APPLIED MICROBIOLOGY, Sept. 1971, p. 309-314
Vol. 22, No. 3

Antibiotic Susceptibilities of *Serratia marcescens* and *Enterobacter liquefaciens*

PATSY GREENUP AND DONNA J. BLAZEVIC

Department of Laboratory Medicine, University of Minnesota, Minneapolis, Minnesota 55455

Received for publication 14 June 1971

Production of 5'-nucleotides by *Serratia marcescens* and *Enterobacter liquefaciens* correlates with deoxyribonuclease production, indicating the close relationship between these two organisms. To determine further relationships, susceptibilities of 279 strains of the tribe *Klebsielleae* were determined by the high-potency disc method, agar-dilution method, or both, by using 14 antibiotics. Ninety-seven per cent of *S. marcescens* (201 of 207 strains) and 100% of *E. liquefaciens* (17 strains) had minimum inhibitory concentration (MIC) of 100 μg/ml or greater with colistin and polymyxin B. With these two antibiotics, 93% of other *Enterobacter* species (28 strains) had MIC values of less than 1.6 μg/ml, and 100% of *Klebsiella* (27 strains) had MIC values less than 1.6 μg/ml. Consistent patterns were not noted with the other antibiotics tested, but the results with colistin and polymyxin B provide additional evidence of the close relationship of *S. marcescens* and *E. liquefaciens*.

In the clinical laboratory, nonpigmented strains of *Serratia marcescens* are more common than pigmented ones, but many laboratories either do not identify or are unable to identify nonpigmented strains correctly (19). Deoxyribonuclease production has been used to identify *Serratia* (2), but strains of *Enterobacter liquefaciens* as well as certain other gram-negative rods also produce this extracellular enzyme (9).

Durand and Blazevic (3) utilized thin-layer chromatography to detect a specific nucleoside phosphotransferase in *Serratia* and *E. liquefaciens*, and the production of 5'-nucleotide correlated with deoxyribonuclease production. On the basis of deoxyribonuclease, biochemical, and phosphotransferase activity, they proposed *E. liquefaciens* to be taxonomically similar to *Serratia*.

In 1968, Ramirez (12) proposed the use of cephalothin and colistin to differentiate *Klebsiella-Enterobacter-Serratia*. He found that most *Klebsiella* were susceptible to cephalothin, and all were sensitive to colistin. *Enterobacter* and *Serratia* were resistant to cephalothin; *Enterobacter* sp. were sensitive to colistin whereas *Serratia* sp. were resistant.

Edmondson and Sanford (4) stated that colistimethate resistance is constant enough to be useful in identification of *Serratia*. Von Gravenitz (18) stated that *Serratia* had no uniform susceptibility pattern but did suggest that certain features of *Serratia* sensitivity could be of help in the bacteriological diagnosis and clinical treatment of *Serratia*. More recent publications (11, 13, 19, 20) also suggested the use of antibiotic susceptibility patterns as a possible aid in classification of members of the *Klebsiella-Enterobacter-Serratia* groups. However, of these papers only the work of Poupard et al. (11) presented an antibiogram for *E. liquefaciens* as a separate species. Since it has been proposed that *E. liquefaciens* and *Serratia* are taxonomically related, it was the purpose of this project to compare the antibiograms of *Serratia* and *E. liquefaciens* with respect to correlation with 5'-nucleotide production.

MATERIALS AND METHODS

The recent isolates of 45 *Serratia*, 4 *E. liquefaciens*, 22 *Enterobacter* sp., and 19 *Klebsiella* tested in this study were isolated from clinical specimens by the Diagnostic Microbiology Laboratory of the University of Minnesota Hospitals from March 1970 to October 1970. Stock cultures of these isolates were added to 157 cultures of *Serratia*, 5 *E. liquefaciens*, 5 *Enterobacter* sp., and 8 *Klebsiella* which had been collected from 1966 to 1969 and maintained on sealed Trypticase soy agar (BBL) slants in the dark at room temperature. Sheep blood-agar plates, eosin-methylene blue plates, or MacConkey agar plates were used for primary isolation. Stock cultures were streaked on MacConkey agar to obtain isolated colonies and to check for purity. Triple sugar-iron-agar (TSI) slants were inoculated from these plates, and biochemical tests were inoculated with growth from these TSI slants. In addition to these clinical isolates, seven *E. liquefaciens* were obtained from the Center for Disease Control, Atlanta, Ga., and one strain from the American Type Culture Collection (*E. liquefaciens* ATCC 14460). Three strains of *Serratia* reported to

be deoxyribonuclease-negative were obtained from J. J. Farmer II, of the National Institutes of Health. Two strains of *Serratia* and one strain of *Enterobacter cloacae* were obtained from Walter H. Traub of Bowman Gray School of Medicine.

Biochemical studies. Media and methods as outlined by Durand and Blazevic (3) were used except for the following. Cytochrome oxidase production was tested for by the method of Edwards and Johnson (5), and deoxyribonuclease production was determined on DNase test base agar (Difco) with 0.005% toluidine blue O (14).

Nucleoside phosphotransferase determination. The thin-layer chromatography method described by Durand and Blazevic (3) was utilized for the nucleoside phosphotransferase determination. Mixtures of reagents and organisms were incubated at 35 C in a dry bath for 2 hr to allow synthesis of the mononucleotide by the phosphotransferase to take place. Tubes containing the reaction mixtures were not mixed before removing the 2-μliter sample to avoid tailing of the guanosine. The development tank was filled daily with 120 ml of freshly prepared solvent.

Antibiotic susceptibility studies. Two-hundred seventy-nine strains were tested by the Bauer et al. (1) single-disc diffusion method of antibiotic susceptibility, by the agar dilution procedure (6) with Steers et al. (15) replicator, or by both. Stock cultures of all organisms were transferred to MacConkey agar; at least five colonies of each strain were inoculated into Mueller Hinton broth (Difco) and incubated a minimum of 4 hr at 35 C. This culture suspension was visually standardized against a 0.5 MacFarland standard by using the device described by Stemper and Matsen (16). Commercially prepared 150-mm plates filled with Mueller Hinton agar (BBL) to a depth of 4 mm were used for the disc method. Plates were incubated in the inverted position 18 to 24 hours at 35 C, and zones of inhibition were measured with a ruler, reading from the bottom of the plate.

Agar-dilution testing was carried out by using a 1:200 dilution of the same standardized suspension used for the disc-diffusion test. The organisms were inoculated onto Mueller Hinton agar plates containing antibiotic concentrations from 100 μg/ml to 0.2 μg/ml by using the replicator. Readings of agar-dilution results were made after 18 to 24 hr incubation at 35 C.

RESULTS

Data obtained from the nucleoside phosphotransferase studies indicated average R_F values for each substance spotted on the chromatogram as follows: guanosine 3'(and 2')-phosphate, 0.43; guanosine 5'-phosphate, 0.58; guanosine, 0.36; *p*-nitrophenylphosphate, 0.52; and *p*-nitrophenol, 0.10.

Organisms were identified as *Serratia* if they liquified gelatin within 2 days and failed to ferment arabinose, raffinose, and rhamnose (5). Of 47 isolates positively identified as *Serratia*, all were indole-negative, grew on Simmon's citrate agar, were lactose-negative, produced an acid reaction throughout TSI slants with little or no gas, produced deoxyribonuclease, were cytochrome oxidase-negative, and lysine decarboxylase-positive. Only one strain was nonmotile; two strains produced arginine dihydrolase. None of the 47 isolates was pigmented on Trypticase soy agar. The 5'-nucleotide was produced by all 47 strains.

The three strains of *Serratia* obtained from NIH produced the 5'-nucleotide. Two of the three strains were pigmented and strongly deoxyribonuclease-positive, and the third was nonpigmented and weakly positive for deoxyribonuclease production after 48 hr of incubation.

Four other organisms gave biochemical reactions similar to *Serratia* organisms except that two strains were lactose-positive, and all four strains fermented arabinose and raffinose. These four organisms would be classified as *E. liquefaciens* by Edwards and Ewing (5). All four strains produced the 5'-nucleotide.

Strains were identified as *Klebsiella* if they produced acid in TSI slants, were indole-negative, citrate-positive, ornithine decarboxylase-negative, nonmotile, and deoxyribonuclease-negative. None of the nineteen strains studied produced the 5'-nucleotide.

Enterobacter species, other than *E. liquefaciens*, produced acid in TSI and were indole-negative and oxidase-negative. Differentiation into species was by the method of Ewing (7). The 23 *Enterobacter* organisms (15 *E. cloacae*, 7 *E. aerogenes*, and 1 *E. hafnia*) tested failed to produce 5'-nucleotide.

Stock cultures collected before March 1970 had previously been identified biochemically and tested for the presence of the nucleoside phosphotransferase. *Serratia* and *E. liquefaciens* produced the 5'-nucleotide, whereas the *Enterobacter* sp. and the *Klebsiella* failed to produce the 5'-nucleotide (3).

The results of the tests for in vitro susceptibility by agar dilution of 279 strains of *Klebsielleae* to 13 antibiotics are summarized in Table 1, and the results of the agar diffusion tests using 11 antibiotics are summarized in Table 2. Gentamicin was the most active of the drugs tested, with approximately 99% of the four groups of organisms being inhibited at a concentration of 3.1 μg/ml. The synthetic compound nalidixic acid was the most active against the four groups. Kanamycin was active against *Serratia*, *E. liquefaciens*, *Enterobacter*, and most *Klebsiella*; however, 29% of the *Klebsiella* were not inhibited at a concentration of 100 μg/ml. Chloramphenicol, streptomycin, and tetracycline all showed variation in ac-

TABLE 1. *Results of agar dilution susceptibility tests with 279 strains of Klebsielleae*[a]

Antibiotic	Strain	Per cent of strains with minimum inhibiting concn of (μg/ml)												
		>100	100	50	25	12.5	6.3	3.1	1.6	0.8	0.4	0.2	≤12.5	≥25
Ampicillin	*Serratia*	18	24	32	18	7	0.5	0	0	0	0	0	8	93
	E. liquefaciens	6	0	6	12	47	29	0	0	0	0	0	76	24
	Enterobacter	43	10	29	10	7	0	0	0	0	0	0	7	93
	Klebsiella	48	0	22	27	3	0	0	0	0	0	0	3	97
Carbenicillin	*Serratia*	2	1	0	0.5	4	58	32	2	0	0	0	96.5	3.5
	E. liquefaciens	6	0	0	0	0	12	41	35	6	0	0	94	6
	Enterobacter	3.5	7	10	0	3.5	26	28	18	0	0	0	79	21
	Klebsiella	63	27	7	3	0	0	0	0	0	0	0	0	100
Cephalothin	*Serratia*	99	0.5	0	0	0	0	0	0	0	0	0	0	100
	E. liquefaciens	70	12	12	6	0	0	0	0	0	0	0	0	100
	Enterobacter	38	10	18	23	3.5	0	3.5	3.5	0	0	0	10.5	89.5
	Klebsiella	15	0	15	6.5	6.5	15	15	27	0	0	0	63.5	36.5
Cephaloridine	*Serratia*	75	24	0.5	0	0.5	0	0	0	0	0	0	0.5	99.5
	E. liquefaciens	6	6	12	58	6	12	0	0	0	0	0	18	82
	Enterobacter	64	14	7	7	0	0	3.5	3.5	0	0	0	7	93
	Klebsiella	15	4	15	0	11	6.5	48	0	0	0	0	65.5	34
Nitrofurantoin	*Serratia*	86	12	1	0	0	0	0	0	0	0	0	0	100
	E. liquefaciens	30	70	0	0	0	0	0	0	0	0	0	0	100
	Enterobacter	14	23	24.5	28	10	0	0	0	0	0	0	10	90
	Klebsiella	22	15	6.5	41.5	15	0	0	0	0	0	0	15	85
Colistin	*Serratia*	97	0	0	0	0	1	1	0.5	0	0.5	0	3	97
	E. liquefaciens	77	23	0	0	0	0	0	0	0	0	0	0	100
	Enterobacter	0	0	3.5	0	3.5	0	0	0	0	67	26	96.5	3.5
	Klebsiella	0	0	0	0	0	0	0	0	11	78	11	100	0
Polymyxin B	*Serratia*	97	0	0	0	0	0.5	0.5	1.5	0	0.5	0	3	97
	E. liquefaciens	41	59	0	0	0	0	0	0	0	0	0	0	100
	Enterobacter	0	0	0	0	0	0	0	0	4	60	36	100	0
	Klebsiella	0	0	0	0	0	0	0	0	3	55	42	100	0
Chloram-phenicol	*Serratia*	6.2	0.9	1	6.2	47	37	0.5	0.5	0	0	0	85.7	14.3
	E. liquefaciens	0	0	0	0	17	66	17	0	0	0	0	100	0
	Enterobacter	0	0	0	0	7	46	43	3.5	0	0	0	100	0
	Klebsiella	22	0	3	0	3	0	45	27	0	0	0	75	25
Gentamicin	*Serratia*	0	0	0	0	0	0	1	3	66	29	0.5	100	0
	E. liquefaciens	0	0	0	0	0	0	0	6	25	52	17	100	0
	Enterobacter	0	0	0	0	0	0	0	3.5	40	50	7	100	0
	Klebsiella	0	0	0	0	0	0	0	0	66	34	0	100	0
Kanamycin	*Serratia*	0.5	0	0	0	2	3.2	38	53	2	0	0	99.5	0.5
	E. liquefaciens	0	0	0	0	0	6	0	41	47	6	0	100	0
	Enterobacter	7	0	0	0	0	3.5	28	52.5	7	0	0	93	7
	Klebsiella	29	0	0	0	0	0	20	51	0	0	0	71	29
Streptomycin	*Serratia*	8	9	9	5	1.5	1.5	9	53	3	0	0	69	31
	E. liquefaciens	6	6	0	0	6	0	0	58	24	0	0	88	12
	Enterobacter	0	0	0	0	0	14	17.5	58	10	0	0	100	0
	Klebsiella	31	0	0	0	0	0	66	0	3	0	0	69	31
Tetracycline	*Serratia*	15	30	19	12	18	6	0.5	0	0	0	0	24.5	76
	E. liquefaciens	6	0	0	6	0	70	18	0	0	0	0	88	12
	Enterobacter	0	0	0	0	0	14	60	26	0	0	0	100	0
	Klebsiella	31	0	0	0	0	0	31	31	7	0	0	69	31
Nalidixic acid	*Serratia*	0	0.5	6	1	3.2	18	66	3.2	0.5	0	0	92.5	7.5
	E. liquefaciens	0	0	0	0	0	0	5	52	38	5	0	100	0
	Enterobacter	0	0	0	0	0	3.5	96.5	0	0	0	0	100	0
	Klebsiella	0	0	0	3	20	22	55	0	0	0	0	97	3

[a] No. used: 207 strains of *Serratia*, 17 *E. liquefaciens*, 28 *Enterobacter*, 27 *Klebsiella*.

TABLE 2. *Results of disc diffusion susceptibility test with 279 strains of Klebsielleae*

Antibiotic	*Serratia* (207 strains)			*Enterobacter liquefaciens* (17 strains)			*Enterobacter* (28 strains)			*Klebsiella* (27 strains)		
	S[a]	I[b]	R[c]	S	I	R	S	I	R	S	I	R
Ampicillin (10 μg)	4	5	91	29	6	65			100			100
Cephalothin (30 μg)			100	6		94	11	3	86	65	4	31
Cephaloridine (30 μg)	1		99	18		82	11		89	52	11	37
Nitrofurantoin (300 μg)	1	13	86	12	59	29	78	14	8	45	29	25
Chloramphenicol (30 μg)	92	1	7	100			100			75		25
Gentamicin (10 μg)	100			100			100			100		
Kanamycin (30 μg)	94	5	1	100			89	3	8	71		29
Streptomycin (10 μg)	67		33	88		12	83	17		45	25	29
Tetracycline (30 μg)	1	4	95	88		12	86	14		65	7	29
Nalidixic acid (30 μg)	92	2	6	100			100			71	29	
Sulfisoxazole (250 μg)	77	14	8	100			93		7	66		31

[a] S, per cent of strains sensitive.
[b] I, per cent of strains intermediate.
[c] R, per cent of strains resistant.

tivity but with a greater number of each group of organisms being inhibited by a concentration of 6.3 μg/ml. Of the penicillins and cephalosporins tested, cephalothin and cephaloridine were active against some of the strains of *Klebsiella*, and carbenicillin had little or no activity against the *Klebsiella*. Cephalothin and cephaloridine had very little activity against *Serratia*, *E. liquefaciens*, and *Enterobacter* sp., whereas carbenicillin inhibited most strains of these three groups at a concentration of 6.3 μg/ml. Ampicillin had little activity against any of the *Serratia*, *Enterobacter* sp., and *Klebsiella*, whereas a slightly greater activity was observed against the *E. liquefaciens*.

The polypeptide antibiotics, polymyxin B and colistin, were studied by the agar-dilution method only. These agents inhibited 93% *Enterobacter* sp. at minimum inhibitory concentration (MIC) values less than 1.6 μg/ml, and 100% *Klebsiella* at less than 1.6 μg/ml. Ninety-seven per cent of the *Serratia* and all *E. liquefaciens* were not inhibited at a concentration of 100 μg/ml.

DISCUSSION

In this study the presence of a nucleoside phosphotransferase in *Serratia* (50 strains) and in *E. liquefaciens* (4 strains) was detected by the production of 5′-guanosine monophosphate by using thin-layer chromatography. These data correlate with those of Durand and Blazevic (3) and further indicate the possibility of a relationship of the production of extracellular deoxyribonuclease and nucleoside phosphotransferase. The addition of the deoxyribonuclease medium to the biochemical protocol for lactose fermenters and nonfermenters would be a rapid screening procedure for *E. liquefaciens*, as well as *Serratia*. However, as indicated by the small numbers available for this study, *E. liquefaciens* is rarely isolated from clinical specimens. Whereas the use of the deoxyribonuclease medium would not differentiate these two groups of organisms as defined by Edwards and Ewing (5), the data with colistin and polymyxin in addition to the production of deoxyribonuclease and 5′-nucleoside phosphotransferase further indicate that *E. liquefaciens* are more closely related to the *Serratia* group than to the *Enterobacter* group.

In examining the antibiograms of 279 strains of *Klebsielleae*, antibiotics were grouped according to their site of activity to look for similarities of these organisms as to their morphological and biochemical components.

Of the antibiotics studied which are known to alter the structure and function of the cell wall,

data agreed with those previously reported (12, 13, 19, 20) for members of the *Klebsielleae*. Values for ampicillin indicated a generally similar pattern for *Serratia*, *Enterobacter* sp., and *Klebsiella*; 29% of *E. liquefaciens* indicated a variant pattern by the disc method. However, this latter value for *E. liquefaciens* is in disagreement with one study in which 100% of 14 strains of *Enterobacter* group C were reported to be resistant to ampicillin (11). *Serratia*, *E. liquefaciens*, and *Enterobacter* sp. presented similar antibiograms with carbenicillin, cephalothin, and cephaloridine. *Klebsiella* sp. were highly resistant to carbenicillin, with 65% of the strains tested inhibited by 12.5 μg/ml or less with cephalothin and cephaloridine. Nitrofurantoin did not permit differentiation of any one group of the *Klebsielleae*.

Agents which impair the translation of genetic information into protein synthesis (chloramphenicol, gentamicin, kanamycin, streptomycin, and tetracycline) indicated similar patterns for *E. liquefaciens* and *Enterobacter* sp., whereas the *Klebsiella* showed a wider variation of susceptibility with chloramphenicol, kanamycin, streptomycin and tetracycline. The *Serratia* were sensitive to chloramphenicol, gentamicin, and kanamycin and highly resistant to tetracycline and less so to streptomycin. Similar data were reported (10, 13, 19) for the high susceptibility of the *Klebsiella-Enterobacter-Serratia* to gentamicin. None of these agents permits distinct separation of any of the groups of the *Klebsielleae*.

Nalidixic acid, which impedes replication of genetic information, and sulfisoxazole, which affects intermediary metabolism, also presented patterns which did not permit the separation of any one group of organisms.

Results with colistin and polymyxin B, agents which restrict the function of the cell membrane, were similar to previous reports for *Klebsiella-Enterobacter-Serratia* (4, 10, 13, 19). MIC values for *E. liquefaciens* were essentially the same as for the *Serratia* so that both groups of organisms demonstrated almost complete resistance. These values were quite different from those obtained with the other *Enterobacter* sp. and *Klebsiella*, which were almost all sensitive. These MIC values for *E. liquefaciens* with colistin and polymyxin B are in agreement with the disc values reported by Poupard et al. (11).

Overall examination of the antibiograms obtained in this study did not indicate distinct differentiation of *E. liquefaciens* from other *Enterobacter* sp. except the MIC values with colistin and polymyxin B. Traub et al. (17) stated that all species of *Enterobacter* yielded essentially identical antibiograms with the exception of atypical *E. cloacae*; however, it is not known whether colistin, polymyxin B, or both, were included in his studies. In partial agreement with Poupard et al. (11), there does not appear to be any specific taxonomic value to the susceptibility patterns of *Klebsiella* and *Enterobacter* sp. other than *E. liquefaciens*. However, it is of interest to note the data obtained in this study and that of Durand and Blazevic (3). These results and the reported characteristic resistance of *Serratia* to polymyxin (8) and to colistin and polymyxin B (10) further suggest the need for examination of the classification of *E. liquefaciens*.

Examination of *E. liquefaciens* and *Serratia* for resistance factors or colicins might indicate why certain antibiograms are produced, and comparative base pairing and cell wall studies of these two groups might be of taxonomic value. The relationship of the location and interaction, if any, of the nucleoside phosphotransferase to observed reactions and comparative studies of deoxyribonuclease activity as to heat lability and antigenicity would also merit investigation. Although the latter procedures are beyond practical clinical application, these tools of microbial physiology might supply additional data for interpretation of the relationship of these two groups of organisms.

ACKNOWLEDGMENT

This study was supported by Public Health Service Allied Health Professions Advanced Traineeship Grant AHT-70-059.

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