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Correlation of Bacterial Lipid Composition with Antibiotic Resistance

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The correlation of bacterial lipid composition with antibiotic resistance was investigated with particular emphasis on those organisms in which resistance may be related to membrane or envelope structure or function, as in resistance to tetracyclines and polymyxin. Chloroform-methanol-extractable lipids, phosphatidyl ethanolamine fractions, and both fatty acids of these lipid fractions and total fatty acids were studied by using thin-layer chromatography, gas chromatography, and infrared spectroscopy. Consistent quantitative differences were found between the fatty acid compositions of sensitive and resistant strains. Most notable was the fact that, in gram-negative organisms, resistant strains showed decreases in cyclopropane acids as compared with sensitive strains. These changes were found to be inherent in the strains and not due to growth stage or culture age. No significant qualitative differences were noted. In contrast, no such variation in fatty acid content was observed in penicillin-sensitive and resistant strains of gram-positive cocci. As significant alterations of fatty acid composition were noted in gram-negative strains resistant to antibiotics, we suggest that resistance is correlated to membrane or envelope lipid composition.

It is well-established that bacterial lipids are major constituents of bacterial membranes and gram-negative bacterial envelopes (5, 32), but little is known of the exact functions of lipids in these structures (23, 30). However, there have been some suggestions that bacterial lipid composition or arrangement might prevent the entrance or binding of certain antibiotics (2, 16, 38-41).

This paper reports our findings on the nature of bacterial lipids obtained from a variety of organisms and the correlation of these lipid compositions with antibiotic resistance. Emphasis was placed on investigating those cases in which the site of antibiotic action is thought to be in the bacterial envelope as in the case of polymyxin (9, 27), in those cases in which antibiotic resistance has been attributed to a decreased permeability of the drug as with the tetracyclines (2, 11, 12, 17, 20, 28, 29), or in those cases in which resistance is associated with an increased lipid content as in the case of penicillin (16).

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MATERIALS AND METHODS

Bacteria. Allen I. Laskin of The Squibb Institute for Medical Research, New Brunswick, N.J., generously supplied us with several strains of *Escherichia coli: E. coli* Sc3552 resistant to tetracycline (TC), *E. coli* Sc8280 resistant to tetracyline (TC), and *E. coli* Sc8190 sensitive to tetracycline. *E. coli* Sc8280 was developed by conjugation of *E. coli* Sc8190 with *Shigella flexneri* carrying R factor 222 which is characterized by sulfonamide-, streptomycin-, chloramphenicol-, and tetracycline-resistant traits. *E. coli* B7350 was a laboratory strain sensitive to tetracycline.

The strain of *Staphylococcus aureus* resistant to penicillin (PR) was a laboratory strain, and the strain of *S. aureus* sensitive to penicillin was strain ATCC 12600.

Both the polymyxin-resistant and -sensitive strains of *Klebsiella pneumoniae* were laboratory strains.

Media and culture methods. All strains of *E. coli* were grown in medium K, a modification of Franklin's medium (11) consisting of: 7.0 g of K_2HPO_4, 3.0 g of KH_2PO_4, 0.2 g of $MgSO_4 \cdot 7H_2O$, 1.0 g of $(NH_4)_2SO_4$, and 12.0 g glucose per liter at a *p*H of 7.0. *K. pneumoniae* strains were grown in the chemically defined medium of Davis and Mingioli (6). *S. aureus* strains were grown in the semisynthetic medium of Lascelles and Woods (19). Antibiotic-resistant strains of *E. coli*,

K. pneumoniae, and *S. aureus* were grown in 10 μg of chlortetracycline hydrochloride, polymyxin B sulfate, and penicillin G (potassium salt) per ml, respectively All cultures were incubated for 24 hr at 37 C in a New Brunswick model G-25 shaking incubator operated at 250 oscillations per min. Cells were harvested by centrifugation at 8,200 × *g* for 10 min in a Lourdes model A-2 centrifuge maintained at 5 C, washed with distilled water, and recentrifuged. The cell pastes were then lyophilized and weighed. Growth of the cultures was measured turbidimetrically with a Klett-Summerson photoelectric colorimeter fitted with a no. 54 green filter.

Antibiotics and antibiotic sensitivity tests. Earl H. Dearborn of the Lederle Laboratories, Pearl River, N.Y., supplied us with chlortetracycline hydrochloride. Polymyxin B sulfate and penicillin G (potassium salt) were purchased from Calbiochem, Los Angeles, Calif. Sensitivity to antibiotics was determined by tube dilution methods by using these antibiotics in the medium specified above for each organism.

Chemicals. The chemicals used in this study were of the highest quality available. All organic solvents were redistilled before use. Authentic samples used in gas-liquid chromatography and thin-layer chromatography were of the highest chromatographic purity available.

Extraction and analysis of readily extractable lipid. Lipids were extracted from the dry cells by the method of Folch, Lees, and Sloane Stanley (10). These lipids (designated FLS lipid or chloroform-methanol-extractable lipid) were then separated by thin-layer chromatography on silica gel G plates prepared from silica gel G obtained from Warner Chilcott Laboratories, Morris Plains, N.J., or on commercially prepared plates of silica gel G obtained from Analtech Inc., Wilmington, Del. All plates had a gel layer of 250 μm and measured 20 by 20 cm. The following solvent mixtures were used for development: (i) chloroform-methanol-water, 65:25:4 (42); (ii) chloroform-methanol-7 N ammonium hydroxide, 35:60:5 (34); and (iii) chloroform-methanol-acetic acid-water, 50:30:8:4 (35). Solvents were allowed to travel 10 cm from the point of sample application. Good separation of lipid samples was also obtained when the thin-layer plates were first developed in solvent system one for 10 cm, dried, and then developed for 14 cm in hexane-ether (4:1).

The bacterial lipid classes separated by thin-layer chromatography were characterized by comparison of their R_F values with the R_F values of standard lipid samples and by the reaction of bacterial lipids with several reagents which were usually applied to the plate in a spray. The reagents used were as follows: iodine vapors (37), ninhydrin (37), 2,7-dichlorofluorescein (37), molybdenum blue (7), rhodamine 6 G (7), periodate-Schiff reagent (33), and diphenylamine reagent (37). Treatment of the chromatograms with iodine vapors, rhodamine 6 G, and 2,7-dichlorofluorescein facilitated the detection of all lipid classes. Ninhydrin treatment was used to identify primary and secondary amines, molybdenum blue treatment was used to detect phosphate ester groups, periodate-Schiff reagent was used to characterize lipids containing alpha glycols, and diphenylamine reagent was used to characterize glycolipids. Permanent spots of all carbon-containing compounds were obtained by charring the plate at 150 C after spraying with 50% sulfuric acid.

Collection of phosphatidyl ethanolamine fractions. Phosphatidyl ethanolamine fractions were collected by preparative thin-layer chromatography on plates with a silica gel G layer (Warner-Chilcott gel) of 500 μm. Chloroform-methanol-extractable lipids were applied to these preparative thin-layer plates in samples of 500 μg, and 20 to 25 samples were applied to each plate. The plates were developed in chloroform-methanol-water (65:25:4). After development, a narrow strip at one side of the plate was sprayed with 2,7-dichlorofluorescein and viewed under ultraviolet light to determine the location of the separated lipids, particularly the phosphatidyl ethanolamine fraction.

Horizontal strips of silica gel containing phosphatidyl ethanolamine fractions were transferred into lipid-free Whatman filter paper Soxhlet thimbles (10 by 50 mm). The suction apparatus used for this transfer consisted of a flask which was connected to a vacuum line and stoppered with a rubber cork through which a glass rod protruded. The Whatman thimble was attached at the inside end of the rod. Only the portion of the gel layer which had not been sprayed with the dye was collected into the thimble. Phosphatidyl ethanolamine was removed from the silica gel by placing the thimble containing the gel in a Soxhlet extractor and refluxing for 2 hr with chloroform-methanol (2:1). The phosphatidyl ethanolamine was then collected by evaporation of the solvent.

Detection of nitrogenous bases. The chloroform-methanol-extractable lipids of *E. coli* B7350 were hydrolyzed with acid to cleave nitrogenous bases from the phospholipids. Lipid (30 mg) was placed in a heavy-wall pyrex digestion tube with 1.5 ml of 1.2 N hydrochloric acid. The tube was evacuated to a pressure of 500 μm of mercury, sealed, and heated at 110 C for 15 hr (24, 25, 31). After heating, water and ether were added to the hydrolysate, the nitrogenous bases were extracted into the water phase, and the water was removed by lyophilization.

The nitrogen-containing residue was characterized by paper chromatography by using Whatman no. 1 paper. Three solvent systems were used: ethyl alcohol-ammonia-water, 90:5:5; ethyl alcohol-glacial acetic acid-water, 90:5:5; and phenol-butyl alcohol-formic acid-water, 50:50:3:10 (13, 14). Paper used in the last developing solvent system was first treated with 1 N potassium chloride and dried. Primary and secondary nitrogenous bases were detected with ninhydrin, tertiary nitrogenous bases with starch and iodine treatment (22), and choline bases with Chargaff's reagent (24).

Extraction and characterization of fatty acids. Total fatty acids were obtained by heating dried cells under nitrogen in a hydrolysis mixture of ethyl alcohol-potassium hydroxide-water (100 ml:9 g:60 ml) refluxed at 79 C for 4 hr. Non-saponifiable material was removed by ether extraction. The water phase was

acidified, and the fatty acids were recovered by extraction into pentane. The pentane was dried over anhydrous sodium sulfate, after which the solvent was removed under reduced pressure. The fatty acids were methylated by the method of Metcalfe and Schmitz (26).

Fatty acids were also liberated from the chloroform-methanol-extractable lipids and phosphatidyl ethanolamine fractions by alkaline hydrolysis and methylated according to the methods described above.

The fatty acid methyl esters were analyzed with a Perkin-Elmer model 154D fractometer fitted with a thermal conductivity detector and two meter columns of diethylene glycol succinate (DEGS) or butanediol succinate (BDS). All analyses were run on both columns, and the retention times of the bacterial fatty acids on each column were compared with the retention times of authentic fatty acid samples. Infrared spectra of the methylated fatty acid samples were obtained prior to gas-liquid chromatography by the use of a Perkin-Elmer model 237 dual-beam grating infrared spectrophotometer over the range of 4,000 cm^{-1} to 667 cm^{-1}.

In some cases, samples of individual fatty acids separated by gas chromatography were collected and further analyzed by infrared spectroscopy.

Methylated fatty acid samples of *K. pneumoniae* G (PM) were hydrogenated under ambient conditions in a solution of ethyl alcohol over a palladium catalyst. A 5% palladium (100 mg) catalyst on charcoal was used for each 5-mg sample of fatty acid. The catalyst was removed by filtration, and the solvent was evaporated under reduced pressure (3, 4, 18). This mild hydrogenation procedure hydrogenates unsaturated bonds and leaves the cyclopropane ring intact.

Methylated samples of fatty acids were brominated after they had been hydrogenated according to the procedure described by Brian and Gardner (4). Treatment with bromine reagent affects cyclopropane-containing fatty acids, causing these acids to have a longer retention time. This procedure enabled the verification of the cyclopropane nature of the fatty acids.

RESULTS

Antibiotic resistance and growth of bacteria. During this experiment, care was taken to grow all strains of the same bacterial species under identical growth conditions. When the bacterial cultures were harvested after 24 hr of incubation, they were at the same point in their growth cycle as is illustrated for *E. coli* strains in Fig. 1. (A Klett reading of 75 obtained after 24 hr of growth corresponds approximately to 10^8 viable cells per cm^3.) This graph also shows that the growth of *E. coli* strain Sc8280 was the same whether grown in the presence or absence of antibiotic. However, the resistant organisms in this study were grown in the presence of antibiotic unless otherwise specified.

Measurements of the growth of *K. pneumoniae* strains and *S. aureus* strains showed that cultures of these bacteria were also harvested at the same point in their growth cycle.

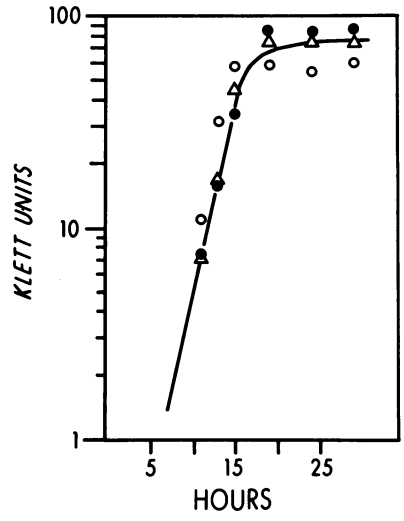

FIG. 1. *Growth curves for* (●) *E. coli Sc8190,* (○) *E. coli Sc8280 grown with chlortetracycline, and* (△) *E. coli Sc8280 grown without chlortetracycline. A Klett reading of 75 obtained after 24 hr of growth corresponds approximately to 10^8 viable cells per ml.*

E. coli strains Sc3552 and Sc8280 were found to be resistant to 90 and 70 μg of chlortetracycline hydrochloride per ml, whereas *E. coli* strains B7350 and Sc8190 would not grow in concentrations of 1 μg of chlortetracycline hydrochloride per ml.

K. pneumoniae G would tolerate greater than 500 μg of polymyxin B sulfate per ml, whereas the polymyxin-sensitive strain of *K. pneumoniae* would not grow in medium containing 1 μg of polymyxin B sulfate per ml, or more.

S. aureus (PR) was resistant to 500 μg of penicillin G per ml, whereas *S. aureus* ATCC 12600 was sensitive to penicillin levels of 1 μg/ml.

Fatty acids. Similar results of fatty acid composition were obtained by using either the DEGS or BDS column as (Table 1) for the total fatty acid composition of *K. pneumoniae* G (PM) grown without antibiotic. Sample chromatograms of methylated fatty acids of *K. pneumoniae* G (PM), *E. coli* B7350, and *E. coli* Sc3552 appear in Fig. 2. The per cent composition of fatty acids in the various fatty acid extracts was determined on the basis of chromatograms such as these. After mild hydrogenation of the total fatty acids of *K. pneumoniae* G (PM), the peaks characterized as C16:1 and C18:1 were altered to behave chromatographically as C16:0 and C18:0, respectively. This hydrogenated sample of fatty

TABLE 1. *Total fatty acid composition of Klebsiella pneumoniae G grown without antibiotic*[a]

Fatty acid	Content[b]	
	BDS	DEGS
	%	%
C12:0	0.7	0.5
C14:0	15.4	15.5
C15cy	1.8	2.3
C16:0	39.9	40.3
C16:1	12.6	11.8
C17cy	6.8	7.7
C18:1	8.3	8.4
C19cy and peak 13	14.7	13.5

[a] Fatty acids were determined by gas chromatography by using the column specified.

[b] BDS, butanediol succinate; DEGS, diethylene glycol succinate.

acid was then treated with bromine reagent, and a chromatogram of this brominated-fatty acid sample showed that only the retention times of C19cy and C17cy peaks were increased. Peaks 6 and 13 are as yet uncharacterized fatty acids.

Infrared analysis of the bacterial fatty acid samples showed typical methylated-fatty acid spectra, including CH_3 stretch at 2,960 cm^{-1}, CH_2 stretch at 2,870 cm^{-1}, ester stretch at 1,750 cm^{-1}, and absorption at 720 cm^{-1}, all characteristic of long-chain fatty acids (1, 8, 36).

Individual components of the total fatty acids of *K. pneumoniae* G were collected after separation on a DEGS column. These fractions were then examined by infrared spectroscopy, and the fractions that were characterized by gas chromatography as containing cyclopropane acids showed absorption at 1,020 cm^{-1}, which is typical of cyclopropane acids (15, 43).

The fatty acids of *S. aureus* strains were found to contain a high proportion of branched-chain acids. The infrared patterns of the fatty acids of *S. aureus* and a representative gram-negative organism, *K. pneumoniae* G, are compared in Fig. 3, and only the spectra of *S. aureus* fatty acids show the strong absorption at 1,370 cm^{-1} characteristic of branched-chain acids (36). The major fatty acid of *S. aureus* is a C15 acid with the same retention time on DEGS and BDS columns as an authentic sample of C15 anteiso branched-chain fatty acid (methyl 12-methyltetradecanoate). This *S. aureus* ATCC 12600 C15 acid was collected after gas-chromatographic separation of the total fatty acids, and was shown to have an infrared absorption peak at 1,370 cm^{-1}.

The composition of the total fatty acids, fatty acids of the chloroform-methanol-extractable lipids, and fatty acids of phosphatidyl ethanolamine fractions of the gram-negative bacteria appear in Tables 2 and 3, and the total fatty acid composition of *S. aureus* strains appear in Table 4.

It was found that in all cases the antibiotic-resistant gram-negative organisms contained a higher concentration of unsaturated acids and a lower concentration of cyclopropane acids than was found in the corresponding sensitive strains. For example, C19 and C17 cyclopropane acids account for 14.2 and 5.8% of the total fatty acids of *K. pneumoniae* G (PM), whereas, in *K. pneumoniae*, these acids account for 37.0 and 9.5% of the total fatty acids, respectively. The sum of unsaturated and cyclopropane acids in the resistant strains is similar to the corresponding sum of unsaturated and cyclopropane acids in the sensitive organisms: e.g., the sum of the cyclopropane acids from the FLS lipids of *E. coli* (TC) is 32.5%, and, in *E. coli* Sc8190, this sum is 33.6%.

In contrast to the gram-negative bacteria, the

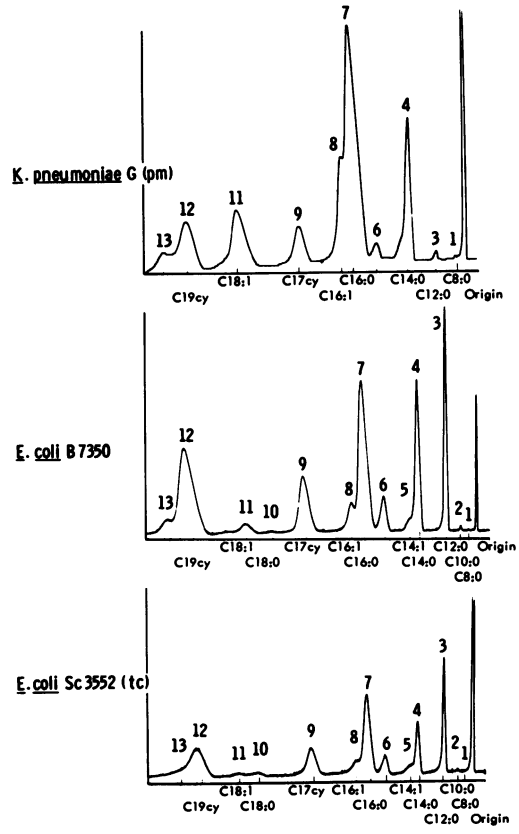

FIG. 2. *Gas chromatogram of the methylated total fatty acids of K. pneumoniae G (PM), E. coli B7350, and E. coli Sc3552 (TC); butanediol succinate column.*

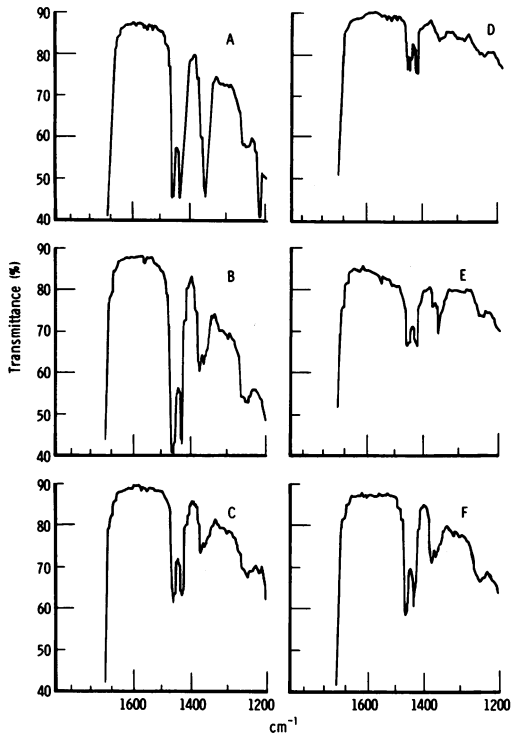

FIG. 3. *Infrared absorption spectra of (A) methylated total fatty acids of S. aureus ATCC 12600, (B) methylated total fatty acids of S. aureus (PR), (C) fraction collected after gas chromatographic separation of the methylated total fatty acids of S. aureus ATCC 12600 and characterized as a C15 branched chain acid, (D) methylated total fatty acids of K. pneumoniae G (PM), (E) authentic sample of methyl 14-methylpentadecanoate, and (F) authentic sample of methyl 12-methyltetradecanoate.*

fatty acids of the penicillin-resistant *S. aureus* are similar both qualitatively and quantitatively to the fatty acids of the antibiotic-sensitive *S. aureus* strain.

Analysis of lipid fractions. The chloroform-methanol-extractable lipids were characterized by thin-layer chromatography in multiple-solvent systems and representative chromatograms are shown in Fig. 4. All lipids could be visualized by treatment with iodine vapors or rhodamine 6 G spray. Those classes of lipids characterized as phosphatidyl ethanolamine and lysophosphatidyl ethanolamine gave a positive reaction with molybdenum blue and ninhydrin reagents, and a negative reaction with periodate-Schiff reagent. The lipids characterized as phosphatidyl glycerol and diphosphatidyl glycerol gave a positive reaction with molybdenum blue and periodate-Schiff reagent and a negative reaction with ninhydrin reagent. The lipids characterized as fatty acid and neutral lipid did not react with molybdenum blue, ninhydrin, or periodate-Schiff reagents. Bacterial lipid classes were also characterized on the basis of comparison of R_F values of standard phosphatidyl ethanolamine, lysophosphatidyl ethanolamine, phosphatidyl glycerol, diphosphatidyl glycerol, fatty acid, and glycerides in solvent systems 1 to 3. Other lipid standards used included phosphatidyl choline, cholesterol, phosphatidyl dimethylethanolamine, phosphatidyl serine, lysophosphatidyl choline, phosphatidyl inositol, and phosphatidic acid. None of the bacterial lipids had R_F values commensurate with the R_F values of this last group of authentic lipid samples.

Thin-layer chromatographic analysis showed that all strains of *K. pneumoniae* and *E. coli* studied have similar lipid components. There was no significant difference in the amount of chloroform-methanol-extractable lipid collected from the antibiotic-sensitive and -resistant gram-negative bacteria. The FLS lipids of *E. coli* B7350 were hydrolyzed with acid, and the nitrogenous bases were collected. Paper chromatography of these bases in three different solvent systems showed only one ninhydrin-positive component which in all cases had the same R_F value as an authentic sample of ethanolamine hydrochloride. The *E. coli* nitrogenous base did not react with Chargaff's reagent (24) or with starch and iodine treatment.

In contrast to the gram-negative bacteria, the lipids of the resistant strain of *S. aureus* contained more lipid than was found in the sensitive *S. aureus* strain. The resistant strain contained 5.45% FLS lipid, whereas the sensitive strain contained 3.41% FLS lipid. In the lipids of the bacterial strains examined in this study, only the *S. aureus* lipids contained a fraction that gave a positive reaction with diphenylamine reagent, indicating that this fraction is glycolipid.

DISCUSSION

Lipids of bacteria resistant and sensitive to antibiotics were characterized in this study. As it is well known (30) that variation in growth conditions can affect lipid composition in bacteria, precautions were taken to insure that bacteria of the same species were grown in the same manner. The resistant organisms were grown in the presence of antibiotic to prevent the emergence of a mutant antibiotic-sensitive strain. It was found, as is shown for the total fatty acid composition of *K. pneumoniae* G (PM), that the fatty acid composition is the same whether the strain is grown with or without antibiotic (Tables 1 and 2).

Three types of fatty acid extracts from gram-

TABLE 2. *Fatty acids of species of Enterobacteriaceae*

Fatty acid[a]	Total fatty acids				Content					
					Fatty acids of chloroform methanol-extractable lipid					
	E. coli, Sc3552 (TC)	*E. coli* B7350	*K. pneumoniae* G (PM)	*K. pneumoniae*	*E. coli* Sc3552 (TC)	*E. coli* B7350	*E. coli* Sc8280 (TC)	*E. coli* Sc8190	*K. pneumoniae* G (PH)	*K. pneumoniae*
					%	%	%	%	%	%
C8:0	0.3	0.1	0.1	0	0	0	0	0	0	0
C10:0	0.6	0.3	0	0	0	0	0	0	0	0
C12:0	16.3	13.6	0.7	1.3	0.1	0.2	0.3	0	0	0
C14:0	10.4	13.7	12.7	12.2	2.0	5.4	7.8	9.8	4.3	5.7
C14:1	6.3	1.3	0.2	1.6	0	0	0	0	0	0
Peak 6	5.4	4.0	1.6	1.9	0	0	0	0	0	1.0
C16:0	21.2	21.5	38.3	20.8	47.2	44.4	56.7	56.7	51.5	42.7
C16:1	2.6	4.7	14.4	3.7	10.7	7.9	3.1	0	15.0	8.2
C17cy	9.8	10.2	5.8	9.5	20.7	23.3	17.0	25.6	9.5	24.0
C18:0	2.0	0.1	0	7.3	0	1.2	0.6	0	0	0
C18:1	1.8	1.8	12.1	4.5	13.0	8.2	8.5	1.7	15.9	7.6
C19cy	16.7	25.3	10.4	37.0	6.3	9.9	3.9	5.3	3.8	10.7
Peak 13	7.2	3.3	3.8	0	0	0	0	0	0	0
Total unsaturated acids	10.0	7.7	26.7	9.9	23.7	16.0	11.6	2.7	30.9	15.8
Total saturated acids (straight chain)	50.5	49.4	51.1	41.6	49.2	51.2	65.4	66.5	55.8	48.4
Total cyclopropane acids[b]	39.1	43.2	21.6	48.5	27.0	33.2	20.9	30.9	13.3	35.7

[a] Number preceding colon indicates number of carbons; number after colon designates degree of unsaturation; cyclopropane acids are represented by the symbol cy.
[b] Includes peaks 6 and 13.

TABLE 3. *Fatty acids from phosphatidyl ethanolamine fractions*

Fatty acid[a]	Content					
	K. pneumoniae	*K. pneumoniae* G (PM)	*E. coli* B7350	*E. coli* Sc3552 (TC)	*E. coli* Sc8190	*E. coli* Sc8280
C12:0	1.0	0	0	0	0	0
C14:0	7.2	5.6	6.9	3.2	11.4	8.9
C14:1	2.1	0	0	0	0	0
C16:0	41.5	48.2	43.5	38.8	43.6	50.2
C16:1	3.9	18.6	7.4	13.6	8.4	7.2
C17cy	27.0	8.6	25.5	23.0	36.6	23.0
C18:1	6.2	13.3	7.1	15.3	0	8.0
C19cy	11.0	5.6	9.6	6.1	0	2.7
Total unsaturated acids	12.2	31.9	14.5	28.9	8.4	15.2
Total saturated acids (straight chain)	48.7	53.8	50.4	42.0	55.0	59.1
Total cyclopropane acids	38.0	14.2	35.1	29.1	36.6	25.7

[a] Number preceding colon indicates number of carbons; number after colon designates degree of unsaturation; cyclopropane acids are represented by the symbol cy.

negative bacteria were studied: fatty acids collected after alkaline hydrolysis of whole cells, fatty acids of chloroform-methanol-extractable lipids, and fatty acids of phosphatidyl ethanolamine fractions. In all fatty acid extracts from the antibiotic-resistant gram-negative organisms, there was a higher concentration of unsaturated acids and a lower concentration of cyclopropane

acids than were found in corresponding extracts from sensitive organisms. The total fatty acids of *S. aureus* resistant to penicillin were similar both qualitatively and quantitatively to the fatty acids of the penicillin-sensitive *S. aureus* strain. This difference in the correlation of the fatty acid composition with antibiotic resistance in gram-negative and gram-positive organisms might be interpreted to suggest that the lipids of these organisms may have different functions.

TABLE 4. *Total fatty acids of Staphylococcus aureus*

Fatty acid[a]	Content	
	S. aureus, penicillin-resistant	*S. aureus* ATCC 12600, penicillin-sensitive
	%	%
C14br	Trace	Trace
C14:0	Trace	Trace
C15br	52.6	50.7
C16br	Trace	Trace
C17br	16.5	18.1
C18:0	4.7	7.6
C19br	12.0	10.4
C20 ?	12.7	11.7

[a] Number preceding colon indicates number of carbons; number after colon designates degree of unsaturation; branched-chain acids are represented by the symbol br.

As mammalian cells do not contain cyclopropane acids (21), it might be hypothesized that a lowering of cyclopropane acid content in the gram-negative bacterial cell results in a fatty acid composition which is more similar to that of mammalian cells, cells which are naturally more resistant to tetracycline and polymyxin than are bacterial cells. Introduction of an unsaturated bond or a cyclopropane ring (especially *cis* configuration) lowers the melting point below that of analogous saturated straight-chain acids or *trans* isomers (15). Thus, the presence of cyclopropane and unsaturated acids in membranes and envelopes may in part be responsible for the nature and integrity of these structures, and may therefore affect the ability of antibiotics to react with or pass through these structures. Further studies on the effects of unsaturated and cyclopropane acids on membrane function would help to elucidate this problem.

The complex lipids of antibiotic-resistant gram-negative bacteria showed no consistent difference from the lipids of the antibiotic-sensitive gram-negative bacteria. However, there was more FLS lipid in a strain of *S. aureus* resistant to penicillin than there was in a penicillin-sensitive strain of

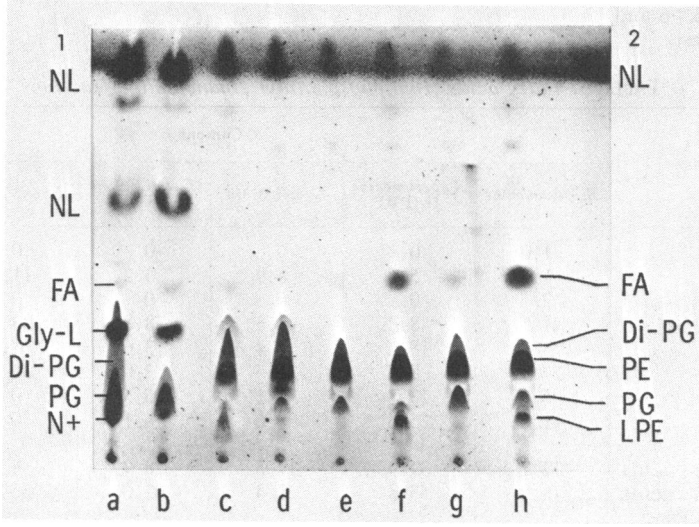

FIG. 4. *Thin-layer chromatogram (Analtech TLC plate) of the FLS lipids of (a) S. aureus (PR), (b) S. aureus ATCC 12600, (c) K. pneumoniae G (PM), (e) E. coli B7350, (f) E. coli Sc3552 (TC), (g) E. coli Sc8280 (TC), and (h) E. coli Sc8190. Development was first in chloroform-methanol-water (65:25:4) for 10 cm and then after drying in hexane-ether (4:1) for 14 cm. The lipids were visualized by charring after treatment with 50% sulfuric acid. S. aureus lipids are characterized in column one, and E. coli and K. pneumoniae lipids are characterized in column two (unlabeled spots are uncharacterized lipid). The abbreviations are: LPE, lysophosphatidyl ethanolamine; PG, phosphatidyl glycerol; PE, phosphatidyl ethanolamine; Di-PG, diphosphatidyl glycerol; FA, fatty acid; NL, neutral lipid; N+, ninhydrin positive lipid; and Gly-L, glycolipid.*

S. aureus. In addition, the resistant strain of *S. aureus* contained a larger concentration of a lipid component tentatively characterized as diphosphatidyl glycerol than was found in the sensitive strain of *S. aureus*.

Bacterial resistance to antibiotics is often attributed to such mechanisms as an enzymatic destruction of the antibiotic, an alteration in the target site of the antibiotic, or to an alteration in the cell which prevents antibiotic uptake. Several reports (2, 16, 38–42) have suggested that, in certain cases, there may be a relation between antibiotic resistance and the lipid content of the bacterial cell. This investigation has explored the correlation of antibiotic resistance with lipid content and has shown a correlation between fatty acid content and antibiotic resistance in the gram-negative bacteria, and between lipid content and antibiotic resistance in the gram-positive bacteria. Whether these findings are limited to the types of organisms and antibiotics so far studied or are still more widespread is yet to be determined.

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