Emergence of Gentamicin-Resistant *Klebsiella* in a General Hospital

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Received for publication 5 August 1976

Gentamicin had been in use in a general hospital for over 7 years before any gentamicin-resistant *Klebsiella* were observed. In 1974 and 1975, nine different gentamicin-resistant serotypes of *Klebsiella* were isolated from 35 patients. The first strain to appear had R-factor-mediated gentamicin resistance, and it infected 19 patients during a period of almost 2 years, spreading largely by case-to-case infection in patients with urinary catheters. It appeared to lose the capacity to transfer its gentamicin resistance after it had infected five of the patients. We had previously isolated on the same ward a gentamicin-susceptible *Klebsiella* of identical type, and it was found to be capable of acquiring an R-factor for gentamicin resistance. All of the other types of gentamicin-resistant *Klebsiella* infected few patients and did not persist in the hospital; four of them had R-factor-mediated resistance to gentamicin and all four, as did the original strain, cotransferred kanamycin, neomycin, and tobramycin resistance. Every gentamicin-resistant *Klebsiella* was susceptible to amikacin and netilmicin.

The appearance of multiresistant strains of *Klebsiella* in hospitals has been clearly associated with the widespread use of antimicrobial agents (12, 13, 16, 17). Strains of *Klebsiella* isolated from healthy individuals who had no association with hospitals were shown by Davis and Matsen (5) to be resistant only to ampicillin and carbenicillin. By contrast, these workers found that strains carried by hospital patients in the same city were frequently resistant to many other antibiotics and often possessed transmissible R-factors.

Until recently even multiresistant hospital strains of *Klebsiella* have retained their susceptibility to gentamicin. There have been few reports of gentamicin-resistant *Klebsiella* causing outbreaks of nosocomial infection (8, 10). In these cases the gentamicin resistance was transferable from the *Klebsiella* to recipient laboratory strains of *Escherichia coli*. In our own hospital, gentamicin was used for 7 years before the first gentamicin-resistant strains of *Klebsiella* appeared. The present report gives an account of their emergence and spread and describes the main characteristics of the gentamicin-resistant strains.

**MATERIALS AND METHODS**

**Identification and typing of *Klebsiella***. All of the *Klebsiella* studied were clinical isolates from inpatients at Sunnybrook Medical Centre, Toronto, which is a 1,000-bed teaching hospital with almost equal numbers of acute and chronic patients. Isolates were first identified in the diagnostic laboratory as *Klebsiella* species by standard methods (7). The capsular serotypes of gentamicin-resistant isolates were determined by quelling reaction by the method reported by Casewell (3). Typing antisera against the 72 recognized capsular types of *Klebsiella* were prepared by immunizing rabbits and were absorbed to remove cross-reacting antibodies (7). The gentamicin-resistant *Klebsiella* were further subdivided by the numerical biotyping scheme that we described previously (14). The results of the two typing methods were combined as the serotype of the strain, and this provided a more discriminating system for differentiating *Klebsiella* than did either typing method used alone.

**Antimicrobial susceptibility testing**. Routine susceptibility tests were done in the diagnostic laboratory by inoculating isolates with a Steers inocula replicator to Mueller-Hinton agar (BBL) plates containing antimicrobials in the following concentrations: chloramphenicol (Ch), 16 μg/ml; tetracycline (Te), 4 μg/ml; cephalothin (Ce), 16 μg/ml; ampicillin (A), 16 μg/ml; carbenicillin (Ca), 100 μg/ml; colistin (Co), 10 μg/ml; nalidixic acid (Na), 12 μg/ml; nitrofurantoin (Ni), 30 μg/ml; sulfamethoxazole (Su), 10 μg/ml; kanamycin (K), 8 μg/ml; and gentamicin (G), 4 μg/ml. Isolates not inhibited at these concentrations were considered resistant for the purposes of clinical reporting. Isolates not inhibited by 4 μg of gentamicin per ml had full minimal inhibitory concentration (MIC) estimations performed for gentamicin, streptomycin (Str), neomycin (Ne), kanamycin, tobramycin (To), sisomycin (Si), amikacin, and netilmicin (an experimental semisynthetic aminoglycoside developed by Schering Corp. and origi-
nally designated Sch 20569). Serial two fold dilutions of the antibiotics were made so that their final concentrations in Mueller-Hinton agar ranged from 0.25 to 128 µg/ml. The inoculum for MIC estimations was an 18-h broth culture diluted in saline to contain $2 \times 10^8$ bacteria/ml; the number transferred by the inocula replicator to each plate was approximately $1 \times 10^6$ organisms.

Cephalosporinase activity of Klebsiella isolates was determined qualitatively by a modification of an iodometric method (9, 11) with cephalothin (2.5 mg/ml) as the substrate. The reaction mixture consisted of 1 ml of cephalothin solution and 1 ml of an overnight broth culture of Klebsiella. After 2 h of incubation at 37°C, 0.4 ml of iodine reagent and 0.1 ml of soluble starch (0.4%, wt/vol) were added; the formation of a blue color that disappeared within 1 min was recorded as a positive test. Appropriate positive and negative controls were included in the assay.

Transfer of gentamicin resistance. Conjugation experiments were performed by mixing diluted (1:100) overnight broth cultures of gentamicin-resistant Klebsiella with a similarly diluted culture of a mutant strain of E. coli K-12 (lac" hve" cys" His") that was resistant only to nalidixic acid (MIC > 1,000 µg/ml) and had a gentamicin MIC of 0.25 µg/ml. This recipient strain was kindly provided by R. B. Grant, Hospital for Sick Children, Toronto. The mating mixtures were made in a 1:5 ratio of donor to recipient. After overnight incubation at 37°C, the mixed cultures were plated on brain heart infusion agar (BBL) and MacConkey agar (Oxoid), each containing 100 µg of nalidixic acid per ml and 4 µg of gentamicin per ml. Neither the donor strain nor Klebsiella nor the recipient E. coli would grow on the selective media, even after 72 h of incubation. Representative colonies from the antibiotic selection plates were picked and purified on antibiotic-free medium. Antimicrobial susceptibility tests and gentamicin MIC estimations were done on transconjugants from these experiments to determine the nature of the resistance determinants that had been transferred by conjugation.

RESULTS

Incidence and epidemiology of gentamicin-resistant Klebsiella. Gentamicin was released for general use in Canada in August 1966, and, as shown in Table 1, use of the antibiotic in our hospital has increased steadily since. In the 6-year period from January 1968 to March 1974, gentamicin-resistant Klebsiella were not isolated from patients in the hospital. Then, from April 1974 to the end of 1975, 98 gentamicin-resistant Klebsiella were found among a total of 2,300 isolates identified as Klebsiella species in our laboratory. These resistant strains were isolated from 35 patients on nine different wards. They were found only in urine specimens in 23 of the 35 cases, only in sputum in 4 cases, in wound specimens in 2 cases, and in blood cultures in 2 cases. In the other four patients, the resistant Klebsiella were isolated from more than one body site, but both urinary and respiratory tracts were involved in each case.

The gentamicin-resistant Klebsiella were subdivided by combined typing into eight distinct serobiotypes (Table 2). The 27 isolates obtained from five patients did not react with any of our 72 monospecific antisera of the international set, but did give positive quellung reactions with an antisera (labeled SK1) prepared by immunizing rabbits with one of these isolates. Two other strains did not type with the standard antisera or with antisera SK1. These two strains, NT:4/3/4 and NT:1/1/1, were differentiated from each other by their biotypes and from other isolates by the combination of biotype and failure to react with any of the antisera. Table 2 shows that four of the resistant serobiotypes were isolated from only one patient each and that serobiotypes SK1:1/1/1, NT:4/3/4, and K45:1/1/2 each infected only a very small number of patients over a relatively short period of time. We also found that these strains did not spread from one ward to another.

By contrast, gentamicin-resistant Klebsiella of serobiotype K22:1/1/2 have persisted in the hospital for almost 2 years since they were first isolated in April 1974. In all, gentamicin-resistant type 22 Klebsiella with indistinguishable biochemical characteristics were isolated from 20 patients on five different wards. One strain of serobiotype K22:1/1/2 differed from the other 19 strains, which were entirely homogeneous in their antibiotic susceptibilities, in having much lower MICs for tetracycline, neomycin, and sulfa- 

<table>
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<th>Year</th>
<th>Amt of gentamicin used*</th>
<th>Total no. of Klebsiella isolates</th>
<th>No. of gentamicin-resistant Klebsiella isolates</th>
<th>No. of patients infected</th>
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<tr>
<td>1968</td>
<td>1,800</td>
<td>1,460</td>
<td>0</td>
<td>0</td>
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<td>1969</td>
<td>1,200</td>
<td>1,160</td>
<td>0</td>
<td>0</td>
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<tr>
<td>1970</td>
<td>2,600</td>
<td>990</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1971</td>
<td>4,700</td>
<td>1,270</td>
<td>0</td>
<td>0</td>
</tr>
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<td>1972</td>
<td>5,300</td>
<td>1,070</td>
<td>0</td>
<td>0</td>
</tr>
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<td>1973</td>
<td>6,200</td>
<td>1,190</td>
<td>0</td>
<td>0</td>
</tr>
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<td>1974</td>
<td>7,200</td>
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<td>46</td>
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</tr>
<tr>
<td>1975</td>
<td>9,900</td>
<td>1,440</td>
<td>52</td>
<td>12</td>
</tr>
</tbody>
</table>

* Expressed as the number of 80-mg vials.
infection. The first, which is illustrated in Fig. 1, affected 14 patients on three wards between April and December 1974. As indicated in Fig. 1, the spread of these type 22 *Klebsiella* from a chronic care ward to a general medical ward and subsequently to an urology ward occurred when patients were transferred from one ward to another. Between January and July 1975, gentamicin-resistant type 22 *Klebsiella* were not isolated from any specimens sent to the diagnostic laboratory. Then, five patients on an orthopedic ward acquired the strain during the remaining 5 months of the year. There was no evidence of patients on other wards being involved during this second limited series of infections.

Table 3 shows the association of bladder catheterization and the acquisition of gentamicin-resistant *Klebsiella*. Of the 27 patients from whom urinary isolates were obtained, 22 had an indwelling catheter in place when the strain was isolated, 1 had been catheterized shortly before, and 4 were seriously debilitated patients with no history of bladder instrumentation. Both patients with bacteremia had bladder catheters in place when the *Klebsiella* was isolated from blood cultures, and this is a probable route of entry of the organisms. However, urine samples were not submitted at the times that would have been necessary to prove a urinary infection by the organism. Two of the four patients with resistant *Klebsiella* in their sputum were intubated and received frequent bronchial suction, one had recent anesthesia with intubation, and the last had no respiratory manipulations but was in the terminal stage of bronchogenic carcinoma. All except 2 of the 35 patients infected with gentamicin-resistant *Klebsiella* had received antibiotics prior to their acquisition of the strain; only 11 of them had received gentamicin.

**Characteristics of the gentamicin resistance.** The range of gentamicin MICs found in strains of *Klebsiella* obtained from the 35 patients is shown in Table 4. Although considerable variation was observed in the MICs of strains of the same serobiotype isolated from different patients, the gentamicin MICs of repeat isolates of the same serobiotype recovered from any particular patient remained constant.
The gentamicin-resistant *Klebsiella* isolated from 15 of the 35 patients transferred their gentamicin resistance to the recipient *E. coli* strain. Table 5 shows that the 10 strains of the four capsular types K37, SK1, K45, and K9 all transferred gentamicin resistance and lists the frequency of transfer and the other antibiotic resistances that were cotransferred in each case. However, only the strains isolated from the first 5 of the total of 19 patients involved in the chain of cross-infection with the type 22 *Klebsiella* transferred their gentamicin resistance. The first strain isolated cotransferred K Ne To Ch Te resistance, those from the next 4 patients infected co-transferred these five plus A Ca, and those from the remaining 14 patients failed to demonstrate any transferable gentamicin resistance. Since we selected only for transferable gentamicin resistance, we do not know whether the strains that did not transfer their gentamicin resistance had any other transferable resistance determinants.

Among all of the *Klebsiella* that were shown to transfer their gentamicin resistance, resistance to kanamycin, tobramycin, and neomycin was cotransferred in each case and resistance to chloramphencol was also cotransferred. Although a number of the strains were resistant to sisomicin and streptomycin (Table 2), these resistances were never cotransferred.

**Origin of gentamicin-resistant *Klebsiella* at Sunnybrook Medical Centre.** About a month before the appearance of the first gentamicin-resistant *Klebsiella*, which was of serotype K22:1/1/2, a gentamicin-susceptible strain (MIC = 0.5 μg/ml) was isolated from a catheter urine and the amputation wound of another patient on the same chronic care ward. The serotype of this strain of *Klebsiella* was also K22:1/1/2; it was resistant to ampicillin, carbenicillin, nitrofurantoin, kanamycin, neomycin, and streptomycin and susceptible to other antimicrobials.

To determine whether the gentamicin-susceptible type 22 *Klebsiella* was capable of acquiring an R-factor, a gentamicin-resistant *E. coli* transconjugant, recovered from the mating of the first resistant type 22 *Klebsiella* with the *E. coli* recipient strain, was used as a donor culture and was mated with the gentamicin-resistant type 22 *Klebsiella*. "Laboratory-derived" gentamicin-resistant type 22 *Klebsiella*, with cotransferred resistance to chloramphenicol, tetracycline and tobramycin, were recovered after the mixed culture was plated on selective media containing gentamicin (4 μg/ml) and ampicillin (16 μg/ml). This laboratory-derived strain was then found to be capable of transferring its gentamicin resistance to the original *E. coli* recipient.

**Activity of other aminoglycosides against gentamicin-resistant *Klebsiella*.** All of the strains had kanamycin MICs > 64 μg/ml, and all had neomycin MICs > 128 μg/ml, except for the single strain of K22:1/1/2 (P) which had an MIC of 8 μg/ml. The tobramycin MIC of every strain was consistently the same as its gentamicin MIC. Resistance to streptomycin varied with different serotypes. Types SK1, K45, K9, and K21 had streptomycin MICs < 8 μg/ml, whereas the MICs of the other five types were all >128 μg/ml. Sisomicin MICs ranged from 4 to 16 μg/ml with all of the types of *Klebsiella*. All serotypes had amikacin MICs of 1 to 2 μg/ml, except for the K22:1/1/2 (P) strain which had an MIC of 16 μg/ml. Netilmicin showed the lowest MICs of all the aminoglycosides tested. All of the gentamicin-resistant *Klebsiella* had netilmicin MICs of 0.25 or 0.5 μg/ml.

**DISCUSSION**

Despite the increasing use of gentamicin in our hospital since it was first introduced about 8 years ago, gentamicin resistance has been slow to develop in strains of *Klebsiella* isolated...
in the institution. Other workers (10) have suggested that the incidence of gentamicin-resistant *Klebsiella* may be directly related to the amount of the antibiotic used in a hospital, but we did not find any obvious association between gentamicin usage and the number of resistant *Klebsiella* we isolated (Table 1). Most of the resistant strains failed to spread in the hospital. The small outbreaks of infection that did occur were largely the result of case-to-case spread of urinary tract infections on individual wards, sometimes following the transfer of an infected patient from one ward to another.

It is probable that the emergence of gentamicin-resistant *Klebsiella* in our hospital resulted from the in vivo acquisition of an R-factor by a strain previously susceptible to gentamicin. This strain was of an identical serotype (K22:1/1/2) to that of the first gentamicin-resistant *Klebsiella*; it was isolated earlier on the same ward, and it was shown to be capable of acquiring an R-factor mediating gentamicin resistance. Only gentamicin-resistant type 22 *Klebsiella* were isolated from the index case (Fig. 1) and, therefore, the exact sequence of events that led to the R-factor acquisition could not be determined. However, transferable gentamicin resistance has been described in other bacteria found commonly in hospitals (2, 15, 18), and it seems likely that the source of the R-factor for gentamicin resistance in this *Klebsiella* was another enteric bacterium.

Of particular clinical significance was the observation that the resistant type 22 *Klebsiella*, the strain with a known gentamicin-susceptible precursor, was the only strain that persisted for more than a few months. This strain was also one in which the R-factor for gentamicin resistance appeared to lose its conjugative capacity and was not detected either after the strain had cross-infected a number of patients or when it reemerged in a different area of the hospital after a latent period of several months. We did not attempt transfer-enhancing techniques and, therefore, we cannot say with absolute certainty that the R-factor in the later type 22 isolates had become nonconjugative. However, even if the R-factor in these later isolates was conjugative, it was only present in low enough levels to be detected by our simple methods, these findings still raise the question of the stability and survival advantage of this kind of strain over other strains possessing R-factors that transfer them at higher frequencies. In another study it was shown, while somewhat artificial, that enterobacteria carrying transferable R-factors survived less readily in the gut than strains which appeared to be R-factor free (1). It may well be that the gentamicin-resistant type

22 *Klebsiella* is peculiar in having a mechanism which assists its ability to survive by suppressing the frequency of R-factor transfer, deletion of an undesirable portion of an R-factor, or stable integration into the chromosome. Further studies will be required to determine whether one of these mechanisms is operating in this strain.

Our resistant *Klebsiella* have not yet been tested for gentamicin-inactivating enzyme activity, and therefore we cannot discount other mechanisms of inactivation of the antibiotic (4). However, an enzymatic mechanism seems the most probable, particularly as kanamycin, tobramycin, and neomycin, but not other aminoglycosides, were cotransferred in every strain that transferred its gentamicin resistance. Since the specificity of an aminoglycoside-3'-transferase has been shown to reside in these substrates (6), it will be necessary to have our strains examined for such an enzymatic mechanism of gentamicin inactivation. The likelihood of finding a single enzyme responsible for R-factor-mediated gentamicin resistance in our *Klebsiella* seems quite high and could have significant epidemiological implications for other enterobacteria in our hospital that are capable of acquiring R-factors.

Of all the aminoglycosides tested, only amikacin and netilmicin inhibited all of the gentamicin-resistant *Klebsiella* at levels that can be achieved in the blood with normal doses. In particular, every strain was highly susceptible to netilmicin. Streptomycin and sisomicin MICs were variable, depending on the strain or serotype isolated. All of the gentamicin-resistant strains were susceptible to colistin and nalidixic acid. However, as both of these have definite therapeutic limitations, the efficacy of amikacin and netilmicin against gentamicin-resistant *Klebsiella* is likely to be of real clinical importance in the future.

ACKNOWLEDGMENTS

This investigation was supported by National Health Grant No. 606-1104-28 awarded by Health and Welfare, Canada.

We wish to thank K. Kruschel for her skilled technical assistance and J. J. Iazzetta, Pharmacy Department, Sunnybrook Medical Centre, for providing the data on gentamicin usage.

LITERATURE CITED

