BIOTYPING AND ANTIBIOTIC SUSCEPTIBILITY PATTERN OF *KLEBSIELLA* SPECIES

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SUMMARY

Two hundred strains of Klebsiella species isolated from clinical specimens over a four-month period were biotyped as ***Klebsiella aerogenes*** *(173 strains),* ***Klebsiella ozaenae*** *(15 strains),* ***Klebsiella edwardsii*** *(5 strains),* ***Klebsiella atlantae*** *(2 strains) and* ***Klebsiella oxytoca*** *(1 strain).* ***Klebsiella aerogenes*** *and* ***Klebsiella ozaenae*** *were more resistant towards antibiotics when compared with other species. Colonial morphology on eosin methylene blue agar (Oxoid) was not found useful for differentiations of* ***Klebsiella*** *biotypes.*

INTRODUCTION

Klebsiella species, in particular, *Klebsiella aerogenes* has become increasingly important as a cause of hospital acquired infection. [1,2,3] Epidemiological investigation of outbreaks has been done by determining the serotypes of the strains concerned by capsular typing, [4] fluorescent antibody technique [5,6] and counter immunoelectrophoresis. [7] *Klebsiella aerogenes* as designated by the taxonomic criteria of Cowan [8,9] is the commonest species isolated in clinical laboratories attached to hospitals. It is often an opportunistic pathogen especially in the urinary and respiratory tracts. Other species of *Klebsiella,* in particular, *Klebsiella pneumoniae* may act as primary pathogens.

The relationship of various biotypes of *Klebsiella* with disease was demonstrated by various authors. [10,11,12] Attempts have also been made to correlate the prevalence of certain serotypes with the site of isolation. [13] Early differentiation of biotype may be useful for management of patients, in particular, the differentiation of *Klebsiella pneumoniae* from *Klebsiella aerogenes,* isolated from the respiratory tract. Methyl red reaction and colonial morphology on eosin methylene blue agar [14] have been used for this purpose. This paper presents the result of biotyping of *Klebsiella* species on the basis of related biochemical tests, derived from the scheme of Cowan and Steel. [9] Susceptibility of the strains towards antibiotics is also described.

MATERIALS AND METHODS

Two hundred strains of *Klebsiella* species isolated from clinical specimens processed in the diagnostic microbiology laboratory, UKM over a four-month period from January to April 1980 were included in this study. *Klebsiella* specie was initially identified as oxidase negative, non-motile Gram-negative

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rods, generally growing as mucoid colonies on MacConkey's agar plate.

Biochemical Tests

The strains were divided into biotypes by conducting the following series of tests on overnight broth culture. Indole production, utilization of citrate and malonate, hydrolysis of urea, methyl red reaction and acetoin production, fermentation of glucose, lactose and dulcitol, and growth in the presence of potassium cyanide. The media and methods used were the same as described by Cowan. [8] The sets of biochemical tests were incubated at 37°C and read after 24 and 48 hours. The sets were further incubated for 5 days, if negative, before discarding.

Colonial morphology on eosin methylene blue agar

Growth characteristics on eosin methylene blue agar (Oxoid) as described by Freeman [14] were evaluated for differentiation of *Klebsiella aerogenes* and *Klebsiella pneumoniae* from other members of the group. *Klebsiella pneumoniae* ATCC 27736 and *Klebsiella aerogenes* NTCC 8172 were included as controls.

Susceptibility towards antibiotics

Antibiotic susceptibility pattern of the strains was determined by modified Stokes [15] method on Diagnostic Sensitivity Test Agar. The strains were tested against 10 ug ampicillin, 30 ug cephalexin, 25 ug chloramphenicol, 25 ug cotrimoxazole, 10 ug gentamicin and 10 ug kanamycin discs. Urinary isolates were tested against 25 ug ampicillin, 25 ug cotrimoxazole, 30 ug cephalexin, 25 ug chloramphenicol, 30 ug nalidixic acid and 200 ug nitrofurantoin discs.

RESULTS

Table I shows the biotypes of *Klebsiella* species isolated from clinical specimens over a four-month period. One hundred and seventy-three out of 200 strains (86.5 percent) belonged to *Klebsiella aerogenes* and 72 of these were isolated from sputum and throat swabs. A total of 56 patients were involved, 16 of them had a positive throat swab as well as positive sputum cultures for *Klebsiella aerogenes*. *Klebsiella ozaenae* was the next major isolate, a total of 15 strains (7.5 percent) majority of which were from sputum and surface swabs, followed by *Klebsiella ewardsii* 5, *Klebsiella pneumoniae* 4 and *Klebsiella oxytoca* 1.

TABLE I
BIOTYPES OF *KLEBSIELLA* SPECIES AND THEIR DISTRIBUTION IN CLINICAL SPECIMENS

Nature of specimen	*Klebsiella aerogenes*	*Klebsiella oxytoca*	*Klebsiella pneumoniae*	*Klebsiella atlantae*	*Klebsiella edwardsii*	*Klebsiella ozaenae*
Pus	28	0	2	0	1	1
Throat swab	33	0	0	1	1	1
Urine	31	0	0	1	0	2
Surface swab	32	0	2	0	2	5
Sputum	39	1	0	0	0	4
Blood	6	0	0	0	1	0
Cerebrospinal	2	0	0	0	0	0
Vaginal swab	2	0	0	0	0	2
Total number	173	1	4	2	5	15

Strains isolated from throat swabs were generally considered to be colonizers rather than pathogens. Thirty patients with positive throat swab culture for *Klebsiella aerogenes* were on broad-spectrum antibiotics. There were six cases of septicaemia caused by *Klebsiella aerogenes* where it was isolated from more than one blood culture bottle within 24 hours of collection of specimens. All six were from the special care nursery and were patients within five days to two weeks of age. Two of these infants also grew *Klebsiella aerogenes* from cerebrospinal fluid culture. There was one case of *Klebsiella edwardsii* septicaemia in an adult.

Table II and III show the antibiotic susceptibility pattern of the isolates. All strains were resistant to ampicillin. *Klebsiella oxytoca* and *Klebsiella edwardsii* were sensitive to cephalosporin, cotrimoxazole, kanamycin and gentamicin. One hundred and thirty-two out of 166 strains of *Klebsiella aerogenes* and 10 out of 13 strains of *Klebsiella ozaenae* were sensitive to cephalexin. One hundred and thirty strains of *Klebsiella aerogenes* were sensitive to gentamicin and 122 were sensitive to kanamycin (Table III).

Urinary isolates were generally more resistant to antibiotics (Table IV) and only 21 out of 31 strains were sensitive to cephalexin, 12 to kanamycin and

TABLE II
REACTION OF 200 STRAINS OF *KLEBSIELLA* SPECIES IN BIOCHEMICAL TESTS.

Species	Total No. of Strains	No. of Positive Strains											
		Motility	Oxidase	Growth in KCN	Utilization of citrate	Indole	Gas in glucose	Methyl Red	Acetoin production	Hydrolysis of urea	Lactose	Malonate	Dulcitol
Klebsiella aerogenes	173	0	0	173	173	0	173	0	173	173	173	173	40
Klebsiella oxytoca	1	0	0	1	1	1	1	0	1	1	1	0	1
Klebsiella pneumoniae	4	0	0	0	4	0	4	4	0	4	4	4	4
Klebsiella atlantae	2	0	0	2	2	0	2	2	1	2	2	0	0
Klebsiella edwardsii	5	0	0	5	5	0	0	4	5	5	5	4	0
Klebsiella ozaenae	15	0	0	15	15	0	14	15	15	15	15	0	9

TABLE III
ANTIBIOTIC SUSCEPTIBILITY PATTERN OF 166 STRAINS OF *KLEBSIELLA* SPECIES ISOLATED FROM CLINICAL SPECIMENS OTHER THAN URINE

Species	Total No. of strains	No. of sensitive strains					
		Ampicillin	Cephalexin	Chloramphenicol	Cotrimoxazole	Gentamicin	Kanamycin
Klebsiella aerogenes	142	0	132	110	129	130	122
Klebsiella oxytoca	1	0	1	1	1	1	1
Klebsiella *pneumoniae*	4	0	4	1	3	4	3
Klebsiella atlantae	1	0	1	1	1	1	1
Klebsiella ewardsii	5	0	5	5	5	4	5
Klebsiella ozaenae	13	0	10	8	10	11	10

23 to gentamicin. Growth on eosin methylene blue agar did not give expected results. One hundred and fourty-five strains of *Klebsiella aerogenes* were tested, but only 3 produced the characteristic metallic sheen. The rest conformed with Type B as described by Freeman and could be mistaken for *Klebsiella pneumoniae*.

DISCUSSION

The isolation of *Klebsiella* species from hospitalized individuals and its role in serious infections is well documented. [1,2,3]

Gut has been recognised as the source of these strains [15] and colonization of the respiratory and urinary tracts is commonly encountered. *Klebsiella aerogenes* was the major isolate in this study, as in that of Cooke *et al.* [16] where 480 out of 501 strains of *Klebsiella* isolated belonged to *Klebsiella aerogenes*. It is also more resistant towards antibiotics than other species of *Klebsiella*, which are likely to act as primary pathogens. Further, urinary isolates had a greater percentage of aminoglycoside resistance than isolates from other sites. This finding is similar to that of Riser *et al.* [13] They also demonstrated that certain serotypes were isolated from a particular type of specimen. Virtually, all strains were resistant to ampicillin and this perhaps is a reflection on the extensive use of this antibiotic in our hospitals. A higher percentage of resistance was also demonstrated towards kanamycin making it unsuitable as a first-line agent for treating *Klebsiella* infections.

The current status of *Klebsiella* taxonomy and regional differences in nomenclature [17] are beyond the scope of this paper. The criteria of Cowan [8] were adhered to and were found useful for the differentiation of various members of *Klebsiella* group, although biotyping took three to five days.

TABLE IV
ANTIBIOTIC SUSCEPTIBILITY PATTERNS OF 34 STRAINS OF *KLEBSIELLA* SPECIES ISOLATED FROM URINE

Species	Total No. of Strains	Number of sensitive strains								
		Ampicillin	Cephalexin	Chloramphenicol	Cotrimoxazole	Gentamicin	Kanamycin	Nalidixic acid	Nitrofurantoin	Sulphonamide
Klebsiella aerogenes	31	0	21	9	15	23	12	28	27	2
Klebsiella ozaenae	2	0	1	0	1	1	1	1	1	0
Klebsiella atlantae	1	0	0	0	1	0	0	1	1	0

It was not possible to differentiate between *Klebsiella aerogenes, Klebsiella pneumoniae* and other species on the basis of growth characteristic on eosin methylene blue agar. Determination of antibiotic susceptibility pattern was useful in recognising particular strains. This, together with biotyping may be used for epidemiological studies on *Klebsiella* infection in the absence of serotyping facilities.

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