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Biochemical characteristics and antibiogram patterns of some gram-negative nonfermentors

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BIOCHEMICAL CHARACTERISTICS AND ANTIBIOGRAM PATTERNS OF SOME GRAM-NEGATIVE NONFERMENTORS

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I. INTRODUCTION

At least 30 species of nonfermentative Gram-negative bacilli have been reported from clinical material. Most of these belong to the genera Achromobacter, Acinetobacter, Alcaligenes, Chromobacterium, Flavobacterium and Pseudomonas. Many of these genera are inadequately characterized, and their affiliation with other bacterial groups is uncertain. The most common and most important clinically is Pseudomonas, a member of the family Pseudomonodaceae and order Pseudomonadales. According to Bergey's Manual of Determinative Bacteriology (1974), there are 235 species in the genus, 29 of which are well characterized, 74 more or less adequately described, and 132 considered "species incertae sedis." The 29 well-characterized species have long been recognized as pathogens for plants, animals and man. Serious attempts to better characterize the pseudomonads, as they are collectively termed, are still being undertaken in a number of laboratories across the country.

The pseudomonads are Gram-negative, straight or slightly curved rods, that occur singly, in small bundles, or short chains. All but one species are motile, possessing one or more polar flagella, at one or both ends. No sporeformers exist and all are catalase positive. They are strict

aerobes with oxidative metabolism, never fermentative. In the presence of nitrate some organisms are able to grow anaerobically. A few species give weak or negative oxidase tests but the majority are strongly oxidase positive. The temperature range depends on the species; for the group, growth occurs over the wide range of 4-42C. Some members of the genus accumulate poly-B-hyroxybutyrate as an intracellular storage form of carbon. A noteworthy characteristic of the pseudomonads is their simple nutritional requirements. All species with the exception of Ps. maltophilia are able to grow in a solution composed of mineral salts, ammonia and a carbon-containing compound. Due to their simple nutritional requirements and their ability to utilize any of a wide variety of organic compounds, many pseudomonads are able to survive and grow in weak, old, or improperly prepared disinfectant solutions.

A trait used as an aid in identification is the production of various pigments, one of which is pyocyanin, a fluorescent pigment produced only by Ps. aeruginosa. It is a deep blue phenazine dye which is chloroform soluble. Another pigment produced by Ps. aeruginosa and a few other pseudomonads is fluorescein, a water but not chloroform-soluble pigment. Fluorescence is seen under an ultraviolet light (wave-length below 260 nm). Fluorescein, also known as pyoverdin, resorcinolphthalein and dihydroxyfluorane, exhibits a yellow-green hue. Other pigments liberated by

Ps. aeruginosa and other species include the water-soluble pyorubin, (bright red) and pyomelanin (brown to black). The production of these pigments is enhanced during propagation in iron-deficient or special media, such as media A and B of King, et al. (1954) or that of Wahba and Darrell (1965). All these pigments may occur individually or in combination in any pigment-producing strain.

Pseudomonas species are widely distributed in nature especially in soil, fresh water and marine environments. However, doubt exists as to whether all members are truly free living organisms. This is due to the fact that water environments not subject to fecal pollution do not harbor Ps. aeruginosa (Reitler and Seligmann, 1957). Moreover, the isolation of Ps. aeruginosa in wild animals and birds is a rare event (Hoadley and McCoy, 1968). Values of fecal carriage in man of this organism vary considerably. In adults, not associated with hospital environments, carriage is reported to be between six and 12 percent (Stoodley and Thom, 1970). Ps. aeruginosa may be cultured from the skin or the upper respiratory tract of 20 percent of hospital in-patients (Grogan, 1966). Yet colonization by the organism of the gut of healthy persons is rare, even with oral ingestion of as many as 10^6 organisms (Buck and Cook, 1969). Ps. aeruginosa thrives best in a moist environment but can tolerate dry conditions. Darrel and Wahba (1964) isolated the organism from 5 percent of samples taken from hospital dust.

Other pseudomonads isolated from clinical material include Ps. putida, Ps. fluorescens, Ps. stutzeri, Ps. cepacia, Ps. maltophilia, Ps. diminuta, Ps. alcaligenes, Ps. pseudoalcaligenes, and Ps. putrefaciens. The habitats of some of these are not known but probably, like most pseudomonads, they are saprophytes in the terrestrial and aquatic environments. Possible exceptions are Ps. pseudomallei, the cause of meliodosis, a glanders-like disease, of humans and animals in S.E. Asia, and Ps. mallei, the cause of glanders (farcy), a natural disease of equines, and occasionally of humans.

Since 1950, interest in the pseudomonads, especially Ps. aeruginosa, has increased. This organism has become one of the most common causes of nosocomial infections. Serious and sometimes fatal infections occur in newborn infants. Serious infections in adults occur when the patient has a lowered resistance which allows Ps. aeruginosa to assume the role of an opportunistic pathogen (Myrvik, et al., 1974). Such infections have increased in incidence because the number of susceptible patients has grown. Susceptible individuals include the elderly, those who are afflicted with chronic and degenerative diseases, persons on prolonged antibiotic treatment, immunosuppressive and anticancer drugs, and victims of serious burns. With an increasing number of infections being attributed to nonfermentors, accurate identification requiring rapid and reliable tests become essential.

The majority of biochemical tests used in the diagnostic laboratory are geared to the identification of the fermentative Enterobacteriaceae. Many of these tests are inappropriate for characterization of Gram-negative nonfermentative bacilli (NFB). Until recently these facts dictated that a nonfermentative bacterial isolate be identified no further than the generic level, often reported as "Pseudomonas sp."

The taxonmic identity of Gram-negative nonfermentative bacilli is still far from being well defined. Significant progress in the identification and classification of these bacteria has been made in recent years (King, 1964; Stanier et al., 1966; Ballard et al., 1968; Gilardi, 1968a, 1968b, 1973; Gilardi et al., 1975; Bauman et al., 1968a, 1968b; Pinter and Bende, 1968; Pickett and Manclark, 1970; Pickett and Pedersen, 1970; Pedersen et al., 1970; Hugh and Gilardi, 1974; Otto and Pickett, 1976; and Oberhofer et al., 1977). Furthermore, identification has been facilitated through the introduction of various commercial kits including the Oxidative Screening Kit and Oxidative Confirmatory Kit (Key Scientific Products Company), API 20E (Analytab Products), and most recently the Oxi/Ferm Tube (Hoffman-LaRoche).

A number of researchers have pointed out that antimicrobial susceptibility patterns may be useful as an aid in the identification and differentiation of bacterial species. As with biochemical characterization, the majority of research involving antimicrobial susceptibility testing

has been done with bacteria possessing fermentative metabolism (Eickhoff et al., 1966; Kock and Rose, 1966; Lerner and Weinstein, 1967; Ramirez, 1968; Zabransky et al., 1969; Washington and Bourgeois, 1969; Washington, 1969; Russell, 1969; Wilfert et al., 1970; Greenup and Blazevic, 1971; Friedman and MacLowry, 1973; Darland, 1975; Klein et al., 1975; Sielaff et al., 1976; Hall, 1976; and more recently Storey, 1977). Efforts to use the antimicrobial susceptibility patterns of Gram-negative, nonfermentative bacilli as an aid in characterization have intensified. Von Graevenitz and Redys (1968) were the first researchers to suggest that antibiotic disk sensitivities be used as an aid in the identification of this group. The susceptibility of Bacterium anitratum (Acinetobacter anitratus) to 16 antimicrobial agents was investigated by Hugh and Reese (1969). Washington (1969) used nine antibiotics to study the susceptibilities of several genera and groups of Gram-negative nonfermentative bacilli. Lowbury et al. (1969) tested the susceptibility of 1452 strains of Pseudomonas aeruginosa isolated from burn patients over a four year period to three antibiotics and observed the emergence of strains highly resistant to carbenicillin. Klastersky (1971) isolated fermentative and nonfermentative bacteria from patients with disseminated malignant disease and studied the relationship between site of isolation and their sensitivity to eight antibiotics. Gilardi (1971c) published the first major study which utilized

antimicrobial susceptibility patterns as a diagnostic aid in the identification of Gram-negative nonfermentative bacteria. Moody et al. (1972) studied the susceptibility patterns of 100 "pseudomonads" other than Pseudomonas aeruginosa which were recovered from cancer patients, and suggested these patterns be used as an aid in preliminary identification of these organisms. Martin et al. (1973) examined the biochemical characteristics and the antimicrobial susceptibilities of 183 strains of Pseudomonas fluorescens and Pseudomonas putida isolated from clinical specimens. Friedman and MacLowry (1973) described a computer program utilizing a Baysean mathematical model to identify bacteria, fermenters and nonfermenters, solely on the basis of their susceptibility patterns to 11 antibiotics. Sielaff et al. (1976) and Buck et al. (1977) employed computers and a semi-automated device for susceptibility testing (autobac I, Pfizer, Inc.) to devise a scheme for the identification of bacterial isolates that are based strictly on susceptibility patterns to various antimicrobial agents. Most recently Oberhofer et al. (1977) reported on the biochemical and antibiotic susceptibility testing of 593 strains of nonfermentative Gram-negative bacteria isolated from clinical specimens.

Of all the Gram-negative nonfermentative bacteria, Pseudomonas aeruginosa is one of the most resistant to chemotherapeutic agents. The polymyxins and colistin, antibiotics produced by species of the genus Bacillus were for many

years the only antimicrobials effective against Ps. aeruginosa (Ayliffe et al., 1969). Subsequently, gentamicin produced from Micromonospora and carbenicillin, a semi-synthetic penicillin, became available to the physician. In the last two to four years two other aminoglycosides were introduced; tobramycin, formerly designated nebramycin factor 6, and amikacin both of which have a high degree of activity against Ps. aeruginosa and especially to those strains that have developed resistance to gentamicin.

The innate ability of bacteria to adapt to new environments creates an ongoing problem for the physician with regard to his selection of effective chemotherapeutic agents and for the medical microbiologist in his attempt to fully identify microorganisms. The antibiotic susceptibility pattern of any species of bacteria is dynamic and is affected by various factors including mutation, excessive use of antibiotics, and the introduction of new antimicrobial agents (Gilardi, 1971c). Thus the reaffirmation of antibiotic susceptibility studies is mandatory.

This study was undertaken, chiefly, for the purpose of characterizing biochemically a random sample of nonfermentative bacilli obtained from local hospitals and determine the prevalence of Ps. aeruginosa among them. A second objective was to compare the antiobiogram patterns obtained in this study with those reported by other investigators, and thirdly to establish a baseline for future comparative studies.

II. MATERIALS AND METHODS

One hundred and thirty-seven isolates of Gram-negative, nonfermentative bacilli were obtained from various clinical materials (Table 1) from in-and-out patients at the laboratories of Dameron and St. Joseph's Hospitals in Stockton, California, between January 1976 and February 1977. The Oxi/Ferm Tube (Roche Diagnostics, Nutley, N.J.) system of identification was used in the initial and presumptive identification of all the isolates. In addition, 14 selected enzymatic and oxidative tests, and growth characteristic studies were performed on each. All clinical isolates were subcultured on Hektoen Enteric Agar (Difco Products, Detroit, Mich.) to obtain well-isolated colonies and ascertain their purity. Individual colonies were then transferred to Tryptic Soy Agar (TSA) slants, incubated for 18-24 hours, then stored at two to eight degrees C. These stock cultures were maintained by repeated subculture every two weeks. Cultures were incubated at 36 $\pm$ 1C (except for one test to determine ability of the organism to grow at 42C) for six hours to six days depending on the test.

The tests listed below were used to investigate the biochemical activities of the organisms in this study:

1. Oxi/Ferm Tube (anaerobic-dextrose fermentation, arginine dihydrolase, nitrogen gas production, hydrogen sulfide gas

production, indole production, xylose oxidation, aerobic-dextrose oxidation, urease activity, and citrate utilization); 2. Confirmatory Tests (oxidase activity, pigment production, growth on SS agar, ONPG, gelatin hydrolysis, gluconate, acetamide, carbohydrate oxidation test (C.O.T.) tablets for fructose, maltose, mannitol, arabinose, lactose, and rhamnose, and finally growth at 42C). In addition to biochemical activities, antimicrobial susceptibility testing was conducted. The reaction of each isolate to 17 antimicrobial agents (polymyxin B 300U; gentamicin 10ug; tobramycin 10ug; neomycin 30ug; carbenicillin 100ug; gantrisin 300ug; penicillin 10U; tetracycline 30ug; kanamycin 30ug; ampicillin 10ug; nalidixic acid 30ug; nitrofurantoin 300ug; streptomycin 10ug; chloramphenicol 30ug; lincomycin 2ug; erythromycin 15ug; and cephalothin 30ug) was studied utilizing the Bauer-Kirby agar disk diffusion technique (Table 2).

To test for oxidase activity Patho Tec test strips (General Diagnostics, Morris Plains, N.J.) were used. ONPG tablets, gelatin hydrolysis strips, gluconate, acetamide, and C.O.T. tablets were purchased from Key Scientific Products Co. (Los Angeles, Calif.). Antimicrobial discs were purchased from Difco.

Tests for indole production, pigment production, growth on SS agar, growth at 42C, and antimicrobial susceptibility testing were performed on media prepared by the author.

Pseudomonas aeruginosa ATCC 27853 was run as a control with all tests. Procedures used in performing and interpreting these tests are given on pages 61-65.

III. RESULTS

Biochemical and Physical Characterization

One hundred and thirty-seven isolates were identified and found to belong to the following species: *Pseudomonas aeruginosa* (118), *Ps. putida* (5), *Ps. fluorescens* (1), *Ps. stutzeri* (1), three different isolates of *Pseudomonas* sp., *Alcaligenes faecalis* (4), *Achromobacter* sp. biotype II (4), and *Acinetobacter anitratus* (1) (Tables 3-7). The results obtained on species which contain a single isolate are self-evident and will not be discussed further.

All 118 strains of *Ps. aeruginosa* tested positive for arginine, oxidase, and growth at 42C. Ninety to 99% of the strains of this group gave positive results for xylose, aerobic-dextrose, urease, citrate, growth on SS, gelatin hydrolysis, gluconate, and arabinose. Negative reactions were demonstrated by all strains for anaerobic-dextrose, H_2S, indole, ONPG, and lactose. Ninety-three percent of all strains gave negative reactions for rhamnose.

The five strains of *Ps. putida* showed positive reactions for arginine dihydrolase, xylose, aerobic-dextrose, citrate, oxidase, arabinose, and growth on SS. All five demonstrated a negative reaction for anaerobic-dextrose, nitrogen gas, H_2S, indole, pigment production, ONPG, gelatin hydrolysis, maltose, lactose and growth at 42C.

All four isolates identified as Alcaligenes faecalis gave positive reactions to nitrogen gas, acetamide, and growth at 42C. All were negative for anaerobic-dextrose, arginine, H_2S, indole, xylose, aerobic-dextrose, urease, pigment, ONPG, gluconate, fructose, maltose, mannitol, lactose and rhamnose.

All four strains of Achromobacter sp. biotype II were positive for urease, growth on SS, gelatin hydrolysis, arabinose, and growth at 42C. All four strains were negative for anaerobic-dextrose, H_2S, indole, pigment production, ONPG, gluconate, lactose and rhamnose.

Antimicrobial Characterization

There was no antimicrobial agent tested which was effective against all 118 strains of Pseudomonas aeruginosa. Ninety to 99% of these strains were susceptible to polymyxin B, gentamicin, and tobramycin. Ninety-four strains (80%) were susceptible and twenty strains (17%) were intermediate in their reaction to carbenicillin. All strains demonstrated resistance to penicillin, nitrofurantoin, lincomycin, and cephalothin. Resistance to tetracycline, kanamycin, ampicillin, nalidixic acid, streptomycin, chloramphenicol, and erythromycin was evident in 90% to 99% of the strains of Ps. aeruginosa. Eighty percent of the strains were susceptible, while 16% gave intermediate reactions to gantrisin, a sulfa drug.

All strains (5) of Pseudomonas putida were susceptible to polymyxin B and tobramycin. Four of the five isolates studied were susceptible to neomycin. An intermediate reaction was shown by 80% of the strains to gentamicin. All strains were resistant to gantrisin, penicillin, ampicillin, nalidixic acid, nitrofurantoin, lincomycin, erythromycin, and cephalothin.

The four strains of Alcaligenes faecalis showed susceptibility to nitrofurantoin. Seventy-five percent of the strains demonstrated susceptibility to gentamicin, tobramycin, and carbenicillin. All were resistant to penicillin, streptomycin, and lincomycin.

The four isolates identified as Achromobacter sp. biotype II showed complete susceptibility to gentamycin, and tobramycin. Three strains (75%) were susceptible to polymyxin B, and streptomycin. All were resistant to penicillin, ampicillin, lincomycin, and cephalothin. Resistance to gantrisin, tetracycline, nalidixic acid, nitrofurantoin and erythromycin was demonstrated by 75% of this group.

IV. DISCUSSION

Biochemical and Physical Characterization

A number of investigators (Stanier et al., 1966; Gilardi, 1968b; Pickett and Pedersen, 1970; Gilardi, 1971a, 1971b; Hugh and Gilardi, 1974; and Oberhofer et al., 1977) have studied the biochemical activities of Pseudomonas aeruginosa and other pseudomonads. The studies of Gilardi (1971a), Hugh and Gilardi (1974) and Oberhofer et al. (1977), as the most recent or most comprehensive studies, have been chosen for comparison with the present investigation (Tables 8-17). The results of such research and those of the present investigation were combined and the percentage of positive tests calculated to give the figures indicated in the two bottom rows of each table. As shown in the footnotes some tests were not performed on all isolates. Microbiologists, by convention, have accepted attributes characterizing 90% or more of a certain bacterial species as "constant," typifying a certain species.

Some characteristics of Ps. aeruginosa are remarkably constant, being positive or negative 90% or more of the time. These tests include: negative anaerobic-dextrose, H_2S, indole, ONPG, maltose, lysine decarboxylase, and positive argine dihydrolase, xylose, aerobic-dextrose, citrate,

oxidase, growth on SS, gluconate, arabinose, and growth at 42C. Other features are variable and some of them at least are probably dependent on the substrate used, cultural and incubation conditions, and kind of medium. An example of this kind of variability is evident in the hydrolysis of gelatin where results are either positive or negative depending upon incubation period (two to seven days), incubation temperature (22C compared with 37C), and the method used. Constant features which characterize Ps. aeruginosa are listed in Table 8.

Ps. putida exhibits a high degree of consistency in its reaction to a number of biochemical tests (Table 9). Negative tests include nitrogen gas, H_2S, indole, pigment, ONPG, gelatin hydrolysis, acetamide, growth at 42C, and lysine decarboxylase. Positive tests are arginine dyhydrolase, xylose, aerobic-dextrose, citrate, oxidase, growth on SS, fructose, and arabinose. Biochemically Ps. putida shows a strong relationship to Ps. aeruginosa but may be distinguished from it by the combined characteristics of a negative nitrogen gas test, absence of a pigment, inability to hydrolyze gelatin or grow at 42C.

Anaerobic-dextrose, nitrogen gas, H_2S, indole, pigment production, ONPG, acetamide, arabinose, growth at 42C, and lysine decarboxylase are negative tests while argine dihydrolase, xylose, aerobic-dextrose, citrate, oxidase, growth on SS, gelatin hydrolysis, and fructose are positive tests

which are typical and constant for 90% or more of Ps. fluorescens strains (Table 10). Ps. fluorescens resembles Ps. putida and Ps. aeruginosa in the biochemical tests of the Oxi/Ferm Tube (the first nine tests in Tables 8-18) but may be distinguished from Ps. putida by a pattern: positive gelatin hydrolysis, negative acetamide, and negative arabinose. From Ps. aeruginosa it may be distinguished by its inability to produce nitrogen gas, utilize acetamide, arabinose, or grow at 42C.

Table 11 compares results for Ps. stutzeri. Most strains are arginine dihydrolase negative, but otherwise the organism gives similar biochemical results on the Oxi/Ferm Tube to Ps. aeruginosa, Ps. putida, and Ps. fluorescens. Tests useful in its identification are negative gelatin hydrolysis, gluconate, acetamide, and ability to grow at 42C.

Ps. cepacia, Ps. maltophilia, Ps. diminuta, Ps. alcaligenes, Ps. pseudoalcaligenes, and Ps. putrefaciens were not encountered in this study. Since these species occur in clinical material they are included for comparison with other pseudomonads.

The combined results of tests run on 60 strains of Ps. cepacia are given in Table 12. This species may be identified by the following tests: negative arginine dihydrolase, no growth on SS, positive ONPG and lactose and the ability to decarboxylate lysine.

The single most important attribute that characterizes 93% of Ps. maltophilia strains is a negative oxidase test. Additional tests useful in its identification are given in Table 13, and include the ability to hydrolyze gelatin, utilize maltose and decarboxylate lysine. All strains tested to date are gluconate negative. Eighty-three percent are ONPG positive.

Only 19 strains of Ps. diminuta have been investigated. Table 14 indicates that this organism is quite inert biochemically except for the ability of 79% of the strains to hydrolyze gelatin. It is oxidase positive and can grow at 42C.

Strains of Ps. alcaligenes also seem to be inert biochemically except for a small percent that gives a positive reaction for arginine dihydrolase, urease, citrate, and gelatin hydrolysis. Typical of most pseudomonads, it is oxidase positive, 86% can grow at 42C and a small number (10%) can grow on SS. Not evident in Table 15 is the alkinization of xylose and aerobic-dextrose, a diagnostic feature of this species.

Ps. pseudoalcaligenes (Table 16) may easily be confused with Ps. alcaligenes. Fructose, however, is utilized by the former and therefore may be used to distinguish the species.

Table 17 shows test results for Ps. putrefaciens. The single most important and unique characteristic feature is H_2S production.

Antimicrobial Characterization

A number of investigators (von Graevenitz and Redys 1968; Pedersen et al. 1970; Gilardi 1971c; Moody et al. 1973, and more recently Oberhofer et al. 1977) have studied the antimicrobial susceptibility patterns of various pseudomonads. All these investigators used the Bauer-Kirby agar disk diffusion technique on Mueller-Hinton medium. It should be pointed out that some antibiotics were tested by some but not others; some reported their results in terms of S (Susceptible) and R (Resistant) whereas others used I (Intermediate) or E (Equivocal) to report a third category. The total number of strains studied was indicated by each investigator. In most cases percent susceptibility was given without any indication of the percentage of strains which were I, E, or R. Gilardi (1971c) reported percent susceptibility which included strains giving Intermediate reactions. For this reason results from his study were not included when a combined percentage of strains susceptible was calculated, except in the case of Ps. putrifaciens (Table 28) where his study is the only one that appears in the literature. The results of such research and those of the present investigation were combined and the percentage of positive tests calculated to give the figures indicated in the two bottom rows of Tables 19-29. It is evident from these tables that some antimicrobial substances were not tested either because they were not considered important

by that particular investigator or because such an antimicrobial was not available at the time. It should also be remembered that the reaction to an antimicrobial by a certain organism may not remain constant because of the emergence of resistance. The use of the antibiogram as an aid in identification must therefore be used cautiously and must always be compared with a baseline study to determine if resistance is emerging in that particular community.

Table 19 indicates that the majority of the strains of Ps. aeruginosa are susceptible to gentamicin, tobramycin, and polymyxin B. It is of interest to note that in the present investigation as well as that of Oberhofer et al, (1977), 80% to 82% of the strains are susceptible to carbenicillin. Resistance to carbenicillin is on the rise. When carbenicillin was introduced four years ago Ps. aeruginosa strains in the Stockton community were uniformly sensitive to this antibiotic (F.M. Nahhas, personal communication).

Table 20 indicates that the majority of Ps. putida strains are still susceptible to polymyxin B. Eighty-four percent are susceptible to kanamycin but only 69% are sensitive to gentamicin.

The antibiogram pattern of Ps. fluorescens (Table 21) shows the organism to have a similar antibiogram to that of Ps. putida with respect to polymyxin B, kanamycin, and gentamicin. Only the present study has investigated

susceptibility to tobramycin but no conclusions should be made on the basis of a single strain.

Ps. stutzeri has a distinctive susceptibility pattern as evidenced in Table 22 which shows that in addition to susceptibility by the majority of the strains to the aminoglycosides (gentamicin, tobramycin, kanamycin, neomycin and streptomycin), polymyxin B and carbenicillin, the organism shows susceptibility to ampicillin (80%), tetracycline (90%), erythromycin (96%), and nalidixic acid (85%).

Only Oberhofer et al. (1977) studied the antibiogram pattern of Ps. cepacia (Table 23). This limited study (seven strains) indicates a 100% susceptibility to chloramphenicol and naladixic acid and relative resistance to the aminoglycosides. If further studies confirm these results Ps. cepacia may also be found to have a distinctive antibiogram. Polymyxin B was not tested.

Table 24 for Ps. maltophilia, based on a study of 161 strains, suggests a characteristic pattern of relative resistance to the aminoglycosides and susceptibility to polymyxin B (96%), nalidixic acid (94%), and chloramphenicol (83%). The pattern is similar to that of Ps. cepacia except that in the latter reaction to polymyxin B is not known.

Table 25 is based on 21 strains of Ps. diminuta. The organism shows susceptibility to most of the aminoglycosides (gentamicin 89%, kanamycin 93%, neomycin 100%), 100% susceptibility to carbenicillin and chloramphenicol, 90%

susceptibility to tetracycline, and 92% to erythromycin.

Table 26 is based on 19 strains of Ps. alcaligenes. The organism shows 100% susceptibility to kanamycin, carbenicillin, tetracycline, and erythromycin, 86% to gentamicin, 84% to chloramphenicol, and 89% to nalidixic acid.

Ps. pseudoalcaligenes (Table 27), based on 51 strains, shows a pattern similar to that of Ps. alcaligenes except for 100% susceptibility to gentamicin and 61% to kanamycin.

Table 28 for Ps. putrefaciens, represented by 12 strains, has been obtained from Gilardi (1971c). A distinctive antibiogram pattern is seen in its susceptibility to nitrofurantoin. However, as indicated earlier in the discussion Gilardi (1971c) combined the categories of Intermediate and Susceptible.

V. CONCLUSION

The present study indicates that a few biochemical tests in combination with an antibiogram may be used in the identification of the ten most commonly occurring pseudomonads. Tables 18 and 29 give summaries of the biochemical activities and antibiogram pattern, respectively, of the ten most common pseudomonads. It is clear from these tables that some of the biochemical tests and antibiotics are not essential for identification. A modified scheme that combines a few biochemical tests and antimicrobial agents is here presented. This scheme suggests the use of a modified Oxi/Ferm Tube to exclude the indole and urease tests. Indole and urease do not seem to be essential in identification of pseudomonads. The additional tests recommended include oxidase, growth on SS, ONPG, gelatin hydrolysis, gluconate, and growth at 42C. In some instances confirmatory tests are essential. These appear under "Remarks" in Table 30. The antimicrobial disks recommended for the scheme include carbenicillin, ampicillin, erythromycin, and nalidixic acid. There are at least two characteristics that distinguish one species from another according to this scheme.

Ps. aeruginosa, Ps. putida, and Ps. fluorescens (the

fluorescent group) are distinguished from the other seven species chiefly by their ability to dehydrolyze arginine.

Ps. aeruginosa according to this study, and several others cited earlier, is the most common pseudomonad in clinical material. It may be distinguished from Ps. putida and Ps. fluorescens by its ability to produce nitrogen gas, grow at 42C, and its sensitivity to carbenicillin.

Ps. putida is very similar to Ps. fluorescens and the two species were considered conspecific by earlier investigators. The studies reported in this paper, however, indicate that Ps. putida may be distinguished from Ps. fluroescens by its inability to hydrolyze gelatin and by being arabinose positive.

Ps. stutzeri may be distinguished from the nonfluorescent group by its ability to produce nitrogen gas. It differs from Ps. cepacia by negative ONPG and lysine decarboxylase tests, and susceptibility to carbenicillin and ampicillin.

Ps. cepacia and Ps. maltophilia are the only two pseudomonads capable of decarboxaylating lysine. Ps. maltophilia is predominantly oxidase negative. Most strains are also citrate and xylose negative.

Ps. diminuta, Ps. alcaligenes, and Ps. pseudoalcaligenes are closely related biochemically. Seventy-nine percent of the strains of Ps. diminuta hydrolyze gelatin and 95% are resistant to nalidixic acid. Ps. alcaligenes and Ps. pseudoalcaligenes are very similar but may be distinguished from

each other chiefly by oxidation of fructose and to a lesser extent by growth on SS.

Ps. putrefaciens has the distinctive characteristic of producing hydrogen sulfide.

VI. SUMMARY

One hundred and thirty-seven strains of Gram-negative nonfermentative bacilli were obtained from clinical materials from in-and-out patients from Dameron and St. Joseph's Hospitals in Stockton, California. The strains were identified by biochemical studies, growth characteristics, and their antibiogram patterns determined by the Bauer-Kirby agar disk diffusion technique. Ps. aeruginosa was represented by 118 strains (86.1%), Ps. putida five (3.6%), and one strain each of Ps. fluorescens and Ps. stutzeri (1.5%). Three other pseudomonads (2.0%) could not be identified to the species level. In addition to the pseudomonads four isolates (2.9%), were identified as Alcaligenes faecalis, four (2.9%), Achromobacter sp. biotype II, and one Acinetobacter anitratus (1.0%).

The four species of pseudomonads encountered in this study with six others (Ps. cepacia, Ps. maltophilia, Ps. diminuta, Ps. alcaligenes, Ps. pseudoalcaligenes, and Ps. putrefaciens) were compared through literature search, and a scheme for their identification is presented.

Table 1[a]

Distribution of Isolates in Clinical Material

Clinical Site	*Pseudomonas aeruginosa* (118)	*Pseudomonas putida* (5)	*Pseudomonas fluorescens* (1)	*Pseudomonas stutzeri* (1)	*Pseudomonas* sp. 1 (1)	*Pseudomonas* sp. 2 (1)	*Pseudomonas* sp. 3 (1)	*Alcaligenes faecalis* (4)	Achromobacter sp. biotype II (4)	*Acinetobacter anitratus* (1)	Total (137)
Urine	16	1	1	0	0	0	1	1	0	0	20
Upper Respiratory Tract	18	1	0	1	1	0	0	1	0	0	22
Lower Respiratory Tract	24	2	0	0	0	0	0	1	0	0	27
Burns	20	0	0	0	0	0	0	0	1	0	21
Ear	17	0	0	0	0	0	0	0	0	0	17
Wound	12	1	0	0	0	1	0	1	1	0	16
Cervix	0	0	0	0	0	0	0	0	1	0	1
Cerebrospinal Fluid	1	0	0	0	0	0	0	0	0	0	1
Groin Incision	1	0	0	0	0	0	0	0	0	0	1
Unknown	9	0	0	0	0	0	0	0	1	1	11

a=Numbers in parentheses and in each column represent number of isolates.

Table 2

Antimicrobial Disk Interpretation

	Disc Potency	Inhibition Zone Diameter to Nearest mm		
		Resistant mm or less	Intermediate mm range	Sensitive mm or more
Polymyxin B	300 U	8	9-11	12
Gentamicin	10 ug	12	-	13
Tobramycin	10 ug	11	10-11	14
Neomycin	30 ug	12	13-16	17
Carbenicillin	100 ug	13	14-16	17
Gantrisin	300 ug	12	13-16	17
Penicillin	10 U	11	12-21	22
Tetracycline	30 ug	14	15-18	19
Kanamycin	30 ug	13	14-17	18
Ampicillin	10 ug	11	12-13	14
Nalidixic Acid	30 ug	13	14-18	19
Nitrofurantoin	300 ug	14	15-16	17
Streptomycin	10 ug	11	12-14	15
Chloramphenicol	30 ug	12	13-17	18
Lincomycin	2 ug	9	10-14	15
Erythromycin	15 ug	13	14-17	18
Cephalothin	30 ug	14	15-17	18

Table 3[a]

Biochemical and Physical Characteristics of the Pseudomonads Encountered in this Study

	Ps. aeruginosa (118)	*Ps. putida* (5)	*Ps. fluorescens* (1)	*Ps. stutzeri* (1)	*Ps.* sp. 1 (1)	*Ps.* sp. 2 (1)	*Ps.* sp. 3 (1)
Anaerobic-Dextrose	0	0	0	0	0	0	0
Arginine Dihydrolase	100	100	0	0	100	100	0
Nitrogen Gas	64	0	0	100	0	0	0
H_2S	0	0	0	0	0	0	0
Indole	0	0	0	0	0	0	0
Xylose	96	100	0	100	0	0	0
Aerobic-Dextrose	99	100	100	100	0	0	0
Urease	92	80	100	0	0	0	100
Citrate	99	100	100	100	100	0	0
Oxidase	100	100	100	100	0	0	100
Pigment (Pyocyanin)	83	0	0	0	0	0	0
Growth on SS	97	100	100	100	100	0	100
ONPG	0	0	0	0	0	0	0
Gelatin Hydrolysis	96	0	100	0	0	100	0
Gluconate	97	60	100	0	0	100	0
Acetamide	82	20	0	0	0	100	100
Fructose	56	80	0	100	0	0	0
Maltose	11	0	0	0	0	0	0
Mannitol	77	20	0	0	0	0	0
Arabinose	96	100	0	0	0	100	0
Lactose	0	0	0	0	0	0	0
Rhamnose	7	40	0	0	0	0	0
Growth at 42C	100	0	0	0	0	0	100

a=Numbers in parentheses represent number of strains. Numbers in columns indicate percent positive.

Table 4[a]

Comparison of Characteristics Among Strains of *Ps. putida*, *Alcaligenes faecalis* and *Achromobacter* sp. biotype II

	Pseudomonas putida					*Alcaligenes faecalis*				*Achromobacter* sp. biotype II			
	A	B	C	D	E	A	B	C	D	A	B	C	D
Anaerobic-Dextrose	-	-	-	-	-	-	-	-	-	-	-	-	-
Arginine Dihydrolase	+	+	+	+	+	-	-	-	-	-	+	+	+
Nitrogen Gas	-	-	-	-	-	+	+	+	+	+	-	+	+
H_2S	-	-	-	-	-	-	-	-	-	-	-	-	-
Indole	-	-	-	-	-	-	-	-	-	-	-	-	-
Xylose	+	+	+	+	+	-	-	-	-	+	-	-	+
Aerobic-Dextrose	+	+	+	+	+	-	-	-	-	+	+	+	-
Urease	+	+	+	+	-	-	-	-	-	+	+	+	+
Citrate	+	+	+	+	+	+	-	-	-	+	-	+	+
Oxidase	+	+	+	+	+	+	+	+	-	+	+	+	-
Pigment (Pyocyanin)	-	-	-	-	-	-	-	-	-	-	-	-	-
Growth on SS	+	+	+	+	+	+	-	+	-	+	+	+	+
ONPG	-	-	-	-	-	-	-	-	-	-	-	-	-
Gelatin Hydrolysis	-	-	-	-	-	-	+	-	+	+	+	+	+
Gluconate	+	+	-	+	-	-	-	-	-	-	-	-	-
Acetamide	-	-	+	-	-	+	+	+	+	-	+	-	-
Fructose	+	-	+	+	+	-	-	-	-	-	-	+	-
Maltose	-	-	-	-	-	-	-	-	-	-	+	-	-
Mannitol	+	-	-	-	-	-	-	-	-	-	-	-	+
Arabinose	+	+	+	+	+	-	+	-	+	+	+	+	+
Lactose	-	-	-	-	-	-	-	-	-	-	-	-	-
Rhamnose	+	+	-	-	-	-	-	-	-	-	-	-	-
Growth at 42C	-	-	-	-	-	+	+	+	+	+	+	+	+

a=Positive reaction,+; negative reaction,-.

Table 5[a]

Biochemical and Physical Characteristics

	Alcaligenes faecalis (4)	*Achromobacter* sp. biotype II (4)	*Acinetobacter anitratus* (1)
Anaerobic-Dextrose	0	0	0
Arginine Dihydrolase	0	75	0
Nitrogen Gas	100	75	0
H_2S	0	0	0
Indole	0	0	0
Xylose	0	50	100
Aerobic-Dextrose	0	75	100
Urease	0	100	100
Citrate	25	75	100
Oxidase	75	75	0
Pigment (Pyocyanin)	0	0	0
Growth on SS	50	100	0
ONPG	0	0	0
Gelatin Hydrolysis	50	100	0
Gluconate	0	0	0
Acetamide	100	25	100
Fructose	0	25	0
Maltose	0	25	0
Mannitol	0	25	0
Arabinose	50	100	100
Lactose	0	0	0
Rhamnose	0	0	0
Growth at 42C	100	100	100

a=Numbers in parentheses indicate number of strains. Numbers in columns indicate percent positive.

Table 6[a]

Antibiograms of the Pseudomonads

Antimicrobial	Pseudomonas aeruginosa (118)			Pseudomonas putida (5)			Pseudomonas fluorescens (1)			Pseudomonas stutzeri (1)			Pseudomonas Sp. 1 (1)			Pseudomonas Sp. 2 (1)			Pseudomonas Sp. 3 (1)		
	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R
Polymyxin B	99	1	0	100	0	0	100	0	0	100	0	0	100	0	0	100	0	0	100	0	0
Gentamicin	95	0	5	0	80	20	100	0	0	100	0	0	100	0	0	100	0	0	100	0	0
Tobramycin	94	4	2	100	0	0	100	0	0	100	0	0	100	0	0	100	0	0	100	0	0
Neomycin	8	36	56	80	20	0	100	0	0	100	0	0	100	0	0	100	0	0	100	0	0
Carbenicillin	80	17	3	40	20	40	0	0	100	100	0	0	100	0	0	100	0	0	100	0	0
Gantrisin	4	16	80	0	0	100	0	0	100	0	0	100	0	100	0	100	0	0	0	0	100
Penicillin	0	0	100	0	0	100	0	0	100	0	0	100	0	100	0	0	0	100	0	0	100
Tetracycline	2	1	97	20	40	40	0	100	0	100	0	0	0	100	0	0	100	0	0	0	100
Kanamycin	1	1	98	60	0	40	100	0	0	0	0	100	100	0	0	100	0	0	0	100	0
Ampicillin	1	0	99	0	0	100	0	0	100	100	0	0	100	0	0	0	0	100	0	0	100
Nalidixic Acid	0	1	99	0	0	100	0	100	0	0	0	100	0	100	0	0	100	0	0	0	100
Nitrofurantoin	0	0	100	0	0	100	0	0	100	0	0	100	0	0	100	0	0	100	0	0	100
Streptomycin	2	1	97	40	40	20	100	0	0	100	0	0	0	0	100	0	0	100	0	100	0
Chlormphenicol	0	4	96	0	40	60	0	0	100	0	0	100	0	0	100	0	0	100	0	0	100
Lincomycin	0	0	100	0	0	100	0	0	100	0	0	100	0	0	100	0	0	100	0	0	100
Erythromycin	0	1	99	0	0	100	0	0	100	0	100	0	0	100	0	0	100	0	0	0	100
Cephalothin	0	0	100	0	0	100	0	0	100	0	0	100	0	0	100	0	0	100	0	0	100

a=Numbers in each column indicate percent Susceptible (S); Intermediate (I); or Resistant (R).
Numbers in parentheses indicate number of strains

Table 7[a]

Antibiograms of *Alcaligenes faecalis* *Achromobacter* Sp. biotype II and *Acinetobacter anitratus*

	Alcaligenes faecalis (4)			*Achromobacter* sp. biotype II (4)			*Acinetobacter anitratus* (1)		
Antimicrobial	S	I	R	S	I	R	S	I	R
Polymyxin B	50	25	25	75	0	25	100	0	0
Gentamicin	75	0	25	100	0	0	100	0	0
Tobramycin	75	0	25	100	0	0	100	0	0
Neomycin	25	25	50	50	25	25	100	0	0
Carbenicillin	75	0	25	50	0	50	0	100	0
Gantrisin	25	0	75	25	0	75	0	0	100
Penicillin	0	0	100	0	0	100	0	0	100
Tetracycline	50	0	50	25	0	75	0	100	0
Kanamycin	25	0	75	50	0	50	100	0	0
Ampicillin	25	0	75	0	0	100	0	0	100
Nalidixic Acid	50	0	50	25	0	75	0	100	0
Nitrofurantoin	100	0	0	25	0	75	0	0	100
Streptomycin	0	0	100	75	0	25	0	0	100
Chloramphenicol	25	25	50	50	0	50	100	0	0
Lincomycin	0	0	100	0	0	100	0	0	100
Erythromycin	25	25	50	25	0	75	0	0	100
Cephalothin	25	0	75	0	0	100	0	0	100

a=Numbers in each column indicate percent Susceptible (S); Intermediate (I); or Resistant (R). Numbers in parentheses indicate number of strains.

Table 8*

Summary of Biochemical and Physical Characteristics of Ps. aeruginosa

	This Study (118)	Oberhofer et al., 1977 (91)	Hugh and Gilardi 1974 (118)	Gilardi 1971c (48)	#Positive/#Tested	% Positive
Anaerobic Dextrose	0	0	0	0	0/375	0
Arginine Dihydrolase	118	91	117	48	374/375	99
Nitrogen gas	76	87	111	30	304/375	81
H_2S	0	0	0	0	0/312	0
Indole	0	0	NT	NT	0/209	0
Xylose	113	96	118	43	361/375	96
Aerobic Dextrose	117	91	118	48	374/375	99
Urease	109	63	27	43	242/375	65
Citrate	117	87	118	NT	322/323	99
Oxidase	118	91	118	48	375/375	100
Pigment (Pyocyanin)	98	72	68	NT	238/327	72
Growth on SS	114	84	51	47	296/312	95
ONPG	0	3	0	0	3/312	1
Gelatin Hydrolysis	113	73	117	29	332/375	89
Gluconate	114	82	118	35	349/375	93
Acetamide	97	85	NT	NT	182/205	89
Fructose	66	(62)	107	46	281/375	75
Maltose	13	0	0	0	13/375	3
Mannitol	91	(63)	109	34	297/375	79
Arabinose	113	NT	118	NT	231/236	98
Lactose	0	0	(41)	0	41/375	11
Rhamnose	8	NT	(90)	15	113/284	40
Growth at 42C	118	NT	118	48	284/284	100
Lysine Decarboxylase	NT	0	0	0	0/257	0

*=Numbers in each column indicate number of positive strains. NT signifies not tested. Parentheses indicate percent positive, delayed 3 or more days.

Table 9*

Summary of Biochemical and Physical Characteristics of *Ps. putida*

	This Study (5)	Oberhofer et al., 1977 (52)	Hugh and Gilardi 1974 (72)	Gilardi 1971c (30)	#Positive/#Tested	% Positive
Anaerobic Dextrose	0	0	0	0	0/159	0
Arginine Dihydrolase	5	52	70	30	157/159	99
Nitrogen gas	0	0	0	0	0/159	0
H_2S	0	0	0	0	0/159	0
Indole	0	0	NT	NT	0/57	0
Xylose	5	50	69	29	153/159	96
Aerobic Dextrose	5	52	72	30	159/159	100
Urease	4	6	37	19	66/159	42
Citrate	5	52	NT	NT	57/57	100
Oxidase	5	52	72	30	159/159	100
Pigment (Pyocyanin)	0	0	0	NT	0/129	0
Growth on SS	5	49	72	30	156/159	98
ONPG	0	0	1	0	1/159	1
Gelatin Hydrolysis	0	0	0	0	0/159	0
Gluconate	3	41	53	22	119/159	75
Acetamide	1	3	NT	NT	4/57	7
Fructose	4	(49)	71	29	153/159	96
Maltose	0	0	25	7	32/159	20
Mannitol	1	(8)	14	6	29/159	18
Arabinose	5	NT	NT	NT	5/5	100
Lactose	0	0	20	6	26/159	16
Rhamnose	2	NT	45	18	65/107	61
Growth at 42C	0	NT	0	0	0/107	0
Lysine Decarboxylase	NT	0	0	0	0/154	0

*=Numbers in each column indicate number of positive strains. NT signifies not tested. Parentheses indicate percent positive, delayed 3 or more days.

Table 10*

Summary of Biochemical and Physical Characteristics of *Ps. fluorescens*

	This Study (1)	Oberhofer et al., 1977 (18)	Hugh and Gilardi 1974 (50)	Gilardi 1971c (12)	#Positive/#Tested	%Positive
Anaerobic Dextrose	0	0	0	0	0/81	0
Arginine Dihydrolase	0	18	49	12	79/81	98
Nitrogen gas	0	0	1	0	1/81	1
H_2S	0	0	0	0	0/81	0
Indole	0	0	NT	NT	0/19	0
Xylose	0	16	49	12	77/81	95
Aerobic Dextrose	1	18	50	12	81/81	100
Urease	1	4	20	11	36/81	44
Citrate	1	18	NT	NT	19/19	100
Oxidase	1	18	50	12	81/81	100
Pigment (Pyocyanin)	0	0	0	NT	0/69	0
Growth on SS	1	16	49	11	77/81	95
ONPG	0	0	2	0	2/81	2
Gelatin Hydrolysis	1	17	50	12	80/81	99
Gluconate	1	10	35	8	54/81	67
Acetamide	0	1	NT	NT	1/19	5
Fructose	0	(15)	49	11	75/81	93
Maltose	0	0	35	10	45/81	56
Mannitol	0	(3)	47	10	60/81	74
Arabinose	0	NT	NT	NT	0/1	0
Lactose	0	1	13	1	15/81	19
Rhamnose	0	NT	42	9	51/63	81
Growth at 42C	0	NT	0	0	0/62	0
Lysine Decarboxylase	NT	0	0	0	0/80	0

*=Numbers in each column indicate number of positive strains. NT signifies not tested. Parentheses indicate percent positive, delayed 3 or more days.

Table 11*

Summary of Biochemical and Physical Characteristics of Ps. stutzeri

	This Study (1)	Oberhofer et al.* 1977 (15)	Hugh and Gilardi 1974 (45)	Gilardi 1971c (24)	#Positive/#Tested	% Positive
Anaerobic Dextrose	0	0	0	0	0/85	0
Arginine Dihydrolase	0	10	0	0	10/85	12
Nitrogen gas	1	13	45	24	83/85	98
H_2S	0	0	0	0	0/85	0
Indole	0	0	NT	NT	0/16	0
Xylose	1	(15)	45	24	85/85	100
Aerobic Dextrose	1	15	45	24	85/85	100
Urease	0	8	12	5	25/85	29
Citrate	1	13	NT	NT	14/16	88
Oxidase	1	15	45	24	85/85	100
Pigment (Pyocyanin)	0	0	0	NT	0/61	0
Growth on SS	1	13	(40)	22	76/85	89
ONPG	0	0	0	0	0/85	0
Gelatin Hydrolysis	0	0	0	0	0/85	0
Gluconate	0	0	1	0	1/85	1
Acetamide	0	2	NT	NT	2/16	1
Fructose	1	(12)	45	24	82/85	96
Maltose	0	(13)	14	24	51/85	60
Mannitol	0	(9)	40	21	70/85	82
Arabinose	0	NT	NT	NT	0/1	0
Lactose	0	0	0	0	0/85	0
Rhamnose	0	NT	32	18	50/70	71
Growth at 42C	0	NT	45	24	69/70	99
Lysine Decarboxylase	NT	0	0	0	0/84	0

*=Numbers in each column indicate number of positive strains. NT signifies not tested. Parentheses indicate percent positive, delayed 3 or more days.

Table 12*

Summary of Biochemical and Physical Characteristics of Ps. cepacia

	Oberhofer et al., 1977 (7)	Hugh and Gilardi 1974 (41)	Gilardi 1971c (12)	#Positive/#Tested	% Positive
Anaerobic Dextrose	0	0	0	0/60	0
Arginine Dihydrolase	0	0	0	0/60	0
Nitrogen gas	0	0	0	0/60	0
H_2S	0	0	0	0/60	0
Indole	0	NT	NT	0/7	0
Xylose	6	41	12	59/60	98
Aerobic Dextrose	7	41	12	60/60	100
Urease	6	41	5	25/60	42
Citrate	5[a]	NT	NT	5/5	100
Oxidase	5	37	10	52/60	87
Pigment (Pyocyanin)	0	0	NT	0/48	0
Growth on SS	0	1	0	1/60	2
ONPG	7	37	12	56/60	93
Gelatin Hydrolysis	(7)	26	7	40/60	67
Gluconate	4	5	1	10/60	17
Acetamide	5[a]	NT	NT	5/5	100
Fructose	6	41	12	59/60	98
Maltose	6	41	12	59/60	98
Mannitol	5	41	12	58/60	97
Arabinose	NT	NT	NT	NT	NT
Lactose	7	41	12	60/60	100
Rhamnose	NT	0	0	0/53	0
Growth at 42C	NT	29	7	36/53	68
Lysine Decarboxylase	7	38	12	57/60	95

*=Numbers in each column indicate number of positive strains. NT signifies not tested. Parentheses indicate percent positive, delayed 3 or more days.

a = Five strains were tested.

Table 13*

Summary of Biochemical and Physical Characteristics of *Ps. maltophilia*

	Oberhofer et al., 1977 (80)	Hugh and Gilardi 1974 (195)	Gilardi 1971c (81)	#Positive/#Tested	% Positive
Anaerobic Dextrose	0	0	0	0/356	0
Arginine Dihydrolase	0	0	0	0/356	0
Nitrogen gas	0	0	0	0/356	0
H_2S	0	0	0	0/356	0
Indole	0	NT	NT	0/80	0
Xylose	2	103	45	150/356	42
Aerobic Dextrose	11	195	81	287/356	81
Urease	47	0[b]	0	47/169	28
Citrate	62[a]	13	NT	75/263	29
Oxidase	22	2	0	24/356	7
Pigment (Pyocyanin)	0	0	NT	0/275	0
Growth on SS	24	0	0	24/356	7
ONPG	32	185	80	297/356	83
Gelatin Hydrolysis	80	195	81	356/356	100
Gluconate	0	0	0	0/356	0
Acetamide	(8)[a]	NT	NT	8/68	12
Fructose	34	193	79	306/356	86
Maltose	79	195	81	355/356	99
Mannitol	0	0	0	0/356	0
Arabinose	NT	NT	NT	NT	NT
Lactose	0	176	75	251/356	71
Rhamnose	NT	0[b]	0	0/122	0
Growth at 42C	NT	148	67	215/276	78
Lysine Decarboxylase	80	195	81	276/276	100

*=Numbers in each column indicate number of positive strains. NT signifies not tested. Parentheses indicate percent positive, delayed 3 or more days.
a=Sixty-eight strains were tested.
b=Forty-one strains were tested.

Table 14*

Summary of Biochemical and Physical Characteristics of Ps. diminuta

	Oberhofer et al., 1977 (9)	Hugh and Gilardi 1974 (6)	Gilardi 1971c (4)	#Positive/#Tested	% Positive
Anaerobic Dextrose	NT	NT	0	0/4	0
Arginine Dihydrolase	0	0	0	0/19	0
Nitrogen gas	0	0	0	0/19	0
H_2S	0	0	0	0/19	0
Indole	0	NT	NT	0/9	0
Xylose	NT	0	0	0/10	0
Aerobic Dextrose	NT	0	0	0/10	0
Urease	0	NT	0	0/13	0
Citrate	0	NT	NT	0/9	0
Oxidase	9	6	4	19/19	100
Pigment (Pyocyanin)	0	NT	NT	0/9	0
Growth on SS	0	0	0	0/19	0
ONPG	0	0	0	0/19	0
Gelatin Hydrolysis	(5)	6	4	15/19	79
Gluconate	0	NT	0	0/13	0
Acetamide	0	NT	NT	0/9	0
Fructose	0	0	0	0/19	0
Maltose	0	0	0	0/19	0
Mannitol	0	0	0	0/19	0
Arabinose	NT	NT	NT	NT	NT
Lactose	0	0	0	0/19	0
Rhamnose	NT	NT	0	0/4	0
Growth at 42C	NT	6	4	10/10	100
Lysine Decarboxylase	0	0	0	0/19	0

*=Numbers in each column indicate number of positive strains. NT signifies not tested. Parentheses indicate percent positive, delayed 3 or more days.

Table 15*

Summary of Biochemical and Physical Characteristics of Ps. alcaligenes

	Oberhofer et al., 1977 (8)	Hugh and Gilardi 1974 (15)	Gilardi 1971c (7)	#Positive/#Tested	% Positive
Anaerobic Dextrose	NT	0	0	0/22	0
Arginine Dihydrolase	7	0	0	7/30	23
Nitrogen gas	0	0	0	0/30	0
H_2S	0	0	0	0/30	0
Indole	0	NT	NT	0/8	0
Xylose	NT	0	0	0/22	0
Aerobic Dextrose	NT	0	0	0/22	0
Urease	1	NT	1	2/15	13
Citrate	2	NT	NT	2/8	25
Oxidase	8	15	7	30/30	100
Pigment (Pyocyanin)	0	NT	NT	0/8	0
Growth on SS	3	0	0	3/30	10
ONPG	0	0	0	0/30	0
Gelatin Hydrolysis	0	1	1	2/30	7
Gluconate	0	NT	0	0/15	0
Acetamide	0	NT	NT	0/8	0
Fructose	0	0	0	0/30	0
Maltose	0	0	0	0/30	0
Mannitol	0	0	0	0/30	0
Arabinose	NT	NT	NT	NT	NT
Lactose	0	0	0	0/30	0
Rhamnose	NT	NT	0	0/7	0
Growth at 42C	NT	12	7	19/22	86
Lysine Decarboxylase	0	0	0	0/30	0

*=Numbers in each column indicate number of positive strains. NT signifies not tested. Parentheses indicate percent positive, delayed 3 or more days.

Table 16*

Summary of Biochemical and Physical Characteristics of *Ps. pseudoalcaligenes*

	Oberhofer et al., 1977 (25)	Hugh and Gilardi 1974 (14)	Gilardi 1971c (6)	#Positive/#Tested	% Positive
Anaerobic Dextrose	NT	NT	0	0/6	0
Arginine Dihydrolase	18	2	0	20/45	44
Nitrogen gas	0	0	0	0/45	0
H_2S	0	0	0	0/45	0
Indole	0	NT	NT	0/25	0
Xylose	1	14	3	18/45	40
Aerobic Dextrose	7	13	6	26/45	58
Urease	1	NT	0	1/31	32
Citrate	8	NT	NT	8/25	32
Oxidase	25	14	6	45/45	100
Pigment (Pyocyanin)	0	NT	NT	0/25	0
Growth on SS	16	12	5	33/45	73
ONPG	0	0	0	0/45	0
Gelatin Hydrolysis	0	0	0	0/45	0
Gluconate	0	NT	0	0/31	0
Acetamide	0	NT	NT	0/25	0
Fructose	(24)	14	6	44/45	98
Maltose	0	2	0	2/45	4
Mannitol	3	0	0	3/45	7
Arabinose	NT	NT	NT	NT	NT
Lactose	0	0	0	0/45	0
Rhamnose	NT	NT	0	0/6	0
Growth at 42C	NT	13	6	19/20	95
Lysine Decarboxylase	0	0	0	0/45	0

*=Numbers in each column indicate number of positive strains. NT signifies not tested. Parentheses indicate percent positive, delayed 3 or more days.

Table 17*

Summary of Biochemical and Physical Characteristics of Ps. putrefaciens

	Hugh and Gilardi 1974 (16)	Gilardi 1971c (6)	#Positive/#Tested	% Positive
Anaerobic Dextrose	0	0	0/22	0
Arginine Dihydrolase	0	0	0/22	0
Nitrogen gas	0	0	0/22	0
H_2S	16	6	22/22	100
Indole	NT	NT	NT	NT
Xylose	1	0	1/22	5
Aerobic Dextrose	16	(6)	22/22	100
Urease	4	3	7/22	32
Citrate	NT	NT	NT	NT
Oxidase	16	6	22/22	100
Pigment (Pyocyanin)	0	NT	0/16	0
Growth on SS	15	6	21/22	95
ONPG	0	0	0/22	0
Gelatin Hydrolysis	15	6	21/22	95
Gluconate	0	0	0/22	0
Acetamide	NT	NT	NT	NT
Fructose	7	(3)	10/22	45
Maltose	5	0	5/22	23
Mannitol	NT	0	0/6	0
Arabinose	NT	NT	NT	NT
Lactose	3	(2)	5/22	23
Rhamnose	3	(2)	5/22	23
Growth at 42C	16	6	22/22	100
Lysine Decarboxylase	0	0	0/22	0

*=Numbers in each column indicate number of positive strains. NT signifies not tested. Parentheses indicate percent positive, delayed 3 or more days.

Table 18*

Summary of Biochemical and Physical Characteristics of the Pseudomonads

	Ps. aeruginosa	Ps. putida	Ps. fluorescens	Ps. stutzeri	Ps. cepacia	Ps. maltophilia	Ps. diminuta	Ps. alcaligenes	Ps. pseudoalcaligenes	Ps. putrefaciens
Anaerobic Dextrose	0	0	0	0	0	0	0	0	0	0
Arginine Dihydrolase	99	99	98	12	0	0	0	23	44	0
Nitrogen Gas	81	0	1	98	0	0	0	0	0	0
H_2S	0	0	0	0	0	0	0	0	0	100
Indole	0	0	0	0	0	0	0	0	0	NT
Xylose	96	96	95	100	98	42	0	0	40	5
Aerobic Dextrose	99	100	100	100	100	81	0	0	58	100
Urease	65	42	44	29	42	28	0	13	32	32
Citrate	99	100	100	88	100	29	0	25	32	NT
Oxidase	100	100	100	100	87	7	100	100	100	100
Pigment (Pyocyanin)	72	0	0	0	0	0	0	0	0	0
Growth on SS	95	98	95	89	2	7	0	10	73	95
ONPG	1	1	2	0	93	83	0	0	0	0
Gelatin Hydrolysis	89	0	99	0	67	100	79	7	0	95
Gluconate	93	75	67	1	17	0	0	0	0	0
Acetamide	89	7	5	1	100	12	0	0	0	NT
Fructose	75	96	93	96	98	86	0	0	98	45
Maltose	3	20	56	60	98	99	0	0	4	23
Mannitol	79	18	74	82	97	0	0	0	7	0
Arabinose	98	100	0	0	NT	NT	NT	NT	NT	NT
Lactose	11	16	19	0	100	71	0	0	0	23
Rhamnose	40	61	81	71	0	0	0	0	0	23
Growth at 42C	100	0	0	99	68	78	100	86	95	100
Lysine Decarboxylase	0	0	0	0	95	100	0	0	0	0

*=Numbers in columns indicate percent positive. NT signifies not tested.

Table 19*

Summary of Antibiograms of
Ps. aeruginosa

	This Study (118)	Oberhofer et al., 1977 (85)	von Graevenitz and Redys 1968 (14)	#Susceptible/ #Tested	% Susceptible
Gentamicin	112	75	NT	187/203	92
Tobramycin	111	NT	NT	111/118	94
Kanamycin	1	20	6	27/217	12
Neomycin	9	NT	6	15/132	11
Streptomycin	2	24	3	29/217	13
Polymyxin B	117	NT	NT	117/118	99
Carbenicillin	94	70	NT	164/203	81
Ampicillin	12	4	0	16/321	5
Penicillin	0	NT	NT	0/118	0
Chloramphenicol	0	19	2	21/217	10
Tetracycline	2	16	0	18/321	6
Cephalothin	0	0	0	0/321	0
Lincomycin	0	NT	0	0/132	0
Erythromycin	0	NT	0	0/132	0
Gantrisin	5	NT	NT	5/118	4
Nalidixic Acid	0	13	0	13/321	4
Nitrofurantoin	0	0	NT	0/203	0

*=Numbers in each column indicate number of positive strains. NT signifies not tested. Susceptibilities are not intended as a therapeutic guide but are used as supplemental data for identification.

Table 20*

Summary of Antibiograms of
Ps. putida

	This Study (5)	Oberhofer et al. 1977 (52)	Moody et al. 1973 (17)	Pedersen et al. 1970 (8)	Von Graevenitz and Redys 1968 (5)	# Susceptible/# Tested	% Susceptible
Gentamicin	0	46	5	NT	NT	51/74	69
Tobramycin	5	NT	NT	NT	NT	5/5	100
Kanamycin	3	45	15	5	5	73/87	84
Neomycin	4	NT	4	NT	5	13/27	48
Streptomycin	2	36	0	NT	0	38/79	48
Polymyxin B	5	NT	16	NT	NT	21/22	95
Carbenicillin	2	0	0	NT	NT	2/74	1
Ampicillin	0	0	0	0	0	0/87	0
Penicillin	0	NT	NT	NT	NT	0/5	0
Chloramphenicol	0	11	0	1	0	12/87	14
Tetracycline	1	32	12	4	0	39/87	45
Cephalothin	0	0	0	0	0	0/87	0
Lincomycin	0	NT	NT	NT	0	0/10	0
Erythromycin	0	NT	NT	NT	0	0/10	0
Gantrisin	0	NT	NT	NT	NT	0/5	0
Nalidixic Acid	0	0	NT	NT	0	0/62	0
Nitrofurantoin	0	0	NT	NT	NT	0/57	0

*=Numbers in each column indicate number of positive strains. NT signifies not tested. Susceptibilities are not intended as a therapeutic guide but are used as supplemental data for identification.

Table 21*

Summary of Antibiograms of
Ps. fluorescens

	This Study (1)	Oberhofer et al. 1977 (17)	Moody et al. 1973 (10)	Pedersen et al. 1970 (11)	# Susceptible/# Tested	% Susceptible
Gentamicin	1	17	7	NT	25/39	64
Tobramycin	1	NT	NT	NT	1/1	100
Kanamycin	1	17	90	10	37/39	95
Neomycin	1	NT	50	NT	6/11	55
Streptomycin	1	13	20	NT	16/28	57
Polymyxin B	1	NT	10	NT	11/11	100
Carbenicillin	0	0	0	NT	0/28	0
Ampicillin	0	0	0	NT	0/28	0
Penicillin	0	NT	NT	NT	0/1	0
Chloramphenicol	0	6	0	11	17/39	44
Tetracycline	0	13	3	11	27/39	69
Cephalothin	0	0	0	9	9/39	23
Lincomycin	0	NT	NT	NT	0/1	0
Erythromycin	0	NT	NT	NT	0/1	0
Gantrisin	0	NT	NT	NT	0/1	0
Nalidixic Acid	0	5	NT	NT	5/18	28
Nitrofurantoin	0	0	NT	NT	0/18	0

*=Numbers in each column indicate number of positive strains. NT signifies not tested. Susceptibilities are not intended as a therapeutic guide but are used as supplemental data for identification.

Table 22*

Summary of Antibiograms of
Ps. stutzeri

	This Study (1)	Oberhofer et al. 1977 (28)	Moody et al. 1973 (13)	Pedersen et al. 1970 (14)	Von Graevenitz and Redys 1968 (25)	# Susceptible /# tested	% Susceptible
Gentamicin	1	28	13	NT	NT	42/40	100
Tobramycin	1	NT	NT	NT	NT	1/1	100
Kanamycin	0	28	6	13	25	72/81	89
Neomycin	1	NT	9	NT	25	35/39	90
Streptomycin	1	27	7	NT	24	59/67	88
Polymyxin B	1	NT	13	NT	NT	14/14	100
Carbenicillin	1	27	13	NT	NT	41/42	98
Ampicillin	1	25	6	8	25	65/81	80
Penicillin	0	NT	NT	NT	NT	0/1	0
Chloramphenicol	0	15	12	10	23	60/81	74
Tetracycline	1	28	7	14	23	73/81	90
Cephalothin	0	1	0	4	25	30/81	37
Lincomycin	0	NT	NT	0	0	0/26	0
Erythromycin	0	NT	NT	NT	25	25/26	96
Gantrisin	0	NT	NT	NT	NT	0/1	0
Nalidixic Acid	0	24	NT	NT	22	46/54	85
Nitrofurantoin	0	0	NT	NT	NT	0/29	0

*=Numbers in each column indicate number of positive strains. NT signifies not tested. Susceptibilities are not intended as a therapeutic guide but are used as supplemental data for identification.

Table 23*

Summary of Antibiograms of
Ps. cepacia

	Oberhofer et al. 1977 (7)	# Susceptible/# Tested	% Susceptible
Gentamicin	4	4/7	57
Tobramycin	NT	NT	NT
Kanamycin	4	4/7	57
Neomycin	NT	NT	NT
Streptomycin	1	1/7	14
Polymyxin B	NT	NT	NT
Carbenicillin	1	1/7	14
Ampicillin	0	0/7	0
Penicillin	NT	NT	NT
Chloramphenicol	7	7/7	100
Tetracycline	0	0/7	0
Cephalothin	0	0/7	0
Lincomycin	NT	NT	NT
Erythromycin	NT	NT	NT
Gantrisin	NT	NT	NT
Nalidixic Acid	7	7/7	100
Nitrofurantoin	0	0/7	0

*=Numbers in each column indicate number of positive strains. NT signifies not tested. Susceptibilities are not intended as a therapeutic guide but are used as supplemental data for identification.

Table 24*

Summary of Antibiograms of
Ps. maltophilia

	Oberhofer et al. 1977 (73)	Moody et al. 1973 (24)	Pedersen et al. 1970 (29)	Von Graevenitz and Redys 1968 (35)	#Susceptible/# Tested	% Susceptible
Gentamicin	27	3	NT	NT	30/97	31
Tobramycin	NT	NT	NT	NT	NT	NT
Kanamycin	11	2	5	28	46/161	29
Neomycin	NT	3	NT	28	31/59	53
Streptomycin	18	2	NT	24	44/132	33
Polymyxin	NT	23	NT	NT	23/24	96
Carbenicillin	14	4	NT	NT	18/97	19
Ampicillin	1	0	6	0	7/161	4
Penicillin	NT	NT	NT	NT	NT	NT
Chloramphenicol	56	19	24	35	134/161	83
Tetracycline	23	3	20	24	70/161	43
Cephalothin	1	0	0	26	27/161	17
Lincomycin	NT	NT	NT	0	0/35	0
Erythromycin	NT	NT	NT	26	26/35	74
Gantrisin	NT	NT	NT	NT	NT	NT
Nalidixic Acid	67	NT	NT	35	102/108	94
Nitrofurantoin	2	NT	NT	NT	2/73	3

*=Numbers in each column indicate number of positive strains. NT signifies not tested. Susceptibilities are not intended as a therapeutic guide but are used as supplemental data for identification.

Table 25*

Summary of Antibiograms of
Ps. diminuta

	Oberhofer et al. 1977 (9)	Pedersen et al. 1970 (6)	Von Graevenits and Redys 1968 (6)	# Susceptible/# Tested	% Susceptible
Gentamicin	8	NT	NT	8/9	89
Tobramycin	NT	NT	NT	NT	NT
Kanamycin	8	NT	6	14/15	93
Neomycin	NT	NT	6	6/6	100
Streptomycin	2	NT	3	5/15	33
Polymyxin B	NT	NT	NT	NT	NT
Carbenicillin	9	NT	NT	9/9	100
Ampicillin	1	NT	0	1/9	11
Penicillin	NT	NT	NT	NT	NT
Chloramphenicol	9	6	6	21/21	100
Tetracycline	9	5	5	19/21	90
Cephalothin	5	NT	2	7/15	47
Lincomycin	NT	NT	0	0/6	0
Erythromycin	NT	5	6	11/12	92
Gantrisin	NT	NT	NT	NT	NT
Nalidixic Acid	0	1	0	1/21	5
Nitrofurantoin	0	NT	NT	0/9	0

*=Numbers in each column indicate number of positive strains. NT signifies not tested. Susceptibilities are not intended as a therapeutic guide but are used as supplemental data for identification.

Table 26*

Summary of Antibiograms of
Ps. alcaligenes

	Oberhofer et al. 1977 (7)	Pedersen et al. 1970 (12)	# Susceptible/# Tested	% Susceptible
Gentamicin	6	NT	6/7	86
Tobramycin	NT	NT	NT	NT
Kanamycin	7	12	19/19	100
Neomycin	NT	NT	NT	NT
Streptomycin	2	NT	2/7	29
Polymyxin B	NT	NT	NT	NT
Carbenicillin	7	NT	7/7	100
Ampicillin	1	6	7/19	37
Penicillin	NT	NT	NT	NT
Chloramphenicol	4	12	16/19	84
Tetracycline	7	12	19/19	100
Cephalothin	0	6	6/19	32
Lincomycin	NT	NT	NT	NT
Erythromycin	NT	12	12/12	100
Gantrisin	NT	NT	NT	NT
Nalidixic Acid	7	10	17/19	89
Nitrofurantoin	0	NT	0/7	0

*=Numbers in each column indicate number of positive strains. NT signifies not tested. Susceptiblities are not intended as a therapeutic guide but are used as supplemental data for identification.

Table 27*

Summary of Antibiograms of
Ps. pseudoalcaligenes

	Oberhofer et al. 1977 (23)	Pedersen et al. 1970 (28)	# Susceptible/# Tested	% Susceptible
Gentamicin	23	NT	23/23	100
Tobramycin	NT	NT	NT	NT
Kanamycin	21	10	31/51	61
Neomycin	NT	NT	NT	NT
Streptomycin	15	NT	15/23	65
Polymyxin B	NT	NT	NT	NT
Carbenicillin	23	NT	23/23	100
Ampicillin	14	14	28/51	55
Penicillin	NT	NT	NT	NT
Chloramphenicol	16	28	44/51	86
Tetracycline	23	27	50/51	98
Cephalothin	0	21	21/51	41
Lincomycin	NT	NT	NT	NT
Erythromycin	NT	27	27/28	96
Gantrisin	NT	NT	NT	NT
Nalidixic Acid	21	22	43/51	84
Nitrofurantoin	0	NT	0/23	0

*=Numbers in each column indicate number of positive strains. NT signifies not tested. Susceptibilities are not intended as a therapeutic guide but are used as supplemental data for identification.

Table 28*

Summary of Antibiograms of
Ps. putrefaciens

	Gilardi 1971c (12)	# Susceptible/# Tested	% Susceptible
Gentamicin	12	12/12	100
Tobramycin	NT	NT	NT
Kanamycin	12	12/12	100
Neomycin	12	12/12	100
Streptomycin	12	12/12	100
Polymixin B	12	12/12	100
Carbenicillin	NT	NT	NT
Ampicillin	6	6/12	50
Penicillin	0	0/12	0
Chloramphenicol	12	12/12	100
Tetracycline	10	10/12	83
Cephalothin	3	3/12	25
Lincomycin	0	0/12	0
Erythromycin	10	10/12	83
Gantrisin	NT	NT	NT
Nalidixic Acid	12	12/12	100
Nitrofurantoin	12	12/12	100

*=Numbers in each column indicate number of positive strains. NT signifies not tested. Susceptibilities are not intended as a therapeutic guide but are used as supplemental data for identification.

Table 29*

Antibiogram Summary for the Pseudomonads

	Ps. aeruginosa	Ps. putida	Ps. fluorescens	Ps. stutzeri	Ps. cepacia	Ps. maltophilia	Ps. diminuta	Ps. alcaligenes	Ps. pseudoalcaligenes	Ps. putrefaciens
Gentamicin	92	69	64	100	57	31	89	86	100	100
Tobramycin	94	100	100	100	NT	NT	NT	NT	NT	NT
Kanamycin	12	84	95	89	57	29	93	100	61	100
Neomycin	11	48	55	90	NT	53	100	NT	NT	100
Streptomycin	13	48	57	88	14	33	33	29	65	100
Polymyxin B	99	95	100	100	NT	96	NT	NT	NT	100
Carbenicillin	81	1	0	98	14	19	100	100	100	NT
Ampicillin	5	0	0	80	0	4	11	37	55	50
Penicillin	0	0	0	0	NT	NT	NT	NT	NT	0
Chloramphenicol	10	14	44	74	100	83	100	84	86	100
Tetracycline	6	45	69	90	0	43	90	100	98	83
Cephalothin	0	0	23	37	0	17	47	32	41	25
Lincomycin	0	0	0	0	NT	0	0	NT	NT	0
Erythromycin	0	0	0	96	NT	74	92	100	96	83
Gantrisin	4	0	0	0	NT	NT	NT	NT	NT	NT
Nalidixic Acid	4	0	28	85	100	94	5	89	84	100
Nitrofurantoin	0	0	0	0	0	3	0	0	0	100

*=Numbers in each column indicate percent susceptible. NT signifies not tested.

Table 30*

Scheme for the Identification of
Clinically Isolated Pseudomonads

	Ps. aeruginosa	Ps. putida	Ps. fluorescens	Ps. stutzeri	Ps. cepacia	Ps. maltophilia	Ps. diminuta	Ps. alcaligenes	Ps. pseudoalcaligenes	Ps. putrefaciens
Anaerobic Dextrose	0	0	0	0	0	0	0	0	0	0
Arginine Dihydrolase	99	99	98	12	0	0	0	24	44	0
Nitrogen Gas	81	0	1	98	0	0	0	0	0	0
Xylose	96	96	95	100	98	42	0	0	40	5
Aerobic Dextrose	99	100	100	100	100	81	0	0	58	100
Citrate	99	100	100	88	100	28	0	25	32	NT
Oxidase	100	100	100	100	87	7	100	100	100	100
Growth on SS	95	98	95	89	2	7	0	10	73	95
Growth on 42C	100	0	0	99	68	78	100	86	95	100
ONPG	1	1	2	0	93	83	0	0	0	0
Gelatin Hydrolysis	89	0	99	0	67	100	79	7	0	100
Gluconate	93	75	67	1	17	0	0	0	0	0
Carbenicillin	81	1	0	98	18	19	100	100	100	NT
Ampicillin	5	0	0	80	0	4	11	37	55	50
Erythromycin	0	0	0	96	NT	74	92	100	96	83
Nalidixic Acid	4	0	28	85	100	94	5	89	84	100

Remarks	Arabinose Positive	Arabinose Negative	Lysine Decarboxylase Positive	Lysine Decarboxylase Negative	Fructose Negative	Fructose Positive	H_2S Positive

Table 30. (continued)

*=Numbers in each column indicate percent positive for biochemical tests and percent susceptible for antimicrobial agents. NT signifies not tested.

VIII. PROCEDURES

Acetamide

One tablet and one ml of distilled water are added to a 16 X 100 mm screw top test tube. The test tube is then boiled for ten minutes and allowed to cool. A heavy suspension of bacteria is used as the inoculum. Incubation is from four to six days with daily inspection. A positive test is seen as a color change from yellow (acidic) to purple (basic).

Antimicrobial Susceptibility Testing

The Mueller-Hinton plates, 150 mm in diameter, are stored in the refrigerator, in plastic bags until used or for a maximum of 14 days. The susceptibility testing procedure is as follows: The test organism is introduced into a nutrient broth tube and incubated until the density of the bacterial suspension, based on visual observation, is approximately equal to that of a 1% $BaCl_2$ in H_2SO_4 standard (0.5 ml of a 1% $BaCl_2$ solution in 99.5 ml of 0.36 N H_2SO_4). If the bacterial suspension is too turbid it is diluted with sterile nutrient broth. The suspension is streaked on a Mueller-Hinton plate with a sterile cotton swab after excess moisture is removed by pressing the swab to the inner wall of the test tube. The plates are evenly streaked

using the "three plane" method to insure equal distribution of bacteria. The surface of each plate is allowed to dry for at least five minutes but not longer than 10 minutes. Antibiotic discs are then placed on the plates using a 12 magazine-150 mm dispenser (Difco). The plates are incubated for 18 to 24 hours. The diameter of each zone of inhibition is measured twice and the average recorded.

Carbohydrate Oxidation

Each carbohydrate (lactose, fructose, maltose, mannitol, arabinose or rhamnose) is tested using the same procedure. One C.O.T. sugar tablet is placed in a 16 X 100 mm screw top test tube and one ml of sterile distilled water is added. The test tube is then boiled for ten minutes and allowed to cool. The tube is inoculated with a thick suspension of bacterial growth. Incubation is for one to four days with readings being made daily. Carbohydrate oxidation is revealed as a color change from red to yellow.

Gelatin Hydrolysis

A single gelatin strip is placed in a 16 X 100 mm screw top test tube containing one ml of sterile distilled water. The test tube is then inoculated with a heavy suspension of bacteria and incubated for 24-72 hours. Proteolysis is indicated by the digestion of the gelatin layer and the exposure of the blue plastic supporting base.

Gluconate

A gluconate substrate tablet is placed into a 16 X 100 mm screw top test tube containing one ml of sterile distilled water. The tube is then heavily inoculated with bacteria. The test tube is then incubated for 18 to 24 hours. After incubation a test for reducing sugars is carried out. A Clinitest Reagent Tablet (Ames Company, Elkhart, Indiana) is dropped into the test tube and allowed to react with the contents. During the reaction and for 15 seconds thereafter the test tube should not be shaken. A positive test for reducing sugars is the appearance of a green, orange or brick-red color. Color changes developing after the 15 second waiting period should be disregarded.

Growth at 42C

Inoculate a 16 x 125 mm screw top test tube of a tryptic soy agar slant with the bacteria to be tested. Incubate the slant in a 42C ($\pm$ 0.5C) water bath for 24 hours, and observe for growth.

Growth on SS Agar

Salmonella-Shigella Agar is prepared by suspending 60 grams of dehydrated media in 1000 ml of distilled water. The solution is heated to boiling and without autoclaving poured into 15 X 100 mm petri plates. The plates are inoculated by streaking with a loop and incubated for 24 hours.

Indole

Five ml aliquot of 1.0% solution of tryptone broth in distilled water is dispensed into 16 X 125 mm screw top test tubes, autoclaved at 121C for 15 minutes, inoculated and incubated for 24 hours. A half ml of Kovacs' Reagent (para-dimethylaminobenzaldehyde 5 g, plus 75 ml isoamyl alcohol, in 25 ml concentrated hydrochloric acid) is added and the tube is gently shaken. The appearance within one minute of a red color at the surface indicates a positive test for indole production.

Oxi/Ferm Tube

A single inoculating needle runs the length of the plastic tube piercing through the center of each of the eight compartments which make up the Oxi/Ferm tube. The compartment labeled 'H_2S/Indole' allows for the simultaneous testing of both of these characteristics. Indole production, however, was determined through separate testing as indicated above. The plastic cap covering the inoculating needle is unscrewed, the needle drawn out of the tube, and introduced into the stock culture slant to obtain a large (visible) inoculum and then, with a twisting motion, re-inserted through all eight compartments. The needle is then pulled back so that its tip is seen in the H_2S/Indole compartment. The portion of the needle extending outside the tube is broken off and discarded into a disinfectant jar. The plastic cap is replaced and aerobic conditions created in the xylose, aerobic-dextrose,

urea, and citrate compartments by removing a strip of tape which covers air holes in these compartments. The Oxi/Ferm tube is then incubated for 48 hours.

After incubation results are determined for most tests by the observation of a color change or lack of a color change in the test compartments. Positive tests for the fermentation of anaerobic-dextrose, oxidative attack of xylose and aerobic-dextrose are seen as a color change in these compartments from green to yellow, and for arginine dihydrolase as a change from green to purpole. Nitrogen gas production is seen as a separation of the wax overlay from the agar surface in the compartment labeled N_2 gas. The H_2S/Indole compartment is observed for the formation of a black precipitate which indicates hydrogen sulfide production. Detection of urease is accomplished by the observation of a color change in the compartment labeled urea from yellow to red-purple. Organisms capable of utilizing sodium citrate as a sole source of carbon turn the green citrate compartment deep blue.

The above tests along with the results of an oxidase test have been processed with a computer using binary code logic developed by Roche Diagnostics. This results in identification of a bacterial species according to a code number which is determined by the outcome of the various tests. The tests are grouped and within each group each test is given a different value. The value of the positive tests within each group are summed and a four-digit 'I.D. Value'

is obtained. The I.D. Value in the code booklet identifies the organism to the generic, specific or "group" level.

Oxidase

To run the test, isolated bacteria from a plate or slant are picked up with a sterile loop and rubbed into the test strip (Patho Tec). A positive test will turn the rubbed area on the test strip blue within 30 seconds. The absence of a blue color is a negative test.

IX. LITERATURE CITED

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