The isolation and characterization of *Klebsiella pneumoniae* from animals and their environment

by

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INTRODUCTION

Infections caused by Klebsiella pneumoniae in domestic animals and man are more frequently diagnosed and therefore, more important in recent years. Also the appearance of strains which are resistant to multiple chemotherapeutic agents has created serious problems in the treatment of such infections and has emphasized the importance of controlling the spread of this organism.

Relatively little research has been done on the ecology, epizootiology, and mechanisms of infection in Klebsiella-caused diseases. Information in veterinary literature on these aspects has been based largely on observations of natural outbreaks. Much of the knowledge available has come through investigations of human diseases associated with this organism.

The taxonomy of the Klebsiella-Aerobacter portion of the family Enterobacteriaceae has been quite clear, but from the nomenclatural standpoint, confusion has existed in this area. That confusion largely was due to the fact that cultures isolated from soil, water, sewage, and animals, which could not fulfill the characteristics of Klebsiella pneumoniae by biochemical or serological methods, usually were classified as Aerobacter aerogenes. Consequently there are many reports in which the authors describe Aerobacter infections, but according to more recent classifications probably would have been reported as Klebsiella infections.

The present investigation was undertaken with the object in view of finding the distribution of the organism among some of the domestic animals and their environment, the determination of biochemical, antibiotic

sensitivity, and virulence characteristics of the strains isolated. A search was made for differences among strains isolated from sources which were not contacted by domestic animals.

The great variation in habitat would in itself suggest some differences in the organism. So it would not be unexpected that an organism living in the extremely variable animal environment, in soil, and on plants would develop noticeable differences from those organisms living in the animal body.

REVIEW OF LITERATURE

Taxonomy

Friedländer (33), in 1882, first called attention to the presence of capsulated bacilli in pneumonia. A number of observers (17)(43) since have described organisms more or less similar in form which have been found in a variety of lesions.

From a nomenclatural standpoint, confusion has existed in the division of the Klebsiella-Aerobacter group. The extensive studies of Orskov (62), Kauffman (49), Edwards and Fife (25), Cowan et al. (16), Slopek and Durlakowa (79), and Ewing (29) have resulted in a complete reorganization of this group of organisms. The genus Klebsiella (Trevisan) remains the same, and those nonmotile, encapsulated strains which were formerly classified with Aerobacter aerogenes have been placed with Klebsiella pneumoniae. The motile, nonencapsulated strains of Aerobacter aerogenes have been assigned to a new genus, Enterobacter (Hormaeche and Edwards) as Enterobacter aerogenes (Kruse). The genus Aerobacter has been abolished. All Klebsiella strains not belonging to K. ozaenae or K. rhinoscleromatis are considered to be K. pneumoniae which agrees with Ewing's (29) ideas on the classification of the group.

Klebsiella pneumoniae can be subdivided into many biotypes. On the basis of the fermentation of dulcitol, sorbose, adonitol, and inositol, urease production, utilization of salts of organic acids (d-tartrate and sodium citrate), indol production, and liquefaction of gelatin, Orskov (64) subdivided 226 K. pneumoniae strains into 32 biotypes, if indol production and/or liquefaction of gelatin were omitted, 23 biotypes were

found.

Klebsiella infections in animals

Klebsiella pneumoniae is responsible for a variety of disease in animals. Horses, cattle, swine, cats, and dogs are among the domestic animals more commonly affected. Mice, guinea pigs, and monkeys are the laboratory animals in which the occurrence is more frequently reported.

There are some descriptions in the older veterinary literature in which the authors describe infections as caused by Bacillus lactis aerogenes, Encapsulatus genitalium, Klebsiella genitalium, and Bacterium friedländeri, when they isolated a nonmotile, encapsulated, gram-negative organism. However, in retrospect, they in general fulfill the cultural and biochemical characteristics of Klebsiella pneumoniae and they will be considered as such in this report.

Gilruth and MacDonald (34), in 1911, described an outbreak of mastitis due to Bacillus lactis aerogenes in a herd of 40 cows. About 30 of 40 cows being milked developed a disease condition in one or more quarters of the udder. Specimens of the secretion from two cases were cultured. A short, ovoid, nonmotile, gram-negative bacillus with the cultural characteristics of B. lactis aerogenes was isolated. Jones (47), in 1918, studied three cases of mastitis associated with coliform organisms. He reported the occurrence in a cow, 4 months after calving, an infection of one quarter with B. lactis aerogenes that persisted throughout the lactation period.

Laigret and Leblois (51), in 1950, reported the recovery of Friedländer's bacilli from an acute case of mastitis involving the right hind quarter of a cow. An unspecific coccus was also present. The condition

was characterized by gross edema, a temperature of 105.8F, and systemic disturbance. Buntain and Field (12), in 1953, described an outbreak of clinical mastitis due to infection with an organism of the Friedländer group. The disease was of chronic nature and caused the production of grossly abnormal milk. Of the 38 cows in the milking herd, 29 became infected in one or more quarters. Calves fed on herd milk developed a severe respiratory infection and four calves died within a period of 5 weeks. There was a high incidence of tuberculosis in the herd and the possible relationship between that disease and susceptibility to the Friedländer organisms was suggested.

Barnes (3), in 1954, reported four cases of acute bovine mastitis from which Klebsiella pneumoniae was recovered in pure culture from the inflamed quarters. The five strains isolated were: K. pneumoniae type 4; K. pneumoniae type 16; and K. pneumoniae, related to but not identical with types 17 and 19. He concluded that the strains were probably of different origin. Hinze (41), in 1956, reported clinical observations of an unusual outbreak of mastitis caused by Klebsiella pneumoniae in a large dairy herd of Holsteins. During the initial outbreak in 1952, mastitis spread in a rather uniform manner, and then it appeared sporadically in various parts of the farm. The organism was routinely isolated from apparently normal udders, and virulence tended to fluctuate with most acute cases occurring in the summer months. He also mentioned that the organism was shed intermittently from individual cows and for some undetermined reason, it occasionally became virulent enough to cause a severe case of mastitis. In his report he attributed over 50% of all mastitis in the

herd to K. pneumoniae. Easterbrooks and Plastridge (21), in 1956, reviewed five selected field cases of acute Klebsiella mastitis and five experimental case histories, accumulated over a period of 10 years. In one of the field cases the inflammation disappeared, but the organism persisted for a long period of time. Klebsiella type 31 and Klebsiella related to types 33 and 35 were isolated, and they established that acute Klebsiella mastitis is not caused by a single serotype of the organism.

White (91), in 1957, described an outbreak of mastitis in a dairy herd comprised largely of Ayrshires and Friesians. Organisms of the Klebsiella group were responsible for four cases of mastitis. The infection spread to three groups of calves. Transmission to two groups occurred by a route other than feeding milk from infected cows, however, the third group was fed with milk which included some milk from an infected animal. Cultures from nasal swabs of the calves gave a pure culture of a gram-negative, capsulated rod. The disease caused two deaths among calves from which Klebsiella organisms were recovered from the lungs. Radostits (73), in 1961, reported the incidence of 978 cases of mastitis treated during a 3 year period, of which 80 (8.2%) were due to coliform bacteria. Fifty-eight of the cases were encountered during the stabling periods (October 1 to May 31) and the remaining 22 were seen in the summer periods when the cows were at pasture. Milk samples from all 80 cases of coliform mastitis studied were submitted for culture. Klebsiella spp. were isolated from nine cases and all were capsulated. In Radostits' opinion "the sporadic occurrence of this disease suggests that these saprophytes occasionally become pathogenic, although they are relatively harmless most of the

time." McDonald et al. (59) reported the isolation of 70 cultures of gram-negative, aerobic rods over a period of 8 years from bovine udders in a herd of 20 dairy cows maintained for mastitis research. Most of the isolates corresponded to E. coli (45.7%). Klebsiella pneumoniae was isolated on nine occasions. Since 84.3% of all infections occurred in rear quarters the authors suggest that the discharges from the urogenital and digestive tracts may be a source of the microorganisms.

The first report of infection in the horse by Klebsiella organisms was made by Dimock and Snyder (18) in 1924. They described the isolation of an encapsulated bacillus from a number of cases of metritis. To this organism they gave the name Encapsulatus genitalium. Dimock and Edwards (19), in 1927, investigated the pathology and bacteriology of the reproductive organs of mares and reported the isolation of 62 cultures of Encapsulatus genitalium from 1,424 cultures made. They also stated that Encapsulatus genitalium was morphologically and culturally indistinguishable from Friedländer's bacillus. Edwards (23), in 1928, found Encapsulatus genitalium in approximately 5% of the total number of barren mares examined and studied the systematic relationship of the capsulated bacilli recovered from mares to encapsulated bacilli from other sources, especially Encapsulatus pneumoniae of Friedländer.

Bruner (10), in 1951, stated that at least eight species of bacteria have been shown to produce genital infections which often lead to abortion in the mare. Of these, Klebsiella pneumoniae sometimes invades the genital tract and once established is readily transmitted from diseased to healthy mares. Peck (67), in 1952, reported that infertility in mares is

the most important of breeding problems and infections of the genital tract usually are responsible in over 50% of barren mares. The organisms responsible are primarily beta-hemolytic streptococci, Klebsiella genitalium and E. coli. Collins (15), in 1964, studied the incidence of cervical and uterine infection in mares. He sometimes encountered growth of the Klebsiella type on primary cultures and labeled such organisms as "coliform organisms resembling Klebsiella", and reported 327 coliform type organisms from 1,015 infections. The report of Lord Porchester's Veterinary Committee (76) in 1965 contains the results of the examination of 3,705 mares of which 694 gave positive cultures of pathogenic organisms, including 49 which were identified as Klebsiella pneumoniae. No evidence came from the survey to substantiate the belief that infection with K. pneumoniae constitutes a venereal infection in the horse. No evidence was available to show that the organism may be passed mechanically by a stallion from one mare to another.

Bain (2), in 1966, stated that the uterine infection in mares is assuming greater importance. In a series of 570 cultures from the genital tract of mares, K. pneumoniae was recovered in 26 cases out of a total of 342 isolations of pathogenic organisms. In one instance, a 5-day-old foal died and on post-mortem, peritonitis and septicemia due to K. pneumoniae were diagnosed. Berthelon and Rampin (5a), in 1970, stated that after one or several coituses with infected mares, the stallion will not transmit infection to a healthy mare covered 12 or 36 hours later. The common genital infections due to Klebsiella and Streptococcus are not transmissible by coitus. Scott et al. (78), in 1971, made a study of the aerobic

bacterial flora of the genitalia of 100 mares of unknown breeding history and reported the isolation of K. pneumoniae in eight cases.

Klebsiella pneumoniae has not been considered an important pig pathogen and its distribution in the swine population is not known. Sutherland (83), in 1942, reported the death of seven young pigs from two sows recently purchased. From a dead pig submitted for examination, pure cultures of the Bacterium friedländeri was isolated from heart's blood and the lungs. Easterbrooks and Plastridge (21), in 1956, observed Klebsiella organisms as the cause of acute mastitis in swine.

Jamkhedkar et al. (46), in 1964, reported infectious mastitis due to Klebsiella pneumoniae in four sows. K. pneumoniae was isolated from nine of 18 mammary glands affected bacteriologically. One of the sows died and the other three were treated successfully. Lake and Jones (52), in 1970, during an investigation of post-parturient disease associated with lactational failure in sows, observed four incidents in which Klebsiella was considered to be the immediate cause of disease. The first was an acute generalized infection and the others were cases of acute mastitis. Klebsiella type 31 was isolated in profuse and pure growth from the lungs, spleen, and mammary gland from the animal which died with the acute generalized infection. Klebsiella capsular types 18, 41, and 28 were isolated from the three acute mastitis cases. Among the litters of affected sows there was a 90% mortality rate, and Klebsiella was isolated from rectal swabs taken from piglets of affected sows. There are reports by Adler (1), Langham and Stockton (53), and Helmboldt et al. (38) who described certain swine diseases associated with Aerobacter aerogenes. It can be noted that the characteristics of those organisms, in so far as they were

reported, are the same as those of Klebsiella pneumoniae.

Diseases caused by Klebsiella pneumoniae in sheep are not of common occurrence. Hindmarsh et al. (40), in 1931, reported K. pneumoniae as the cause of infectious pneumonia.

In household pets, infections by K. pneumoniae are present with relative frequency and mostly associated with urogenital infections (65). Gray (36), in 1942, reported K. pneumoniae as cause of canine cystitis, and Ludford and Stevens (55), in 1958, reported the isolation of K. pneumoniae from a case of canine pneumonia.

Laboratory animals are frequently infected with K. pneumoniae in the form of respiratory disease. Webster (88)(89) reported two cases of explosive and fatal epidemics of respiratory infections in mice. Perkins (68), in 1899, reported a laboratory epizootic among guinea pigs associated with gaseous emphysema. Branch (7), in 1927, reported an epidemic which occurred in a colony of about 400 guinea pigs in which the portal of entry appeared to be the upper respiratory tract. Hunt et al. (44), in 1968, reported an acute Klebsiella infection in a rhesus monkey colony in which ten animals died. Snyder et al. (82), in 1970, reported infection by K. pneumoniae in a colony of monkeys in which septicemia was the predominant pathologic finding.

Klebsiella in foods

There are only two reports in which K. pneumoniae has been incriminated as a cause of food poisoning outbreaks. Horváth et al. (42), in 1964, reported a food-borne outbreak in which 190 persons were affected. Ormay and Novotny (61), in 1970, reported K. pneumoniae as one of the

pathogens incriminated in food poisoning outbreaks in Hungary.

Klebsiella in soil and water

It is generally recognized that Klebsiella pneumoniae is widespread in soil and water, but there are no reports available describing the prevalence and characteristics of the organisms found. Edwards (23), in 1928, reported the isolation of some virulent types from soil and water.

Biochemical identification

Orskov (64) on the basis of reaction on adonitol, dulcitol, inositol, sorbose, indol, gelatin, urea, and salts of organic acids (d-tartrate, sodium citrate and mucate) divided the genus Klebsiella into 36 biochemical types. Cowan et al. (16) divided Klebsiella strains into six categories based on the following tests: presence of fimbriae; gas from glucose at 37C; lactose and dulcitol fermentation; methyl red; acetoin production; citrate; urease; gluconate; malonate; lysine decarboxylase; growth in KCN; and ability to produce gas from lactose at 44C. Edwards and Ewing (26) found differences in the biochemical reactions of types 1, 2, and 4 which detracted from the uniformity of reactions when all Klebsiella are grouped together.

Eitckhoff et al. (28) used a battery of 24 biochemical tests for identification of Klebsiella organisms, and found that the following tests were useful in the precise identification: indol; methyl red; Voges-Proskauer; citrate; motility; urease; phenylalanine; lysine decarboxylase; arginine dehydrolase; ornithine decarboxylase; and the production of gas from glucose, adonitol, dulcitol, cellobiose, inositol, and glycine. Kauffman (50) established biochemical types in the genus Klebsiella, and

found that types 1:3 and 1:10 ferment dulcitol, whereas the other types were unable to attack this alcohol. The types 8, 9, and 10 may also be differentiated by using dulcitol and sodium citrate. He stated that K. rhinoscleromatis occupies a special position as it is unable to decompose urea and organic acids. Slopek and Durlakowa (79) differentiated Klebsiella bacilli into six species based primarily on the following characteristics and tests: presence of fimbriae; glucose (gas); lactose fermentation; indol; methyl red; Voges-Proskauer; citrate; urease; malonate; and lysine decarboxylase. Dulcitol fermentation and growth in KCN proved to be of little differential value.

Durlakowa et al. (20) used 29 biochemical properties for differentiation of Klebsiella members, and established 16 biochemical types (biotypes) based primarily in the following reactions: glucose (gas); lactose; dulcitol and sucrose fermentation; indol; methyl red; Voges-Proskauer; and citrate utilization. Ewing (30) advocated the use of the following biochemical characteristics and tests for differentiation among members of the genus Klebsiella: gas from glucose; lactose and dulcitol fermentation; urease; methyl red; Voges-Proskauer; citrate; salts of organic acids (d-tartrate, sodium citrate); malonate; mucate; and lysine decarboxylase.

Serological characterization

Toenniessen (85)(86) was the first to demonstrate that two distinct antigens were present in capsulated Klebsiella cultures, one in the capsule and the other in the soma of the bacteria. Julianelle (48) established three serologic types among Klebsiella cultures, A, B, and C.

Goslings and Snijders (35) extended the number of capsular types to six by the addition of types D, E, and F. Kauffman (49) described eight new capsular types, redesignated the types previously established and published the first antigenic classification of the Klebsiella group based on the recognition of somatic "O" and capsular "K" antigens. Three "O" groups corresponding to three "O" antigens were established and 14 "K" antigens were recognized. Based on the presence of these antigens the organisms were divided into 18 types. Worfel and Ferguson (92), Brooke (9), Edwards and Fife (24), Edmunds (22), and Slopek and Durlakowa (79) have brought the number of known "K" antigens to 80, and the "O" antigens to 11. Since the number of "O" antigens is small compared with the "K" antigens and their determination is difficult because of the heat-stable "K" antigens, the serological typing is primarily based on the determination of "K" antigens by the Quellung reaction (24)(28).

A group-specific precipitating antigen present in almost all Klebsiella strains has been reported by Pickett and Cabelli (69).

Bacteriophage typing

Isolation of phages acting on various Klebsiella strains has been reported by several authors: Caublot (13b); Hadley (37); Prasek and Prika (70); Rakieten et al. (74); Park (66); Milch and Deák (60); and Przondo-Hessek and Slopek (71)(72). More recently Slopek et al. (81) established a scheme for typing Klebsiella by using 15 selected phages from the Hungarian and Polish collections. The proposed set of 15 phages permits typing about 66% of strains of the Klebsiella genus.

Pneumocin typing

Maresz-Babczyszyn et al. (56) isolated 49 bacteriocins produced by Klebsiella strains and classified them into six groups. Slopek and Maresz-Babczyszyn (80) proposed a scheme for typing Klebsiella by means of pneumocins.

Antibiotic susceptibility

There are several reports concerned with the susceptibility of Klebsiella to antibiotics, and primarily refer to strains isolated from human beings (27)(28)(75)(87)(93). While the methods employed for testing were not uniform, there is general agreement that Klebsiella pneumoniae is susceptible to cephalothin, cephaloridine, gentamicin, kanamycin, polymyxin-B; less susceptible to chloramphenicol, streptomycin, and tetracycline; and resistant to ampicillin and penicillin (4)(11)(39)(54)(84).

Pathogenicity in laboratory animals

In general the capsulated strains are considered to be pathogenic to laboratory animals. Mice are quite susceptible to intraperitoneal inoculation (3)(12)(55); guinea pigs less so (34)(43)(83); and rabbits are considered to be somewhat resistant although some strains are able to kill them (17)(34).

MATERIALS AND METHODS

The following culture media were used in the study. All media were prepared following the procedures indicated in the references.

Enrichment media:

- Bacto enterobacteriaceae enrichment broth Mossel[1]

Selective media:

- Bacto Levine eosin methyl blue agar[1]
- Bacto Tergitol-7 agar[1] plus 0.1 g of triphenyltetrazolium (TTC) per 1,000 ml of medium, added after sterilization

Differential media:

- Carbohydrate fermentation media[2]
- Bacto decarboxylase base Moeller[1]
- Peptone broth[1]
- Methyl red - Voges-Proskauer broth[1]
- Phenylalanine agar[1]
- Nitrate agar[1]
- Urea broth[1]
- Simmons' citrate agar[1]
- Nutrient gelatin[1]
- Triple sugar iron agar[1]
- Kligler iron agar[1]

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[1]Difco Laboratories, Detroit, Michigan.

[2]Source: (5b).

Antibiotic sensitivity medium:

- Mueller-Hinton agar[1]

Other media:

- Brain heart infusion[1]
- Blood agar 5% bovine erythrocytes[1]
- Worfel-Ferguson broth[1]

Collection of samples

Samples were collected using sterile cotton swabs. Surface areas were swabbed with the swabs previously moistened in the enrichment broth (EE Mossel broth). Nasal secretions were collected after cleaning well the area around the nasal orifices. Feces were collected directly from the rectum. The swab heads were deposited in the vials containing the enrichment broth. The tubes with the samples were properly identified and taken to the laboratory immediately after collection.

Soil samples were collected in sterile plastic bags with the aid of sterile wood tongue blades. The samples were properly identified and taken to the laboratory the same day as collected.

Processing of soil samples

Samples were processed the same day they were collected. A 50-gram portion of each sample was processed. Each portion was transferred to a wide-mouth flask with the aid of a sterile wood tongue blade. Sterile 0.1 M phosphate buffer solution was added to make a total volume of approximately 100 ml. The contents were shaken by hand and then mechanically

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[1]Difco Laboratories, Detroit, Michigan.

shaken for 30 minutes at moderate speed. The flasks were allowed to stand about 15 minutes or long enough to permit the sedimentation of the heavier soil particles. Ten ml of the supernatant fluids were transferred to 10 ml of double-strength enrichment broth.

Culture procedure

The tubes of the enrichment broth inoculated with the samples were thoroughly shaken and then incubated for 18 to 24 hours at 37C. A loopful from the enrichment broth was then inoculated on EMB agar and Tergitol-7 with TTC (triphenyltetrazolium chloride). The plates were incubated for 24 hours. The enrichment broth cultures were incubated for 18 to 24 additional hours and a second inoculation on selective media was made if the culture gave negative results at 24 hours.

The growth of organisms was examined on the streaked plates. Isolated colonies resembling those of suspected to be Klebsiella were inoculated on Kligler iron agar by stabbing to the base of the butt and by streaking the slant. Klebsiella-like colonies growing confluently with other colonies were picked up and streaked on EMB agar or Tergitol-7 with TTC for further purification, and then individual colonies were inoculated on Kligler iron agar.

The Kligler iron agar tubes were incubated at 37C for 18 to 24 hours. Tubes which exhibited an acid butt and slant without H_2S production were selected and inoculated into tryptose broth. The tryptose broth tubes were incubated 4 to 8 hours at 37C and a motility test using the hanging-drop method was performed. Motile cultures were excluded from consideration as Klebsiella.

Biochemical reactions

The growth in the tryptose broth used to perform the motility test was used as inoculum for the biochemical reactions. Each of the following media received two drops of the culture delivered by means of a sterile 1 ml pipette:

Dextrose	Xylose
Lactose	Cellobiose
Sucrose	Salicin
Dulcitol	Esculin
Sorbitol	Peptone broth
Mannitol	MR-VP broth (two tubes)
Adonitol	Phenylalanine agar
Inositol	Nitrate agar
Raffinose	Tryptose agar slants (two tubes)
Arabinose	

All the above tubes were incubated at 37C for 24 hours with the exception of the peptone broth and MR-VP broths. The sugar tubes showing no change at 24 hours were incubated for an additional 15 days and were checked daily for any change in their reaction.

Indol test

The test for indol production was performed after 40 to 48 hours of incubation of the peptone broth at 37C. About 0.5 ml of Kovac's reagent was added and the tube was shaken gently. A deep red color developed in the presence of indol.

Methyl red test

A tube of MR-VP broth was incubated at 37C for no less than 48 hours. Five to six drops of the test reagent (methyl red 0.1 g; ethyl alcohol 95-96% 300 ml) were added to the culture. The reaction was read immediately. A bright red color indicated a positive reaction and a negative reaction was shown by yellow color. An orange red color was indicative of a weakly positive test, and the test was repeated with cultures that had been incubated for 4 to 5 days.

Voges-Proskauer test

A second tube of MR-VP broth was incubated for 48 hours. The test reagent (O'Meara, modified) consisted of potassium hydroxide 40 g, creatine 0.3 g, and distilled water 100 ml. One ml of the reagent was added to 1 ml of the culture. The test was placed at room temperature and final readings taken at 4 hours. Development of a pink or red color indicated a positive reaction.

Phenylalanine deaminase

Phenylalanine agar slants were incubated at 37C for 24 hours. Four drops of a 10% w/v solution of ferric chloride was allowed to run over the growth on the slants. The presence of phenylpyruvic acid was indicated by development of a green color in the syneresis fluid and in the slant.

Nitrate reduction

Tubes of nitrate agar were incubated at 37C for 24 hours. The test reagent consisted of a sulfanilic acid reagent (5 g of sulfanilic acid in 1,000 ml 5 N acetic acid) and ∝-naphthylamine reagent (5 g of ∝-naphthylamine dissolved in 1,000 ml 5 N acetic acid). A few drops of each reagent

were put into the tube to be tested. A distinct pink or red color indicates the presence of reduction of nitrate to nitrite.

Citrate utilization

Utilization of citrate as the sole source of carbon was determined by growing the cultures at 37C in Simmons' citrate agar. The agar slope was inoculated with a straight wire inoculating needle from the culture in tryptose broth. Observations and records of growth were made for 4 days. Colony formation usually accompanied by a color change was indicative of growth.

Gelatin liquefaction

Liquefaction of gelatin was determined by making a stab inoculation of the culture into nutrient gelatin. The cultures were incubated at 20C for 30 days. Cultures were considered positive if upon inversion of the inoculated culture tube liquefaction was observed.

Hydrogen sulphide production

The production of hydrogen sulphide was determined by stabbing the butt and streaking the slant of TSI agar. The medium was incubated at 37C for 7 days. Production of H_2S was shown by blackening of the medium.

Lead acetate paper strip test for H_2S was used to serve as checks when the test on TSI agar gave suspicious results, showed by the development of a dark brown color.

Urease production

The test for urease production was determined by inoculation of Stuart, Van Stratum, and Rustigan urea broth with a loop from 18 to 24 hour tryptose agar slant culture. The tubes were incubated at 37C. The

results were recorded daily for 4 days. Urease positive cultures produced an alkaline reaction in the medium evidenced by a red color.

Decarboxylation of amino acids

Tubes containing 3 to 4 ml amounts of each amino acid (L-lysine, L-arginine, and L-ornithine) in screw-cap tubes were inoculated lightly from a culture on tryptose agar, and 4 to 5 mm layer of sterile mineral oil was added. The tubes were incubated at 37C for 4 days, examining daily. Positive reactions were indicated by alkalinization of the media and a consequent change in the color of the indicator system from yellow to violet or reddish-violet.

Demonstration of capsule

Production of a capsule was determined by growing the bacteria in Worfel-Ferguson broth. The broth was incubated for 4 to 6 hours at 37C. A drop of the material to be tested was placed on a slide, near it was placed a drop of Pelikan India ink. Using a sterile loop the India ink and the organism suspension were mixed. The mixture was covered with a cover slip and examined with an oil-immersion objective after 10 to 15 minutes. The capsular material appeared as clear zone surrounding the organism.

The cultures which in general reacted as follows were identified as Klebsiella pneumoniae:

Dextrose - Fermented, usually with gas production

Lactose - Fermented

Sucrose - Fermented

Dulcitol - Fermented or not fermented

Sorbitol - Fermented

Mannitol - Fermented

Adonitol - Usually fermented

Inositol - Fermented

Raffinose - Fermented

Arabinose - Fermented

Xylose - Fermented

Cellobiose - Fermented

Salicin - Fermented

Esculin - Positive

Arginine - Negative

Lysine - Positive

Ornithine - Negative

Indol - Negative

Voges-Proskauer - Positive

Methyl red - Negative

Simmons' citrate - Positive

Urease - Positive

Phenylalanine - Negative

Nitrate - Positive

Gelatin - Negative

Hydrogen sulphide - Negative

Motility - Negative

Capsule - Usually present

Antimicrobial susceptibility

Except for a few stock laboratory strains, all bacteria tested were fresh isolates. The studies were carried out by the standardized single disk method (14). The antimicrobial agents employed and their concentrations are shown in table 1.

A few colonies (3 to 4) of the organism to be tested were picked with a wire loop from a culture plate in which the purity was checked, and introduced into a tube containing 4 ml of trypticase soy broth. The tube was then incubated for 2 to 8 hours at 37C to produce a bacterial suspension of moderate cloudiness. The suspension was diluted with saline solution when it was considered necessary, to a density visually equivalent to that of standard prepared by adding 0.5 ml of 1% $BaCl_2$ to 99.5 ml of 1% H_2SO_4 (0.36 N). For the sensitivity plates, plastic petri dishes[1] (100 x 15 mm) were used with Mueller-Hinton agar (4 to 5 mm in depth). Plates were dried for about 30 minutes before inoculation and were used within 4 days of preparation.

The bacterial suspension was streaked evenly in three directions onto the surface of the medium with a cotton swab. Surplus suspension was removed from the swab before the plates were seeded by rotating it against the upper inside wall of the tube.

After the inoculum had dried for about 5 minutes, but not more than 30 minutes, the disks were placed on the agar by a single disk dispenser, so that centers were at least 24 mm apart. Penicillin, cephalosporin,

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[1]Falcon Plastics, Los Angeles, California.

cephaloglycin, and cephalothin disks were placed so that their centers were at least 30 mm apart from the other antibiotic disks. After disks had been placed on the agar they were pressed down with sterile forceps to make complete contact with the surface. The plates were immediately incubated at 37C for 14 to 18 hours.

After incubation, the zone diameters were measured with a ruler on the undersurface of the petri dish. The zone diameters were recorded and interpreted according to table 1.

Standard control organisms, Staphylococcus aureus ATCC 25923 and Escherichia coli ATCC 25922, were employed to check the activity of the disks from each cartridge and on the reproducibility of the test.

Pathogenicity for mice

The pathogenicity of 12 selected strains for mice was determined. Except for "Aerobacter aerogenes" Schalm 1236 and C-59, all bacteria tested were recently isolated and selected randomly. The source of the strains is shown in table 2.

Two different procedures were used for the preparation of the inoculum:

Method "A" (82) Six-hour culture grown in Worfel-Ferguson broth was centrifuged at 2,000 rpm for 15 minutes and the sedimented bacteria washed with isotonic saline and centrifuged again. Washing was repeated twice in order to remove soluble capsular polysaccharide and endotoxin that may have accumulated in the medium. Washed bacteria were resuspended to the original volume.

Table 1. Antimicrobial agents - zone size interpretative chart[a]

Antimicrobial agent	Disk potency	Inhibition zone diameter in mm		
		Resistant	Intermediate	Sensitive
Ampicillin[b]	10 mcg	11 or less	12-13	14 or more
Bacitracin[b]	10 U	8 or less	9-12	13 or more
Carbenicillin[b]	50 mcg	17 or less	18-22	23 or more
Cephaloglycin[b]	30 mcg	14 or less	-	15 or more
Cephaloridine[b]	30 mcg	11 or less	12-15	16 or more
Cephalothin[b]	30 mcg	14 or less	15-17	18 or more
Chloramphenicol[b]	30 mcg	12 or less	13-17	18 or more
Gentamicin[b]	10 mcg	12 or less	-	13 or more
Kanamycin[b]	30 mcg	13 or less	14-17	18 or more
Neomycin[b]	30 mcg	12 or less	13-16	17 or more
Nitrofurantoin[b]	300 mcg	14 or less	15-16	17 or more
Penicillin-G[b]	10 U	11 or less	12-21	22 or more
Polymyxin-B[b]	300 U	8 or less	9-11	12 or more
Streptomycin[b]	10 mcg	11 or less	12-14	15 or more
Triple sulfa[c]	250 mcg	12 or less	13-16	17 or more
Tetracycline[b]	30 mcg	14 or less	15-18	19 or more

[a]Food and Drug Administration, Committee on Anti-infective Drugs (14).

[b]Difco Laboratories, Detroit, Michigan.

[c]Baltimore Biological Laboratories, Baltimore, Maryland.

Table 2. Cultures used for pathogenicity test

Strain	Original source
"Aerobacter aerogenes" Schalm 1236[a]	Cow, mastitis
C-59[a]	Cow, mastitis
531[b]	Cat, urogenital infection
526[b]	Stallion, urethral infection
520[b]	Cow, mastitis
7	Cow, nasal secretion
119	Horse barn, drinking water
187	Swine, nasal secretion
175	Swine barn, drinking water
223	Sheep barn, manger
248	Public park, soil
266	Corn farm, soil

[a]Provided by Dr. John McDonald from the National Animal Disease Laboratory, Ames, Iowa. Classification of strain 1236 as Aerobacter aerogenes is uncertain. It appears identical with strain 2414-2 isolated by Carroll (13a).

[b]Provided by Dr. R. Allen Packer from the Veterinary College, Iowa State University, Ames, Iowa.

Method "B" (3)(12) Twenty-four hour cultures were grown in brain heart infusion broth without further treatment.

A group of three young white mice fed with a diet free of antibiotics was inoculated with 0.1 ml from each different suspension. The animals were observed daily for symptoms and death and the results were recorded. All dead mice were necropsied and cultures on EMB agar and blood agar were made in order to recover the organism.

RESULTS

Three hundred and fifty-nine samples from animals and their environment, and 33 samples from soil were examined for the presence of Klebsiella pneumoniae. A total of 45.1% and 63.3%, respectively, gave positive cultures of the organism. The percent of positive cultures by samples, farms, and animal species are tabulated in tables 3 and 4, which show that the organism is more prevalent in drinking water than in any other source examined, in swine environment than in those of other species, and in soil from farms without livestock than in soil from public parks.

Results of the differential tests used for the identification of 275 K. pneumoniae strains are summarized in table 5. Some variations of the K. pneumoniae pattern for the set of differential tests used were shown by strains isolated from beef cattle (mannitol and arginine), horse (indol), sheep (ornithine and H_2S), and public parks (indol and H_2S).

In vitro antibiotic sensitivity was determined for 107 strains isolated from animals and their environment, 28 strains from soil, and 20 strains from animal infections (tables 6, 7, and 8). Most of the strains show resistance to ampicillin, bacitracin, carbenicillin, and penicillin G; variable resistance to nitrofurantoin, streptomycin, triple sulfa, and tetracycline; and susceptibility to cephaloglycin, cephaloridine, cephalothin, chloramphenicol, gentamicin, kanamycin, neomycin, and polymyxin-B. Strains isolated from swine show more resistance to the set of antibiotics used (10 of 16) followed by dairy cattle (8 of 16), horse (7 of 16), sheep (6 of 16), and beef cattle (4 of 16), table 6. Those isolated from farms

without livestock (6 of 16) show more resistance than the strains from public parks (4 of 16), table 7. Isolates from infections in dogs and cats (9 of 16) appear to be more resistant than to those from horses (8 of 16) and dairy cattle (7 of 16), table 8. Comparison of isolates from different sources show that strains isolated from animal infections apparently are more resistant (10 of 16) than those from farms with livestock (9 of 16) and soil from public parks and farms without livestock (6 of 16), table 9.

The standard control organisms, Staphylococcus aureus ATCC 25923 and Escherichia coli ATCC 25922, were within the acceptable limits for the antimicrobial agents used.

Pathogenicity as determined by lethal effect shows that the whole culture (method B) is more virulent than washed cells (method A) when used as inoculum. Recently isolated strains from animal diseases show a high degree of cirulence. Isolates from healthy animals and their environment, and from places without livestock, show a variable degree of virulence for white mice, table 10.

Table 3. Number of samples collected from animals and their environment, and number of isolates of *Klebsiella pneumoniae* obtained

Samples		Source								
		Dairy cattle			Beef cattle			Horse		
		A	B	C	D	E	F	G	H	I
Nasal secretion	Exam.	10								
	Pos.	5								
Skin	Exam.	10								
	Pos.	6								
Feces	Exam.	10	4	3	5	4	4	10	5	5
	Pos.	0	2	1	1	0	1	0	0	2
Drinking water	Exam.	10		2	5	4	2	10	5	5
	Pos.	9		1	1	2	1	5	4	5
Manger	Exam.	10	4	3	5	4	4	10	5	5
	Pos.	9	2	1	2	4	3	5	3	3
Floor	Exam.	10			5					
	Pos.	5			0					
Wall	Exam.	10	4	3				10	5	5
	Pos.	3	2	2				0	2	4
Total by farms	Exam.	70	12	11	20	12	10	40	20	20
	% Pos.	52.9	50.0	45.5	25.0	50.0	50.0	25.0	45.0	70.0
Total by species	Exam.		93			42			80	
	% Pos.		51.6			35.7			41.3	

Source						Total by Samples	
Swine			Sheep				
E	D	J	D	K	F	Exam.	% Pos.
10				6	4		
						30	50.0
10				0	0		
		4					
						14	64.4
		3					
7	4	12	4	6	4		
						87	16.1
3	1	1	0	1	1		
7	4	12		2	3		
						71	69.0
6	4	8		1	2		
3		12	4	3	3		
						73	57.3
1		5	1	2	2		
			4				
						19	26.3
			0				
4	4	12	4	2			
						63	42.9
4	2	7	1	0			
31	12	52	16	19	14		
77.4	58.3	46.1	12.5	21.1	35.7	359	45.1
	95			49			
	57.9			22.5			

Table 4. Number of soil samples collected and number of isolates of *Klebsiella pneumoniae* obtained

Source	Number of samples	Number of positives	Percent of positives
Public parks, surface	7	5	71.4
Farms without livestock			
Surface	20	16	80.0
1 m below surface	2	0	00.0
3 m below surface	2	0	00.0
7 m below surface	2	0	00.0
Total	33	21	63.6

Table 5. Percent of isolates of Klebsiella pneumoniae from different sources that were positive to selected differential tests

Test or substrate	Source and number of strains							
	Dairy cattle 79	Beef cattle 23	Horse 38	Swine 79	Sheep 19	Public park 9	Farms W/L[a] 28	Total 275
Dextrose (gas)	+[b]	+	+	+	+	+	+	+
Lactose	+	+	+	+	+	+	+	+
Sucrose	+	91.3	+	98.7	+	+	+	98.9
Dulcitol	34.2	+	63.1	45.6	36.8	44.4	21.4	37.8
Sorbitol	+	+	+	98.7	+	+	+	99.7
Mannitol	+	69.6	+	+	94.7	+	+	97.1
Adonitol	91.1	78.3	65.8	93.7	68.4	+	46.4	81.4
Inositol	+	+	92.1	98.7	+	+	+	98.6
Raffinose	+	+	+	+	+	+	+	+
Arabinose	+	+	+	+	+	+	+	+
Xylose	+	+	+	+	+	+	+	+
Cellobiose	+	+	+	+	+	+	+	+
Salicin	+	+	+	+	+	+	+	+
Esculin	93.7	+	+	+	+	+	+	98.2
Arginine dihydrolase	1.3	17.4	-[c]	-	-	-	-	1.8
Lysine decarboxylase	+	+	+	98.7	89.5	+	+	98.9
Ornithine decarboxylase	1.3	-	-	5.1	26.3	-	-	3.6
Indol	19.0	-	23.7	5.1	-	33.3	-	11.3

[a] W/L = without livestock.

[b] + = 100% positive.

[c] - = 0.0% positive.

Table 5. (Continued)

Test or substrate	Source and number of strains							
	Dairy cattle 79	Beef cattle 23	Horse 38	Swine 79	Sheep 19	Public park 9	Farms W/L 28	Total 275
Voges-Proskauer	77.5	+	+	+	+	+	96.4	93.1
Methyl red	22.5	-	-	-	-	-	3.6	6.9
Simmons' citrate	+	+	94.2	+	+	+	+	99.3
Urease	99.9	+	96.3	+	+	+	+	98.2
Phenylalanine	-	-	-	-	-	-	-	-
Gelatin	-	-	-	-	-	-	-	-
Nitrate	+	+	+	+	+	+	+	+
H_2S (TSI)	1.3	-	-	-	26.3	22.2	7.1	3.6
Motility	-	-	-	-	-	-	-	-
Capsule	91.1	95.7	92.1	92.4	+	+	89.3	92.7

Table 6. Percent of in vitro antibiotic susceptibility of randomly selected isolates of Klebsiella pneumoniae from animals and their environment

Antimicrobial agent	Source					
	Dairy cattle 34 strains			Beef cattle 10 strains		
	R[a]	I[b]	S[c]	R	I	S
Ampicillin	70.6	11.8	17.6	70.0	10.0	20.0
Bacitracin	100.0			100.0		
Carbenicillin	91.2	8.8		100.0		
Cephaloglycin			100.0			100.0
Cephaloridine			100.0			100.0
Cephalothin			100.0			100.0
Chloramphenicol			100.0			100.0
Gentamicin			100.0			100.0
Kanamycin	2.9	2.9	94.2			100.0
Neomycin		26.7	73.3		20.0	80.0
Nitrofurantoin	8.8	26.7	64.5		60.0	40.0
Penicillin G	100.0			100.0		
Polymyxin-B		5.8	94.2			100.0
Streptomycin	29.4	35.3	35.3		40.0	60.0
Triple sulfa		14.7	85.3			100.0
Tetracycline	17.6	32.4	50.0		30.0	70.0

[a]R = resistant.

[b]I = intermediate.

[c]S = sensitive.

Source								
Horse 21 strains			Swine 30 strains			Sheep 13 strains		
R	I	S	R	I	S	R	I	S
66.6	29.6	4.8	83.3	16.7		76.9	7.7	15.4
100.0			100.0			100.0		
95.2	4.8		100.0			84.6	15.7	
		100.0			100.0			100.0
	23.8	76.2		10.0	90.0		7.7	92.3
		100.0			100.0			100.0
		100.0			100.0			100.0
		100.0			100.0			100.0
		100.0	13.3		86.7			100.0
	33.3	66.7	13.3	20.0	66.7		30.8	69.2
	52.4	47.6	6.7	40.0	53.3		7.7	92.3
100.0			100.0			100.0		
	9.5	90.5		10.0	90.0			100.0
23.8	47.6	29.6	56.7	20.0	23.3	38.4	30.8	30.8
29.6	33.3	37.1	16.7	23.3	60.0		38.4	60.6
23.8	42.9	33.3	60.0	26.7	13.3	38.4	30.8	30.8

Table 7. Percent of in vitro antibiotic susceptibility of randomly selected isolates of Klebsiella pneumoniae from soil

Antimicrobial agent	Source					
	Public parks 4 strains			Farms without livestock 14 strains		
	R[a]	I[b]	S[c]	R	I	S
Ampicillin	100.0			42.8	28.6	28.6
Bacitracin	100.0			100.0		
Carbenicillin	100.0			78.6	21.4	
Cephaloglycin			100.0			100.0
Cephaloridine			100.0			100.0
Cephalothin			100.0			100.0
Chloramphenicol			100.0			100.0
Gentamicin			100.0			100.0
Kanamycin		25.0	75.0			100.0
Neomycin		100.0			42.9	57.1
Nitrofurantoin		25.0	75.0		14.3	85.7
Penicillin G	100.0			92.9	7.1	
Polymyxin-B			100.0			100.0
Streptomycin		100.0		7.1	85.8	7.1
Triple sulfa		25.0	75.0	7.1	21.5	71.4
Tetracycline		25.0	75.0		28.6	71.4

[a]R = resistant.

[b]I = intermediate.

[c]S = sensitive.

Table 8. Percent of in vitro antibiotic susceptibility of Klebsiella pneumoniae isolates from animal infections

Antimicrobial agent	Source								
	Dairy cattle 10 strains			Horse 6 strains			Dog and cat 4 strains		
	R[a]	I[b]	S[c]	R	I	S	R	I	S
Ampicillin	90.0	10.0		66.7		33.3	100.0		
Bacitracin	100.0			100.0			100.0		
Carbenicillin	100.0			83.3	16.7		100.0		
Cephaloglycin			100.0			100.0			100.0
Cephaloridine			100.0			100.0		100.0	
Cephalothin			100.0	16.7		83.3		100.0	
Chloramphenicol			100.0	16.7		83.3		100.0	
Gentamicin			100.0			100.0			100.0
Kanamycin		10.0	90.0			100.0	100.0		
Neomycin		60.0	40.0		66.7	33.3	100.0		
Nitrofurantoin		30.0	70.0		16.7	83.3		100.0	
Penicillin G	100.0			100.0			100.0		
Polymyxin-B			100.0		16.7	83.3			100.0
Streptomycin	70.0	20.0	10.0	66.7	33.3		100.0		
Triple sulfa	10.0	10.0	80.0		16.7	83.3	100.0		
Tetracycline	10.0	10.0	80.0	33.3		83.3	100.0		

[a]R = resistant.

[b]I = intermediate.

[c]S = sensitive.

Table 9. Percent of in vitro antibiotic susceptibility of Klebsiella pneumoniae isolates from different sources

Antimicrobial agent	Source: Animals and their environment 108 strains			Source: Soil surface from P/P and FW/L[a] 18 strains			Source: Animal infections 20 strains		
	R[b]	I[c]	S[d]	R	I	S	R	I	S
Ampicillin	74.1	15.7	10.2	55.6	22.2	22.2	85.0	5.0	10.0
Bacitracin	100.0			100.0			100.0		
Carbenicillin	94.5	5.5		83.3	16.7		95.0	5.0	
Cephaloglycin			100.0			100.0			100.0
Cephaloridine		8.3	91.7			100.0		20.0	80.0
Cephalothin			100.0			100.0	5.0	20.0	75.0
Chloramphenicol			100.0			100.0	25.0		75.0
Gentamicin			100.0			100.0			100.0
Kanamycin	4.6	0.9	94.5		5.6	94.4	20.0	5.0	75.0
Neomycin	3.7	22.6	73.7		55.6	44.4	20.0	50.0	30.0
Nitrofurantoin	4.6	36.1	59.3		16.7	83.3		40.0	60.0
Penicillin G	100.0			94.4	5.6		100.0		
Polymyxin-B		6.5	93.5			100.0		5.0	95.0
Streptomycin	34.3	33.3	32.4	5.6	88.8	5.6	75.0	20.0	5.0
Triple sulfa	10.2	22.2	67.6	5.6	22.2	72.2	25.0	10.0	65.0
Tetracycline	31.5	32.4	35.1		28.6	71.4	35.0	5.0	60.0

[a]P/P = public parks; FW/L = farms without livestock.

[b]R = resistant.

[c]I - intermediate.

[d]S = sensitive.

Table 10. Pathogenicity in mice (determined by lethal effect) of randomly selected Klebsiella pneumoniae strains

Strain	Original source	Method A		Method B	
		Animals inoculated	Total deaths	Animals inoculated	Total deaths
Schalm 1236	Cow, mastitis	3	0	3	0
C-59	Cow, mastitis	3	0	3	2
531	Cat, urinary infection	3	0	3	2
526	Stallion, urethral infection	3	3	3	3
520	Cow, mastitis	3	0	3	2
7	Cow, nasal secretion	3	0	3	2
119	Horse barn, drinking water	3	2	3	2
187	Swine, nasal secretion	3	0	3	0
175	Swine barn, drinking water	3	0	3	0
223	Sheep barn, manger	3	1	3	2
248	Public park, soil	3	0	3	0
266	Farm without livestock, soil	3	2	3	2
Total		36	8	36	17

DISCUSSION

Klebsiella pneumoniae was isolated from animals (dairy cattle, beef cattle, horses, swine, and sheep), their environment, soil from public parks, and farms without livestock. This information establishes that the organism is widely distributed in nature. Heretofore the widespread presence of K. pneumoniae in the environment outside the animal host has been a matter of speculation. While the existence of K. pneumoniae in soil, water, grains and other plant materials, and the animal environments has been mentioned for many years in most of the bacteriology textbooks (8) (32)(45), studies of the frequency in which the organism is found or the characteristics of the isolates has not been reported.

In all dairy cattle and swine farms, two beef cattle and one horse farms, the frequency of recovery was above the average when all samples were computed together. On the other hand, on all sheep farms, two horse and one beef cattle farms, the recovery was below average.

The higher incidence among normal swine, dairy cattle, and horses seems to correlate with reports of a greater frequency of Klebsiella infections in those same animal species (2)(3)(21)(52)(67)(73) than in sheep (40) and beef cattle. Although K. pneumoniae was present in most of the samples from beef cattle, its prevalence does not correlate with the occurrence of Klebsiella infections in this species. These results may be explained by the fact that in this study only young beef cattle and their environment were examined. Of the Klebsiella infections which occur in cattle most are found in the udder of the dairy cow.

Of the different sources examined, K. pneumoniae was isolated more frequently from drinking water (69.0%), skin (64.4%), manger (57.3%), and nasal secretions (50.0%), less from floors (42.9%) and walls (26.3%), and occasionally from feces (16.1%). Although the number of K. pneumoniae in each specimen was not determined it was observed that cultures of nasal secretions usually yielded large numbers of organisms whereas only one to several colonies would appear on cultures from most other sources.

The presence of the organism in drinking water, manger, and nasal secretions may be explained by the fact that the organism is able to survive and even multiply on grains, other plant materials, and water containing organic material (8)(32). It frequently is found colonizing the nasal cavity (6)(63), and since the water bowls or other water containers and the manger are infrequently cleaned and seldom disinfected, probably the organism is passing from the drinking water and manger to the nasal cavity and vice versa. The failure to isolate the organism from sheep nasal secretions may be explained by the low number of samples examined or perhaps by the apparent resistance of these animals to the organism.

Klebsiella pneumoniae was consistently isolated from the skin of the udder but only in small numbers. The source of the organism might be nasal secretions of the cow herself or floor of the stable. Whether Klebsiella can survive or multiply on the udder skin was not determined. However, McDonald (58) found that "Aerobacter aerogenes", strain 1236, did not colonize the udder skin when directly applied and furthermore it seldom survived for as long as 15 hours on that site. The walls and floors probably are contaminated by the nasal secretions, feed, and feces.

Occasional finding of K. pneumoniae in feces may be related to the fact that growth of K. pneumoniae is restricted when Escherichia coli is present and therefore appear in substantial numbers only when the number of E. coli is low, such as in the treatment with antibiotics by the oral route as is found in man (31)(90). Also it is possible that the organism can pass with the nasal secretions to the intestinal tract and then be eliminated in the feces in low numbers. Often it is not possible to detect Klebsiella in cultures because it is covered by the growth of E. coli or other members of the family Enterobacteriaceae that are present in higher numbers. Since there are no reports of the incidence of K. pneumoniae in the feces of domestic animals, the only source for comparison are those of the findings in human beings which show that the incidence of the organism varies from 5 to 15% (57)(63).

Klebsiella pneumoniae was isolated from soil surfaces from public parks (71.4%) and farms without livestock (80.0%), but not detected in six samples of soil taken from 1 to 7 m below the surface on farms without livestock. These results are in agreement with the general concept that K. pneumoniae is widespread in surface soil, although in low numbers. The failure to isolate the organism from samples below the surface may be explained by the fact that K. pneumoniae may not be able to multiply in this environment and that the soil may act as a filter not permitting the passage of the organism to the subsurface.

The finding that Klebsiella was quite prevalent in surface soils not contacted by livestock would suggest that this organism is able to live in nature apart from animals and is not dependent on them as a host. How-

ever, when K. pneumoniae infects or colonizes an animal the organism is shed in large numbers thereby increasing the population in the environment.

The fact that K. pneumoniae is able to grow in both animals and in environments not contacted by animals suggests that the organism may originate from any one of several sources.

Two hundred and seventy-five strains isolated during the study were identified as K. pneumoniae by using 28 differential characteristics and tests. Most of the isolates showed the pattern of K. pneumoniae as established by the Subcommittee of Enterobacteriaceae (77) and reported by Cowan et al. (16), Edwards and Ewing (26), Eitckhoff et al. (28), Kauffman (50), Slopek and Durlakowa (79), Durlakowa et al. (20), and Ewing (30). Slight variations were found in mannitol fermentation (beef cattle), arginine dehydrolase (beef cattle), ornithine decarboxylase (sheep), indol production (horse, public parks), and H_2S (sheep, public parks).

The results mentioned above may suggest the probability of the development of definite biotypes in these environments, but since K. pneumoniae is found widely distributed in nature as well as the animal environment, and they are constantly subject to changes in their bacterial flora it is not possible to establish biotypes or serotypes specific for particular environment or animal species.

The occurrence of more than one biotype or serotype in one disease is reported by Barnes (3) who found three different serotypes in four cases of bovine mastitis occurring in the same barn. Easterbrooks and Plastridge (21) established that bovine mastitis is not caused by a single

serotype. Lake and Jones (52) were able to isolate four different serotypes in an outbreak of postparturient disease in sows.

In human hospitals the prevalence of specific serotypes has been reported (57)(63). Because the environment of hospitals is not subject to as severe changes as occur in animals, this may contribute to the persistence of given serotypes.

In vitro antibiotic susceptibility of randomly selected strains isolated from various sources show that strains from swine, dairy cattle, and horses appear to be generally more resistant than strains originating from sources unrelated to animals. These results may be related to the use of antibiotics in the treatment of infections in the animals and the development of resistant strains as has been reported. This statement can be supported by the fact that K. pneumoniae isolated from human patients with Klebsiella infections and hospitals where the organism is found permanently, are more resistant than those found in healthy carrier persons (4) (31).

Among the isolates from animal infections those from dogs and cats show uniform resistance to nine of the 16 antibiotics used, which include Kanamycin and Neomycin, and appear to be more resistant than those from horses and dairy cattle which did not show this characteristic. It seems as dogs and cats are more frequently treated with broad spectrum antibiotics they more frequently develop resistant strains.

The strains isolated from soil surfaces not contacted by livestock showed resistance to not more than six antibiotics, and seem to be less resistant than those from animal infections and the animal environment.

It is possible to attribute this result to the fact that these strains show only the natural resistance to antibiotics which is a general characteristic of the Klebsiella organisms (27)(87).

Pathogenicity as determined by lethal effect in white mice was tested for 12 strains, five corresponding to isolates from animals that had suffered disease caused by K. pneumoniae, one of them "Aerobacter aerogenes" Schalm 1236, was an old culture of noncapsulated organism which produces rough colonies and did not kill mice. The other four strains, which were recently isolated were encapsulated and produced smooth colonies, were pathogenic for mice. These results are in agreement with those obtained by other workers (23)(82)(83)(89) who have stated that old, noncapsulated organisms which produce rough colonies are either nonpathogenic or less pathogenic than recent isolates from animals.

Those strains from soil surfaces not contacted by livestock appear to have variable degrees of virulence as is shown by the fact that four of seven strains tested were able to kill mice. This demonstrated that strains from various sources can be pathogenic, which agrees with the observation of Edwards who found isolates from soil to be pathogenic (23).

The inoculums used were prepared in two different ways, washed cells and whole culture, in order to establish if cells alone are able to kill mice as reported by Snyder et al. (82). According to the results, white mice were susceptible to both inoculums, but a higher mortality rate was obtained when whole cultures were injected as is shown in table 10. This is probably due to the actions of some substances formed during the growth such as the "free endotoxin", or by the accumulation of endotoxins by the

lysis of some cells, which lower resistance of the mice.

The death of only some mice in the groups inoculated with the same dose and inoculum, as is shown in table 10, may suggest some variation in host susceptibility to K. pneumoniae.

SUMMARY

Samples from animals and their environment (dairy cattle, beef cattle, horse, swine, and sheep farms) and soil from areas not contacted by livestock were examined culturally for the presence of K. pneumoniae.

Isolates positively identified as K. pneumoniae were obtained from all the farms, soil surface of five public parks, and 16 farms without livestock. No isolations were obtained from any samples taken from subsurface soil on farms without livestock. The organism was found in 45.1% of samples from animals and their environment and in 63.6% of samples from surface soil not contacted by livestock. Two hundred and seventy-five strains obtained were identified by using 28 differential characteristics and tests. Although some deviations from the characteristic pattern of K. pneumoniae for the differential tests and characteristics were noted in some strains, almost all the isolates have the same pattern.

Antibiotic susceptibility by the single disk agar diffusion method to 16 antimicrobial agents was determined for 98 isolates from animals and their environment, 18 from soil not contacted by livestock, and 20 from animal infections. Those strains from animal infections appear to be more resistant than those from animals and their environment and soil not contacted by livestock.

Pathogenicity, as determined by lethal effect in mice using washed cells as inoculum, was shown to be less virulent than when whole broth culture was used. K. pneumoniae organisms recently isolated from animal infections were shown to be virulent for mice and the virulence of those isolates from animals and their environment and soil surface not contacted by animals were of variable virulence.

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