the results obtained with the extracts of several species of Brassica and numerous varieties of Brassica oleracea L. indicates that the reactions are rather specific (50).

Dealcoholized and aqueous extracts of the seeds of Cassia tora L. and leaves of Cassia obovata Colladon. had been studied by Patel et al. (1957) for their antibacterial action and compared with penicillin sodium and dihydrostreptomycin sulphate. All the extracts gave positive test for anthraquinone derivatives. The dealcoholized extracts of Cassia tora seeds and Cassia obovata leaves are found effective on all the organisms tested while the aqueous extract of Cassia tora seeds was not effective on any organisms. The aqueous extracts of Cassia obovata leaves was only effective on Salmonella typhosa (64).

The antibacterial activities of various extractives of leaves and seeds of Diospyros montana Roxb. were reported by Goutan and Puruhit (1973). The test was against the following microorganisms: Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, Bacillus subtilis, Proteus vulgaris, Corynebacterium pyogenes,

Salmonella pullorum, Pasteurella multocida. The results show that petroleum ether, carbon tetrachloride, benzene and chloroform extractives were generally effective against most of the microorganisms tested. Solvent ether, acetone and water extractives showed negative activity against all the microorganisms tested. Thus, the most susceptible test organisms appear to be B. subtilis and C. pyogenes (31).

Southam (1946) had studied the activity of water extract from Western red cedar heartwood (Thuja plicata D. Don) against microorganisms pathogenic to man in vitro. The results showed that the extract inhibits growth of a wide varieties of bacteria and fungi. Inhibition is due to stasis, not to mortality of the organisms. The extract does not lose its antibiotic activity when subjected to prolong boiling or considerable changes in pH. It is not inactivated by urine or gastric residue, but is inactivated by blood, serum and cysteine (83).

Tokin (1944) reported discovery of "Phytoncides", the name applied to volatile substances produced by plants. The bactericidal and protistocidal properties of over 150 plants were investigated. Innumerable experiments on various forms of plant life established that onions and garlic contain powerful bactericidal properties (89).

Toroptsev and Filatova (1944) began experiments to determine the effect of onion and garlic vapours upon the regenerative processes of experimentally infected wounds and the regeneration of aseptic wounds. The experiments performed on laboratory animals not only proved the antiseptic value of phytoncides in treating infected wounds but also demonstrated their somewhat stimulating effect upon the re-

generation process of aseptic wounds. They, therefore, decided to apply their laboratory findings to the individual patient and to use phytoncides in treating infected human wounds (90).

The wide application of Alpinia galanga Willd. and Acorus calamus Linn. for the treatment of infectious diseases in Indian indigenous medicine has prompted the study of their antibacterial spectra by Chopra et al. (1957). The results indicate that the oils of both show marked antitubercular action in in vitro studies. Both oils inhibit the growth of Gram-negative organisms and have no inhibitory effect on the growth of Gram-positive organisms. The LD_{50} of the oil of Alpinia galanga and Acorus calamus for guinea pigs were determined (23).

Low et al. (1974) presented a work aimed at evaluating the antibacterial activity of essential oils produced by Australian Myrtaceae. Special attention has been given to the possible interactions between the several components of the oil of Eucalyptus citriodora Hook. with regard to the enhancement of its antibacterial activity. One oil, from Eucalyptus citriodora, was shown by gas liquid chromatography to consist of citronellal, citronellol and cineole in the ratio of 90:7.5:2.5. It was found that the oil of Eucalyptus citriodora exerted its antimicrobial activity through the synergistic action of citronellal and citronellol (48).

Jain et al. (1974) have reported their investigations on some of the freshly collected volatile oils from indigenous plants in India. Efforts were made to study the antigunfal, antibacterial and

antiwormal responses separately of the oils. Six more oils were thus subjected to test studies to evaluate their antibacterial activity. Some of these oils have been also subjected to their clinical trials in form of topical applications made out of them which were tested on the approaching volunteer-patients having mild skin manifestations. The results are yet awaited for declarations. Meanwhile, in vitro studies are also performed to visualise the general range of effectivity of these oils. The oils used are of Piper cubeba Linn, Acorus calamus Linn., Litsea chinensis Lam., Colubrina asiatica Brongn., Hyptis suaveolens Point., and Blumea laciniata DC. (37).

The aglycone of glucotropaeolin benzyl isothiocyanate (BITC) of Nigerian Carica papaya L. seeds was studied by Tayes et al. (1974) for its antibiotic and pharmacological activity as well as its toxicity. The large number of microorganisms sensitive to BITC would also suggest its possible use as a bacteriostatic and a bactericidal agent in cases of some intestinal and urinary infections. The toxicity of the seed in the experiments is low and that at the usual therapeutically effective single dose of 4-5 g. of seeds (20-30 mg. BITC) would not be harmful (86).

Vajchanakup (1951, B.E. 2494) had conducted an experiment in order to test the antibacterial activity of 20 Thai medicinal plants against Staphylococcus aureus. The results showed that water extract of 8 genera can inhibit the growth of this organism (93).

The investigations mentioned illustrate the desired point that antibiosis is not uncommon among the plants and the potential for

finding useful chemotherapeutic agents there must be regarded as reasonable.

Purpose of the Study

It has been known that there are many interesting medicinal plants in Thailand. A large number of plants have been used in Thai indigenous medicine by Thai folks in the treatment of infectious diseases. Preparations made from these plants are applied locally to abscesses, sores and ulcers and are also administered orally for treatment of systemic infections of the body in spite of the medical advances. Since these Thai medicinal plants have evidently the power to cure diseases, it is worthwhile to make an investigation for the presence of antibacterial properties of these plants. This field offers innumerable possibilities for research. However, to investigate all the Thai medicinal plants will be very time consuming and expensive, so some criteria on selecting the plants must be clarified.

It was, therefore, decided that plants are not chosen at random. Many plants which are commonly found in each preparation of local remedies are reasonably believed to be active against bacteria. These plants have been given special attention for studying. For example, plants which are found common in different formula of Ya Kheow (green medicine) by Phya Bishnhuprasatvej (B.E. 2512) (6), Table 1, on furuncle and antiskin diseases poultices by Phya Bishnhuprasatvej (B.E. 2512) (7) and by Khun Sobhitbarnalakshana (B.E. 2504) (81), Table 2, on antidysentery drugs by Phya Bishnhuprasatvej (B.E.

2512) (7) and by Khun Sobhitbarnalakshana (B.E. 2504) (80), Table 3, on antidiarrhoea drugs by Phya Bishnhuprasatvej (B.E. 2512) (7) and by Khun Sobhitbarnalakshana (B.E. 2504) (80), Table 4, are shown in the following tables.

The purpose of this study is to investigate the antibacterial properties of certain Thai medicinal plants which are commonly found in each preparation of Thai folk remedies against eight representative mecroorganisms which are suspected to be the cause of many diseases as dysentery, diarrhoea, typhoid fever, dental caries, infectious skin diseases. They are Bacillus subtilis (Ehrenberg) Cohn, Escherichia coli (Migula) Castellani and Chalmers, Lactobacillus fermentum Beijerinck, Pseudomonas aeruginosa (Schroeter) Migula, Salmonella typhi (Schroeter) Warren and Scott, Shigella dysenteriae (Shiga) Castellani and Chalmers, Staphylococcus aureus Rosenbach, Streptococcus faecalis Andrewes and Horder.

Hopefully, when this study had been done we will have a scientific foundation by which Thai medicinal plants can be promoted and standardized as modern medicines. By using Thai medicinal plants as raw materials in manufacturing modern medicines in stead of imported ones, the costs minimized or saved will, in one or another way, contribute to the economic progress of Thailand.

Table 1

Ya Kheow by Phya Bishnhuprasatvej

Scientific Names	Part Used	Family	English Name	Thai Name
Phyllanthus distichus Muell. Arg.	Leaf	Euphorbiaceae	Star Gooseberry	มะยม
Averrhoa carambola L.	Leaf	Oxalidaceae	Carambola	มะเฟือง
Cynodon dactylon Pers.	Whole plant	Gramineae	Bermuda Grass	หญ้าแพรก
Sesbania aegyptiaca Pers.	Leaflet	Papilionaceae	-	สมี
Rhinacanthus nasutus Kurz.	Leaf	Acanthaceae	-	ทองพันชั่ง
Tiliacora triandra Diels.	Whole plant	Menispermaceae	-	ย่านาง
Momordica charantia L.	Whole plant	Cucurbitaceae	Bitter Gourd	มะระ
Momordica cochinchinensis Spreng.	Leaf	Cucurbitaceae	-	ฟักข้าว
Curcuma zedoaria Rosc.	Rhizome	Zingiberaceae	Zedoary	ขมิ้นอ้อย
Curcuma longa L.	Rhizome	Zingiberaceae	Turmeric	ขมิ้นชัน
Cassia tora L.	Leaflet	Caesalpiniaceae	Foetid Cassia	ชุมเห็ดไท
Pogostemon cablin Benth.	Leaf	Labiatae	Cablin	พิมเสน
Angiopteris evecta Hoffm.	Rhizome	Angiopteridaceae	-	ว่านกีบแรด
Globba sp.	Rhizome	Zingiberaceae	-	ว่านร่อนทอง
Nelumbo nucifera Gaertn.	Anthers	Nymphaeaceae	Sacred lotus	บัวหลวง
Azadirachta indica Juss. Var. siamensis Valeton.	Leaf	Meliaceae	-	สะเดา

Table 2

Furuncle, Antiskin diseases poultices by Phya Bishnhuprasatvej and by Khun Sobhitbarnalakshana.

Scientific Names	Part Used	Family	English Name	Thai Name
Citrus hystrix DC.	Leaf	Rutaceae	Kaffir Lime	มะกรูด
Acorus calamus Linn.	Rhizome	Araceae	Sweet Flag	ว่านน้ำ
Curcuma zedoaria Rosc.	Rhizome	Zingiberaceae	Zedoary	ขมิ้นอ้อย
Phyllanthus distichus Muell. Arg.	Leaf	Euphorbiaceae	Star Gooseberry	มะยม
Averrhoa carambola L.	Leaf	Oxalidaceae	Carambola	มะเฟือง
Allium sativum Linn.	Bulb	Liliaceae	Garlic	กระเทียม
Pouzolzia pentandra Benn.	Whole plant	Urticaceae	-	ขอบชะนาง(แดง)
Eclipta alba Hassk.	Whole plant	Compositae	-	กระเม็ง
Azadirachta indica Juss. var. siamensis Valeton.	Leaf	Meliaceae	-	สะเดา
Rhinacanthus nasutus Kurz.	Leaf	Acanthaceae	-	ทองพันชั่ง
Alpinia sp.	Rhizome	Zingiberaceae	-	ข่าตาแดง
Cymbopogon citratus Stapf.	Stem	Gramineae	Lemon Grass	ตะไคร้
Gastrochilus panduratus Ridl.	Rhizome	Zingiberaceae	-	กระชาย

Table 2

Furuncle, Antiskin diseases poultices by Phya Bishnhuprasatvej and by Khun Sobhitbarnalakshana.(cont.)

Scientific	Part Used	Family	English Name	Thai Name
Allium ascalonicum Linn.	Bulb	Liliaceae	Shallot	หอม (แกง)
Sesbania aegyptiaca Pers.	Leaf	Papilionaceae	-	สมี
Bridelia siamensis Craib.	Leaf	Euphorbiaceae	-	มะกา
Vitex trifolia L.	Leaf	Verbenaceae	-	คนทีสอ
Vitex trifolia L. var. repens Ridls.	Leaf	Verbenaceae	Indian wild pepper	คนทีสอ (ทะเล)
Cassia alata L.	Leaf	Caesalpiniaceae	Candle Bush	ชุมเห็ดเทศ
Cassia tora L.	Leaf	Caesalpiniaceae	Foetid Cassia	ชุมเห็ดไท
Oxalis repens Thumb.	Whole plant	Oxalidaceae	Wood - Sorrel	ส้มกบ
Alpinia officinarum Hance	Fruit	Zingiberaceae	Galangal Cardamom (for fruit)	พริกเทศ
Momordica charantia L.	Leaf	Cucurbitaceae	Bitter Gourd	มะระ
Acanthus ebracteatus Wall.	Leaf	Acanthaceae	Sea Holly	เหงือกปลาหมอ (ดอกขาว)

Table 3

Antidysenteric Drugs by Phya Bishnhuprasatvej and by Khun Sobhitbarnalakshana.

Scientific Names	Part Used	Family	English Name	Thai Name
Allium ascalonicum Linn.	Bulb	Liliaceae	Shallot	หอม (แกง)
Allium sativum Linn.	Bulb	Liliaceae	Garlic	กระเทียม
Alpinia sp.	Rhizome	Zingiberaceae	-	ข่าตาแดง
Angiopteris evecta Hoffm.	Rhizome	Angiopteridaceae	-	ว่านกีบแรด
Bixa orellana L.	Flower	Bixaceae	Annatto	คำไท
Curcuma longa Linn.	Rhizome	Zingiberaceae	Turmeric	ขมิ้นชัน
Curcuma zedoaria Rosc.	Rhizome	Zingiberaceae	Zedoary	ขมิ้นอ้อย
Cassia alata L.	Leaf	Caesalpiniaceae	Candle Bush	ชุมเห็ดเทศ
Cassia tora L.	Leaf	Caesalpiniaceae	Foetid Cassia	ชุมเห็ดไท
Cynodon dactylon Pers.	Whole plant	Gramineae	Bermuda Grass	หญ้าแพรก
Eclipta alba Hassk.	Leaf	Compositae	-	กระเม็ง
Gastrochilus panduratus Ridl.	Rhizome	Zingiberaceae	-	กระชาย
Globba sp.	Rhizome	Zingiberaceae	-	ว่านร่อนทอง
Nelumbo nucifera Gaertn.	Anthers	Nymphaeaceae	Sacred Lotus	บัวหลวง
Piper betle Linn.	Leaf	Piperaceae	Betel Vine	พลู
Plumbago indica L.	Root	Plumbaginaceae	Rose-colored Leadwort	เจตมูลเพลิงแดง
Vernonia elliptica DC.	Leaf	Compositae	-	ตานหม่อน
Vitex trifolia L.	Leaf	Verbenaceae	-	คนทีสอ
Zingiber cassumunar Roxb.	Rhizome	Zingiberaceae	-	ไพล

Table 4

Antidiarrhoea Drugs by Phya Bishnhuprasatvej and by Khun Sobhitbarnalakshana.

Scientific Names	Part Used	Family	English Name	Thai Name
Allium ascalonicum Linn.	Bulb	Liliaceae	Shallot	หอม (แกง)
Allium sativum Linn.	Bulb	Liliaceae	Garlic	กระเทียม
Alpinia sp.	Rhizome	Zingiberaceae	-	ข่าตาแดง
Areca catechu Linn.	Seed	Palmae	Betel Nut	หมาก
Acorus calamus Linn.	Rhizome	Araceae	Sweet Flag	ว่านน้ำ
Bridelia siamensis Craib.	Leaf	Euphorbiaceae	-	มะกา
Cassia alata L.	Leaf	Caesalpiniaceae	Candle Bush	ชุมเห็ดเทศ
Cynodon dactylon Pers.	Whole Plant	Gramineae	Bermuda Grass	หญ้าแพรก
Curcuma longa Linn.	Rhizome	Zingiberaceae	Turmeric	ขมิ้นชัน
Curcuma zedoaria Rosc.	Rhizome	Zingiberaceae	Zedoary	ขมิ้นอ้อย
Globba sp.	Rhizome	Zingiberaceae	-	ว่านร่อนทอง
Gastrochilus panduratus Ridl.	Rhizome	Zingiberaceae	-	กระชาย
Pogostemon cablin Benth.	Leaf	Labiatae	Cablin	พิมเสน
Tiliacora triandra Diels.	Root	Menispermaceae	-	ย่านาง
Zingiber cassumumar Roxb.	Rhizome	Zingiberaceae	-	ไพล

Methodology of Routine Tests

At the present time, there is no single standard method in use in the routine diagnostic mecrobiology laboratory for testing the susceptibility of microorganisms to the antibiotics, sulfonamides, or other chemotherapeutic antimicrobial agents. Various techniques and modifications of each have been devised. Each has its merit. Among the methods available for susceptibility testing, certain procedures are most frequently used and may be, therefore, considered to be "accepted" methods. Certain of these "accepted" procedures have been selected for description herein. It should be understood that, regardless of the method that is used, the end result should be the same within narrow limits with the same microorganism and antimicrobial agent.

Measurement of the susceptibility of microorganisms to antibiotics and chemotherapeutics is of great importance in the rational use of chemotherapy, in the evaluation of new agents, and in epidemiological studies. However, the results of such measurements are not absolute values, because they are influenced, sometimes markedly, by the test conditions implied. Differences in such factors as inoculum size, medium constituent, pH value, atmosphere, and incubation time may all affect the amount of antibiotic required to inhibit the microorganism in vitro. Thus, the Minimum Inhibitory Concentration (MIC) of an antibiotic for a microorganism has to be defined according to the conditions of test.

An antimicrobial test is a determination of the least amount of an antimicrobial agent that will inhibit the growth of a microorganism in vitro. The test is read by taking the least amount of antibiotic giving complete inhibition as judged by the naked eye as the Minimum Inhibitory Concentration (MIC).

The choice of technique to employ in a susceptibility test will be dependent upon the application of the results. If the object of the test is to direct the treatment of a patient rapidly and practicability in the busy laboratory are the formost considerations and accuracy within narrow limits must be secondary, though reproducibility is important. On the other hand, for the requirements of a survey, rapidity is of no consequence, but accuracy and reproducibility are of prime importance (98).

The three methods that are most widely used to determine the susceptibility of microorganisms to antimicrobial agents are the agar diffusion method, the agar plate dilution method, and the broth test tube dilution method.

Agar Diffusion Method

The principle of any diffusion method as stated by Blair et al. (1970), is dependent upon the inhibition of the growth of a microorganism on the surface of, or in, an inoculated agar plate, by the antimicrobial agent that diffuses into the surrounding medium from a depot on the surface of, or in, the medium. The depot containing the antimicrobial agent may be a cylinder or cup, a filter-paper disc,

a compressed tablet, or a ditch or well cut into the medium and filled with the agent. The area of inhibition or zone that results is dependent both upon the diffusibility of the agent in the medium and the degree of susceptibility of the microorganism. Thus, for a given agent, the diameter of the zone of inhibition of bacterial growth can be related to the MIC as determined from dilution tests. It is well known that the concentration of agent within the area of a few millimeters from the depot can be many times the content of the depot. Therefore, the single presence of a zone of inhibition around a depot containing, for example, 10 µg of an antibiotic does not imply that the microorganism is just susceptible to 10 µg per ml. Such an interpretation would be confering disc content with the concentration of antibiotic in the medium surrounding the depot and could lead to serious error. The zone does require correct interpretation, and the result must never be reported as indicating susceptibility to the content of the depot (8).

Agar Plate Dilution Method

The principle of the agar plate dilution method, as stated by Blair et al. (1970), is the inhibition of growth on the surface of the agar by the antimicrobial incorporated into the medium. Plates are usually prepared in a series of increasing concentrations of the agent. The microorganisms to be tested is grown in broth overnight and diluted in broth to contain 10^5 to 10^6 microorganisms per ml. Some workers recommend the inoculum density to be 10^8 microorganisms

to provide a margin of safety. A loopful of the diluted culture is spotted with a standardized milk loop that delivers 0.001 ml or with a mechenical replicator onto the surface of a segment or templated area.

In contrast to the agar disc diffusion method which qualitatively measures the susceptibility of one microorganism to many antimicrobials, the agar plate dilution method is a quantitative procedure which measures the specific MIC of one agent against many microorganisms. It is also possible with the agar plate technique to get an impression of the homogeneity of the tested culture, because a small proportion of more resistant cells are readily detected (8).

Broth Dilution Test Tube Method

The broth dilution test tube method as stated by Blair et al. (1970), is a quantitative technique for determining the MIC in micrograms or units of antimicrobial agent that will inhibit the growth of a microorganism in vitro. The principle of the tube dilution method is the inhibition of the growth of the tested microorganism by an antimicrobial incorporated in a broth medium. Media that are not optimal for growth or th at neutralize the agent must not be used. As with the agar disc diffusion procedure, there are modifications of the broth test tube dilution method.

The broth dilution method has one advantage over the agar plate dilution method, in that it permits the determination of both the MIC and the minimum bactericidal concentration. The latter can

be determined by subculturing to appropriate culture media from those tubes in the series which show gross inhibition of growth.

The broth dilution method is often recognized as a standard method, particularly because the susceptibility of a microorganism is defined in specific micrograms or units. As in the disc diffusion method, a governing factor affecting the end point of the test is the inoculum density. In general, a 1:1,000 dilution of an overnight broth culture, preferably in the logarithmic phase of growth and which will contain 10^5 to 10^6 viable cells per ml, can safely be used in the routine diagnostic laboratory in preparing the inoculum for the broth tube dilution susceptibility test. With slow-growing and other bacterial species that are considered not to approach the above population density after dilution, it is advisable to dilute the culture 1:100. No single dilution criterion to cover all situation can specifically be given (8). Ericsson and Sherris (1971), considered that there are a number of acceptable approaches to the performance of routine tests. Whatever method is used should, however, be described in full detail and thoroughly documented as to its performance under routine conditions (28).

In agar dilution methods, plates which have been prepared for a week with the antibiotic may be used. Inoculum replicator equipment simplifies their use and makes them more appropriate for day to day testing. The requirement for the inoculum is that it should conform to the "just short of confluent" description of growth used for the agar diffusion method. The inoculum could be applied with a loop,

fine pipette, or replicator apparatus, as long as the end result is correct. Complete inhibition is read (28).

Broth dilution methods promise to become more practicable for routine purposes with the development of mechanized procedures using relatively small volumes and cupped plastic plates in place of conventional glassware. The accuracy of the dilutions must be completely checked, as must be the relationship of the results to those of an orthodox broth dilution reference procedure. Tests are read after overnight incubation, the results recorded as complete inhibition (28).

Broth and agar dilution tests are considered fully acceptable as routine methods, and their use is facilitated by semi-mechanized procedures. Such procedures must be checked against the reference methods. However, their practicability would be enhanced by the ready availability of ampoules of standard antibiotic powders of appropriate content (28).

It is reasonable and more practical for the busy laboratory routinely to employ standardized quantitative screening tests, based on quantitative studies, which most accurately indicate the approximate MIC and give results which indicate that a microorganisms is susceptible or resistant to a given antimicrobial agent. Disc diffusion tests satisfy this requirement reasonably well and, of course, are widely used. This method will probably continue to be the mainstay of routine susceptibility test methods for sometimes in the future because of their simplicity, speed, flexibility and economy as well as degree of accuracy and reproducibility. They are easily

performed and it is possible to maintain discs of all agents against which susceptibility tests may be needed. It is also possible to read preliminary results after only a few hours of incubation (28).

For all the reasons stated above Disc Diffusion Method has been chosen for our study.

Microorganisms Used

Healthy human-beings, as well as other animals, are infected with a variety of bacteria from infancy through old age. This is not surprising when one considers the ubiquitous distribution of bacteria in the environment. In fact, most of the air we breathe is heavily contaminated. Soon after birth our body surfaces become colonized with bacteria. The surprising fact is that we can live in peaceful coexistence with microorganisms capable of producing overt disease. We are able to do so only because our tissues possess efficient natural mechanisms of antibacterial defense which restrict the microbes to areas where they can be tolerated. When the defenses are penetrated and bacteria gain access to tissues not normally infected, disease usually results.

Eight microorganisms used :

1. Bacillus subtilis (Ehrenberg) Cohn
2. Escherichia coli (Migula) Castellani and Chalmers
3. Lactobacillus fermentum Beijerinck
4. Pseudomonas aeruginosa (Schroeter) Migula
5. Salmonella typhi (Schroeter) Warren and Scott

6. Shigella dysenteriae (Shiga) Castellani and Chalmers

7. Staphylococcus aureus Rosenbach

8. Streptococcus faecalis Andrewes and Horder

1. Bacillus subtilis (Ehrenberg) Cohn is a saprophyte, found in soil, water, dust and air. Being ubiquitous, frequent contaminant of culture medium in the laboratory. It is usually non-pathogenic on experimental inoculation into laboratory animal (26).

Weinstein and Colburn showed that Bacillus subtilis is frequently isolated from old sinuses or chronically infected wounds, and there are some reports of the isolations from meninges and from the blood stream (95).

Subtilisin, a microbial hydrolase for tendering of meat or ovalbumin is produced by Bacillus subtilis. Penicillinase for destroying Penicillin is also produced by some strains of Bacillus subtilis (27).

It was found that Bacillus subtilis sporulated more profusely in Bacto-peptone broth. When a small quantity of activated charcoal or kaolin, ferric hydroxide or aluminium hydroxide were added to the medium (69).

Bacillus subtilis may occasionally cause human eye infections and it may become an opportunistic invader in the presence of a foreign prosthetic material in the human body. A strain of Bacillus subtilis produces the polypeptide antibiotic bacitracin (27).

2. Escherichia coli (Migula) Castellani and Chalmers (also known as Bacterium coli (Migula) Lehmann and Neumann, Bacillus coli Migula and as the colon bacillus) possesses O, H and K antigens (10). K antigen is a group of antigens designated L, A or B on the basis of physical characteristics, these are somatic antigens which occur as envelopes or capsules and prevent O-agglutination of living strains by their homologous O antisera. The pathogenicity of many strains of Escherichia coli is related to the possession of K antigens. Serotype distribution studies reveal that strains isolated from pathological material usually belong to a relatively few O groups and the majority are O-inagglutinable due almost invariably to the possession of L antigens; by contrast, strains isolated from healthy faeces are distributed over many more O groups and only a minority are O-inagglutinable, and is due to the presence of L antigen in only 50 percent of such strains (26).

Sears et al. (1950) noted that Escherichia coli strains predominate among the aerobic commensal organisms present in the healthy gut (73).

The pathogenic role of a particular serological type of Bacterium coli (Migula) Lehmann and Neumann var. neapolitanum Topley and Wilson (sucrose and salicin-fermenting strains of the highly heterogeneous group of bacteria commonly classified as Bacterium coli) in infantile gastroenteritis was first emphasized by Bray (1945)(9).

Noyes et al. (1964) have determined the kinds and distribution of enteropathogenic Escherichia coli isolated from acute diarrhoeas in Thailand (61)

Escherichia coli also be capable of causing endogenous and exogenous infections. In urinary tract infection, the microorganism being introduced during diagnostic or therapeutic catheterisation. In gastroenteritis, the majority has occured among infants under 18 months of age (26).

Most strains of Escherichia coli are sensitive to sulfonamide, ampicillin, cephalosporins, tetracyclines, carbenicillin, nitrofurantoin, naldixic acid and methenamine mandelate (27).

3. Lactobacillus fermentum Beijerinck. The lactobacilli constitute a group of acid-resistant, Gram positive, non-sporing bacilli, which occur in the intestine of mammalian animals and are particularly numerous during the stage of suckling. Microorganisms of this group form one of the main elements of the commensal flora of of the human alimentary canal, including the mouth, stomach and intestine, and occur also in the vagina and on the skin, they are found in cow's milk, silage and bran (26).

From paper of Green and Weisenstein (1959) it is clear that an increased incidence of lactobacilli may be found in most persons in whom caries are active in comparison to noncaries-active persons (32).

4. Pseudomonas aeruginosa (Schroeter) Migula is the only one species of Pseudomonas genus that is pathogenic to man. It is the most dangerous pathogen that can survive and multiply in water with

minimal additions of nutriment. It is also more resistant to many forms of chemical disinfection than most other vegetative organisms (26).

Most strains produce a bluish-green phenazine pigment pyocyanin (blue pus), as well as fluorescein, which is greenish-yellow and fluoresces; the pigments diffuse into and color the medium surrounding the colonies. About 10% of strains do not form pigment. The mechanism of pathogenicity is not clear, but the organism produces endotoxin and a number of extracellular products (lecithinase, collagenase, lipase, hemolysin) that may be of pathogenic significance (27).

Individual strains of Pseudomonas aeruginosa is frequently present, in the normal intestinal flora of men and animals and thus can be isolated from sewage. It is usually associated with pyogenic cocci or with a member of the family Enterobacteriaceae. It is sometimes incriminated in urinary tract infections; and commonly found in infected wounds, burns and in chronic otitis media. Infection is usually localized but in infants or debilitated persons it may invade the blood stream and give rise to fatal generalised infection; this risk is greater in persons receiving anti-neoplastic drugs or in radiation therapy (26).

The outbreak of postoperative infection with Pseudomonas aeruginosa which Ayliffe et al. (1966) reported was traced to the use of contaminated physiological saline solution in the operating-theatre. 15 out of 25 patients who had intraocular operations at

an eye hospital over a period of 6 days developed infection; from 10 of the infected eyes Pseudomonas aeruginosa was isolated (5).

5. Salmonella typhi (Schroeter) Migula is the major enteric pathogen belonging to the Enterobacteriaceae. The serum of animals immunized with Salmonella typhi contains agglutinating antibodies which are specific for the characteristic antigens, somatic (O) and flagellar (H), of the organism, and agglutination reactions are therefore employed in the identification of this species (26).

Infection is by ingestion and the bacteremic phase will be in the first seven to ten days of the disease. Haemorrhage of varying degree may occur and, less frequently, perforation through a necrotic Peyer's patch may complicate the illness (74).

Observations by phase microscopy of Showacre et al. (1961) showed that Salmonella typhi located within L 929 mouse fibroblast cells multiplied at the same rate as extracellular organisms. Multiplication of Salmonella typhi ceased promptly when streptomycin, chloramphenicol, penicillin or synnematin was added to infected tissue cultures. Moreover, division of extra and intracellular organisms stopped simultaneously (74).

Hopps et al. (1961) noted that cultures of L 929 mouse fibroblast cells infected with the Ty 2 strain of Salmonella typhi were regularly freed of infection by treatment for prolonged periods of time with synnematin and occasionally by treatment with other antibiotics. Failure to eradicate Salmonella typhi from the tissue cultures resulted when the experiment was terminated pre-

maturely because of non-specific death of the host cells or overgrowth of the cultures by antibiotic resistant microorganisms. Several hypotheses were discussed concerning the capacity of Salmonella typhi to persist for long periods of time in antibiotic-treated cultures. Previously, emphasis has been placed on the unique properties of the living cell in protecting the invading organism in the presence of antibiotic. The metabolic state of the organism in the treated infected culture may also be an important factor in its survival in the presence of drug (35).

6. Shigella dysenteriae (Shiga) Castellani and Chalmers. The Shigellae are much less invasive than the Salmonellae, rarely causing bacteremia. They produce natural disease in man and higher apes, and inhabit only the intestinal tracts of primates, or rarely of dogs. Other properties that distinguish them from most Salmonellae (biochemical properties) include lack of motility, failure to produce gas during fermentation, and lack of lysine decarbosylase. They possess specific polysaccharide O antigens, but since they are nonmotile they have no H antigens. Certain smooth (s) strains have heat labile K (envelope) antigens and hence agglutinate more readily in homologous anti-O antiserum after they are heated.

The four species of Shigella are differentiated from each other by biochemical and antigenic characteristics (Table 5*). All fail to ferment lactose, except Shigella sonnei Weldin which does so slowly (27).

Table 5*

Simplified Outline of Shigella Classification.

Species	Serological subgroup	Serological type (S)**	Mannitol	Ornithine decarboxylase
Shigella dysenteriae (Shiga) Castellani and Chalmers	A	1 - 10	-	-
S. flexneri Castellani and Chalmers	B	1 - 6	+	-
S. boydii Ewing	C	1 - 15	+	-***
S. sonnei Weldin	D	1	+	+

* From Davis et al. (1973) Microbiology, 2ed, Harper & Low, Inc.

** The serotypes are separately numbered for each species; cross-reactions between those with corresponding numbers of different species are absent or minimal.

*** Cultures of S. boydii 13 are positive.

\+ = 90% or more positive in 1 or 2 days.

\- = 90% or more negative.

Infection occurs by ingestion; after reaching the large intestine the Shigellae multiply locally with resultant inflammation of the mucosa which may, progress to ulcer formation and sloughing of large areas of mucous membrane. The cellular response is characterized by polymorphonuclear leucocytes and these are readily noted on microscopic examination of the dejecta (26).

Streptomycin in adequate dosage give satisfactory clinical and bacteriological cure, but inadequate dosage or too brief a treatment period allows Shigella sonnei to develop resistance in vivo. The tetracyclines or chloramphenicol should be reserved for such cases (26).

Davis et al. (1973) pointed out that Shigellae are usually, but not always, sensitive to ampicillin, tetracyclines, streptomycin, sulfonamides, kanamycin, chloramphenicol, naldixic acid, and colistin; but resistance develops quickly. Presently ampicillin is considered the drug of choice (27).

Shigella dysenteriae type 1 strains were resistant to the sulfonamides, streptomycin, the tetracycline, and chloramphenicol. Large parenteral doses of ampicillin were effective in curing patients in the Central America epidemic. Controlled studies in Vietnam indicate that oral ampicillin significantly shortens the clinical course of the disease and effectively eliminates Shigellae from the intestinal tract within 24 to 48 hours in about 75% of patients (27).

7. Staphylococcus aureus Rosenbach is the causative agent of most superficial lesions, e.g. boils, styes and wound infections. Less frequently a more extensive infection involving deeper tissues may develop, e.g., broncho-pneumonia, osteomyelitis and pyaemia; and occasionally septicaemia and death may result from widespread dissemination of the organism from a focus (26).

The Staphylococci show a marked degree of variation in their biological characters. This is reflected in the variable reaction

of many strains to antibiotics and chemotherapeutic agents so that the antibiotic resistance of strains of Staphylococcus aureus has become of great epidemiological and therapeutic importance (26).

There are protein antigens common to both pathogenic and non-pathogenic Staphylococci, but sera prepared against pathogenic strains do not agglutinate non-pathogenic strains, and the converse is also true. Agglutination tests using sera prepared against a selection of coagulase-positive strains can be used to distinguish a number of groups or types of Staphylococcus aureus (26).

The virulent Staphylococci apparently owe their disease producing power to their capacity to produce the coagulase factor, hemolysins, and other mildly injurious substances. Other products of virulent Staphylococci include leukocidin (a material which kills white blood cells), staphylokinase, a substance which can activate the natural proteolytic (fibrinolytic) enzyme of the blood so that fibrin clots are digested, and the spreading factor (hyaluronidase)(11).

Numerous staphylococci are found in the air and dust and on clothing and fomites. A variable proportion of these will be Staphylococcus aureus, number of this organism is greater in the hospital environment than in the home. The term "hospital staphylococcus" is used to define those strains of Staphylococcus aureus which, with increasing frequency in recent years, have caused infections of surgical wounds, and penumomia and gastroenteritis in patients after their admission to hospital. These Staphylococci are almost

invariably resistant to penicillin, and often to streptomycin and the tetracyclines as well. Wallis and Turner (1963) examined 503 strains of Staphylococcus aureus isolated in a general hospital. The results showed that most of those that were resistant to penicillin, streptomycin and tetracycline (i.e., "multiple-resistant") were also resistant to mercuric chloride. These strains were the predominant cause of staphylococcal sepsis in hospital in-patients, were uncommon causes of staphylococcal infections in out-patients, and were not isolated from the noses of normal healthy individuals. Most multiple-resistant strain isolated from in-patient infections formed yellow-pigmented or orange colonies on glycerol monoacetate agar, a characteristic rarely seen in sensitive strains (97).

In a minority of cases pyemia, septicemia and malignant endocarditis may result from spread from a primary focus. Cases of food poisoning are frequently due to the enterotoxin produced by Staphylococci growing in certain articles of food. The production of the toxin depends upon suitable conditions for the growth of the organism in the food.

Owing to the variable susceptibility of strains of Staphylococcus aureus to antibiotics, it is advisable to carry out antibiotic sensitivity tests on the causative organism whenever possible. Benzyl penicillin is effective in the majority of staphylococcal infections in the general population which require antibiotic therapy but infections occuring in hospital are not usually amenable to penicillin therapy. Combinations of antibiotics have been

recommended by several workers for the treatment of patients infected with strains resistant to multiple antibiotics. The best combinations can be determined only by laboratory tests (26).

8. Streptococcus faecalis Andrewes and Horder is non-haemolytic or gamma (γ) type faecal streptococci. A variant of Streptococcus faecalis (Lancefield group D) may be actively haemolytic on blood agar, although it does not produce a soluble hemolysin (26).

Streptococcus faecalis is found in the human faeces and the intestine of many warm-blooded animals. Occasionally encountered in urinary infections and in the blood stream and heart lesions in subacute endocarditis cases. Associated with European foul-brood of bees found in milk and dairy products, has been associated with mild outbreaks of food poisoning, intestines of human beings and many other warm-blooded animals.

. Characteristically belong to sero-group D, in contrast to other aerobic streptococci, Streptococcus faecalis is capable of growing on media containing bile salts and in the presence of 6.5 percent NaCl; fermentation of mannitol with gas production also differentiates enterococci from other streptococci (26).

Lead an essentially commensal existence in the human and animal intestines but are not infrequently incriminated in urinary tract infections, sometimes alone but more often in association with Escherichia coli, etc. They are also rarely causative micro-organisms in subacute bacterial endocarditis (26).

Alpha-type Streptococci have been responsible for some cases of gastroenteritis following the eating of contaminated food. This type of gastroenteritis appears to be an infection and not due to performed toxins. The responsible organism is Streptococcus faecalis, but only a few strains have this property. Symptoms arise when large numbers of the organisms are ingested with the contaminated food. In the adult the upper part of the small intestine contains few microorganisms. Streptococcus faecalis and staphylococci are usually the only ones found (43).

In the treatment of disease due to Streptococcus faecalis Penicillin and streptomycin or its combination are the drug of choice. In addition, many strains encountered in clinical practice are highly resistant to antimicrobial drugs, making the treatment of enterococcal endocarditis especially difficult (26).

The Gram-staining, morphology and cultural characters of test microorganisms are summarized in Table 6.

Table 6

Gram-staining, Morphology and Cultural Characters of the Test Microorganisms.

Test Microorganisms	Gram-staining	Morphology	Cultural characters
Bacillus subtilis	positive	aerobic spore forming bacilli; peritrichous flagella	optimum temperature about 20°C, some types 30° and 37°C
Escherichia coli	negative	non-sporing bacillus, most strains are flagellate	aerobe and facultative anaerobe, temperature range, 15°-41°C, optimum temp. 37°C
Lactobacillus fermentum	positive	rods, variable in size, non-motile	microaerophilic, optimum temp. 37°C
Pseudomonas aeruginosa	negative	non-sporing bacillus, actively motile by polar flagella non-capsulate	essentially aerobic optimum temp. 37°C, produce a musty odor like trimethylamine
Salmonella typhi	negative	non-sporing bacillus, motile	aerobe and facultative anaerobe, optimum temp. 37°C
Shigella dysenteriae	negative	non-sporing bacillus, non-motile, does not possess capsule	aerobe and facultative anaerobe, optimum temp. 37°C
Staphylococcus aureus	positive	spherical cocci	aerobe and facultative anaerobe, optimum temp. 35°C - 37°C
Streptococcus faecalis	positive	oval in shape, non-motile, non-sporing may be capsulate	aerobe and facultative anaerobe; optimum temp. 39°C