Relationship of Indole Production and Antibiotic Susceptibility in the *Klebsiella* Bacillus

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Received for publication 14 November 1974

Of 2,442 *Klebsiella* strains isolated from clinical specimens at the University of Minnesota hospitals, 18.3% were found to be indole positive. A randomly selected equal number of indole-positive and indole-negative control isolates, characterized by 27 biochemical tests and by serotyping against 72 antisera, were tested against 14 antibiotics. The results indicated a greater incidence of multiple drug resistance among the indole-negative strains than among those that produced indole. The organisms in the former group, in comparison to their indole-positive counterparts, were significantly more resistant to nitrofurantoin, tetracycline, chloramphenicol, neomycin, streptomycin, nalidixic acid, and kanamycin. Both groups of organisms were similar in their degree of resistance to ampicillin, carbenicillin, cephalothin, sulfisoxazole, colistimethate, polymyxin B, and gentamicin. The biochemical properties of the two indole groups were essentially identical. Correlation between serotype and multidrug resistance was inapparent.

Fife et al. (7) reported that 6.0% of the *Klebsiella* strains isolated from patients between 1948 and 1964 gave positive indole reactions. Eickhoff et al. (5) found a similar incidence among their isolates collected during a 6-month period in 1964. On the other hand, there is evidence that the incidence of indole-positive *Klebsiella* among clinically isolated *Klebsiella* strains is greater than had been observed previously. Between September 1966 and July 1972, 18.3% of 2,442 *Klebsiella* strains were indole positive upon initial isolation from patients at the University of Minnesota hospitals, and Martin et al. (10) noted that the indole reaction was positive among 16.9% of the *Klebsiella* strains obtained clinically during a 3-month study in 1970. A report by Duncan and Razzell (3) indicates that, although only 2.4% of *Klebsiella* isolated from forest environments were indole positive, there was a striking 27.6% positive reaction for indole among isolates from farm produce. More recently, Davis and Matsen (2) found indole was produced by one-third of *Klebsiella* strains isolated from a variety of hospitalized and non-hospitalized human subjects. The fact that *Klebsiella*, along with other gram-negative bacilli, have become increasingly im-

portant as agents of serious infections and that there appears to be significant increase in the proportion of indole-positive *Klebsiella* has prompted us to investigate the relationship of indole-positive *Klebsiella* to their serological, biochemical, and, in particular, antibiogrammatic patterns in comparison to the patterns of indole-negative strains.

MATERIALS AND METHODS

During arbitrarily selected periods between September 1966 and July 1972, 2,442 *Klebsiella* strains were isolated from clinical specimens and stored in agar deeps at room temperature. Among this population there were 466 organisms which were indole positive upon initial isolation from the patient. The organisms were arranged in chronological order of isolation and approximately every third indole-positive organism was selected for further study until an arbitrarily predetermined number of 125 was reached. The corresponding group of 125 indole-negative *Klebsiella* was chosen by selecting the indole-negative organism which chronologically preceded each indole-positive strain selected. The total study population was 250 organisms, which approximated 10% of the total *Klebsiella* isolated. Only one isolate from any single patient was included in the study population.

All study organisms were recovered from storage in agar deeps by streaking to sheep blood agar plates from which agar slants were subcultured and subsequently used for testing. Biochemical tests were performed according to the procedures of Edwards

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and Ewing (4) with the exception of the deoxyribonuclease reaction which was done according to the method of Schreier (13). Indole production was determined using tryptophan broth incubated for 48 h, with the subsequent addition of Kovac reagents as described by Edwards and Ewing (4). Antibiotic susceptibility testing was performed by the high content disk method of Bauer et al. (1). Interpretation of zone sizes as sensitive, intermediate, or resistant was based on the recommendations of the Food and Drug Administration (6) and upon determinations by Matsen et al. (11). Serological typing was done according to the procedure outlined by Edwards and Ewing (4) with 72 capsular antisera produced in the Diagnostic Microbiology Research Laboratory at the University of Minnesota.

RESULTS

More than 80% of the indole-negative and indole-positive isolates were from urine, sputum, and wound specimens (Table 1). The percentage of distribution of the organisms among the various sources was consistent with those reported by other investigators (7, 9, 10, 12). There was no apparent difference in the distribution of the two indole reaction groups with regard to the sources.

The biochemical reactions are listed in Table 2. With the exception of the gelatin liquefaction reaction, there was virtually no difference in the biochemical properties between the two groups. In the test for gelatin liquefaction, 25.6% of the indole-positive strains gave a delayed positive reaction after 28 to 30 days, whereas only one positive reaction occurred among the indole-negative *Klebsiella*.

Antibiotic susceptibility and extent of multidrug resistance, on the other hand, varied markedly between the two groups of organisms. Compared with the indole-negative *Klebsiella*, the indole-positive group exhibited a significant shift in decreased resistance to nitrofurantoin, tetracycline, chloramphenicol, neomycin, streptomycin, nalidixic acid, and kanamycin (Table 3). The population distribution graph (Fig. 1) illustrates the extent to which both groups of organisms responded to the action of nitrofurantoin. Responses of both populations are monophasic, but the indole-positive group tends to be distributed over a narrower range than the indole-negative group and is shifted to the right towards the higher sensitivity values. Figure 2 shows the manner in which the two populations are distributed in response to tetracycline. In this case, there is a diphasic distribution of each population in addition to the differences between the two populations in regard to their range and shift. At the 6-mm zone, the number of indole-positive strains is significantly less than the number of indole-negative strains.

TABLE 1. Clinical sources of 250 *Klebsiella* strains

Source	Isolates			
	Indole negative (125 strains)		Indole positive (125 strains)	
	No.	%	No.	%
Urine	43	34	46	37
Sputum	37	30	47	38
Wound	21	17	11	9
Stool	8	6	4	3
Blood	2	2	5	4
Bile	2	2	2	2
Nasæ'	1	1	1	1
Chest	1	1	0	0
Other	10	8	9	7

Similar responses were observed with the other five antibiotics in this first category of significantly differing resistance. On the other hand, the two groups of organisms did not significantly differ in their susceptibilities to the actions of ampicillin, carbenicillin, cephalothin, sulfisoxazole, colistimethate, polymyxin B, and gentamicin (Table 3). For example, the distribution responses of both populations against gentamicin (Fig. 3) and sulfisoxazole (Fig. 4) were superimposable, respectively, but the distributions were monophasic in the former case and diphasic in the latter case. Against sulfisoxazole, 25 strains of each indole reaction group were resistant at the 6-mm zone. The other antibiotics in this second category of insignificant differences evoked similar responses.

The indole-negative *Klebsiella* also demonstrated a significantly higher incidence of multidrug resistance than did the indole-positive group (Table 4). The binomial test was applied as the test for the significance of data on the hypothesis that there is no correlation between indole production and drug susceptibility ($H_0 P_1 = P_2$). The low P values (levels about 0.001) obtained indicate that there are certain antimicrobial agents for which the hypothesis is invalid ($P_1 \neq P_2$) and which, therefore, indicates a probability correlation. An organism was considered multidrug resistant when it demonstrated resistance to three or more antibiotics. Ampicillin and carbenicillin were considered as 1 U as were both of the combinations, polymyxin B-colistimethate and kanamycin-neomycin. Under this definition of multiple resistance, there was an incidence of 13.6% (17 of 125) indole-positive *Klebsiella* and 32% (40 of 125) indole-negative strains. Furthermore, the indole-negative strains possessed multidrug resistance to a greater number of antibiotics than their indole-positive counterparts (Table 4). Cor-

TABLE 2. Twenty-seven biochemical reactions of 125 indole-positive and 125 indole-negative *Klebsiella pneumoniae* strains

Test	Indole positive		Indole negative	
	No. positive	% Positive	No. positive	% Positive
Indole	125	100	0	0
Methyl red	3	2.4	8	6.4
Voges-Proskauer	124	99.2	123	98.4
Simmons citrate	125	100	123	98.4
Lysine decarboxylase	123	98.4	121	96.8
Arginine dihydrolase	1	0.8	4	3.2
Ornithine decarboxylase	0	0	2W ^a	1.6
Malonate	124	99.2	122	97.6
Mucate	125	100	125	100
Urease	124	99.2	112	89.6
Gelatin liquefaction	32	25.6	1	0.8
Nitrate	125	100	125	100
Oxidase	0	0	0	0
Deoxyribonuclease	0	0	0	0
Phenylalanine deaminase	0	0	0	0
Motility	0	0	0	0
Lactose	123	98.4	125	100
Adonitol	125	100	118	94.4
Inositol	125	100	122	97.6
Glycerol	125	100	125	100
Cellobiose	124	99.2	125	100
Arabinose	124	99.2	125	100
Raffinose	125	100	125	100
Rhamnose	125	100	125	100
Salicin	125	100	123	98.4
Sorbitol	125	100	124	99.2
Glucose	125	100	125	100

^a W, Weak positive reaction.

relation between multidrug resistance and gelatin liquefaction was inapparent among those indole-positive organisms capable of hydrolyzing gelatin.

The frequency of occurrence of *Klebsiella* serotypes is shown in Fig. 5. Among the indole-positive strains there were 37 different serotypes of which types 3, 8, 19, 47, 55, 68, and 69 represented 46% of the total strains and contained 53% (9 of 17) of the multidrug organisms (Fig. 5A). There were 45 different serotypes among the indole-negative strains of which types 8, 11, 19, 30, 33, and 56 collectively represented 38% of the strains and contained 65% (26 of 40) of the organisms bearing multidrug resistance (Fig. 5B). The data were insufficient for the determination of the propensity of any *Klebsiella* serotype for multidrug resistance in most instances. However, there is a significant relationship with serotypes 3 and 54 for indole production, and with serotype 30 for the absence of indole production.

DISCUSSION

This study indicates a prevalence of indole-

producing *Klebsiella* from clinical sources in greater numbers than had been seen previously by other investigators (2, 9, 10, 12). Furthermore, it appears that the ability for indole production tends to render the organism more susceptible to the action of antibiotics than when this biochemical trait is absent. Martin et al. (10) reported that the indole-positive strains were more susceptible to tetracycline, streptomycin, chloramphenicol, cephalothin, ampicillin, nalidixic acid, and nitrofurantoin, but not to gentamicin, kanamycin, or polymyxin.

We have shown that biochemical and serological characterization of the organisms within the two indole reaction groups do not reveal any distinguishing differences other than the observation that 25% of the indole-positive *Klebsiella* were also able to hydrolyze gelatin. Fife et al. (7) also reported that 52% (22 of 42) of their indole-positive *Klebsiella* were gelatinase positive, whereas only 1 indole-negative organism from among 663 *Klebsiella* possessed this enzyme.

The ability of an organism to produce indole in a suitable medium is presumably due to the presence of the enzyme, tryptophanase (8, 14),

TABLE 3. Antibiotic susceptibility and multidrug resistance

Antibiotics	Resistant (%)		
	Indole negative (125 strains)	Indole positive (125 strains)	Significance of difference (P)
Nitrofurantoin	27	0	<0.001
Tetracycline	30	6	<0.001
Chloramphenicol	20	2	<0.001
Neomycin	18	2	<0.001
Streptomycin	23	11	<0.005
Nalidixic acid	11	2	<0.01
Kanamycin	17	10	<0.01
Ampicillin	89	95	NS ^a
Carbenicillin	94	91	NS
Cephalothin	14	6	NS
Sulfisoxazole	21	20	NS
Colistimethate	2	0	NS
Polymyxin B	0	0	NS
Gentamicin	0	0	NS

^a NS, Not significant. FIG. 1. Population distribution response of indole-positive (shaded area) and indole-negative (unshaded area) strains of *Klebsiella* against nitrofurantoin.

and its hydrolytic activity against the substrate tryptophan. Since *Klebsiella* chromosomes do not normally produce indole (4), they are not apt to carry the tryptophanase gene, and it follows that such genes are acquired either by transforming, transducing, or conjugational processes. It would appear that the ability of an organism to produce indole interferes with antimicrobial activity either by chemical or genetic means.

Indeed, we have observed in our laboratory that the organisms, upon prolonged storage, spontaneously lose either the antibiotic resist-

FIG. 2. Population distribution response of indole-positive (shaded area) and indole-negative (unshaded area) strains of *Klebsiella* against tetracycline. FIG. 3. Population distribution response of indole-positive (shaded area) and indole-negative (unshaded area) strains of *Klebsiella* against gentamicin. FIG. 4. Population distribution response of indole-positive (shaded area) and indole-negative (unshaded area) strains of *Klebsiella* against sulfisoxazole.

TABLE 4. Extent of multiple drug resistance in 17 indole-positive and 40 indole-negative strains of multidrug-resistant *Klebsiella*

Indole reaction	Number of strains resistant to:						
	3 drugs	4 drugs	5 drugs	6 drugs	7 drugs	8 drugs	9 drugs
Positive	9	7	1	0	0	0	0
Negative	4	11	5	5	4	8	3

FIG. 5. Distribution of serotypes among the 125 indole-positive (A) and 125 indole-negative (B) *Klebsiella* having multidrug resistance against less than three (unshaded area) or three or more (shaded area) antimicrobial agents.

ance determinants or the ability to produce indole. Thus, the transitory nature of these tryptophan. Since *Klebsiella* chromosomes do genotypes suggests that these markers may well be located on mutually exclusive plasmids.

Since multiple antibiotic resistance is a continuing clinical problem, it is pertinent to consider whether or not the acquisition of the capacity to produce indole by the *Klebsiella* produces in and of itself an interference to the acceptance of episomally mediated antimicrobial resistance. Further studies are needed to define this.

The clinical implications of this phenomenon are unclear. Though the indole-producing strains of *Klebsiella* as a group are less likely to be antibiotic resistant, they are similar to non-indole-producing strains in other ways. An understanding of the mechanism of acquiring the ability for indole production and its relationship to the development of antibiotic resistance

could conceivably assist in the further approach to the considerable clinical dilemma of antibiotic resistance.

ACKNOWLEDGMENTS

We gratefully acknowledge the technical assistance of Doris Goldman, William Spring, and Rosemary Wilson, and the secretarial assistance of Marian Wallfred and Debbie Guevara.

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