The changing ecology of hospital bacteria and the selective role of cephalosporins

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SUMMARY

More than 12800 clinical isolates from 115373 in-patient specimens obtained at the Sir Charles Gairdner Hospital, Perth, Western Australia, were identified and analysed statistically for relationships with usage of three generations of cephalosporins over the 5-year period from July 1984 to June 1989. A positive relationship between cephalosporin usage and significantly increasing isolation rates for those species capable of producing chromosomal β-lactamases was observed. Simultaneously, a small increase in the isolation frequency of non-chromosomal β-lactamase-producing strains was noted and no correlation with cephalosporin usage was demonstrated. The trend toward predomination in the hospital environment of strains possessing substantial cephalosporin resistance has implications for future antimicrobial policy, choice of empiric therapy and the predictive value of standard antimicrobial susceptibility tests.

INTRODUCTION

The emergence of resistance to cephalosporins, during the treatment of infections caused by certain members of the Enterobacteriaceae family and *Pseudomonas aeruginosa*, has been widely addressed in the literature since the early 1980s [1–4].

Several mechanisms of resistance have been postulated. Initially, induction or unstable derepression of chromosomal β-lactamase was thought to be of clinical significance [5]. A second mechanism is selection of stably derepressed hyper-producers of chromosomal β-lactamase by β-lactam antibiotics [1, 3, 6–9]. Of these two mechanisms, which frequently occur together, only the latter is now being proposed as having clinical importance [10]. The ability of some bacterial species to display both these mechanisms of resistance has lead to confusion [12]. Species exhibiting these two resistance mechanisms include *P. aeruginosa*, *Acinetobacter calcoaceticus*, *Morganella morganii*, indole-positive *Proteus* species and members of the genera *Serratia*, *Citrobacter* and *Enterobacter*. A third mechanism is that of mutation of plasmid-borne genes encoding TEM or SHV-type β-lactamases and increasing the substrate spectrum to include third generation cephalosporin and monobactam antibiotics [13]. This mechanism has recently been reported in Australian isolates of *Klebsiella pneumoniae* but was not found in chromosomal β-lactamase-producing species [11].
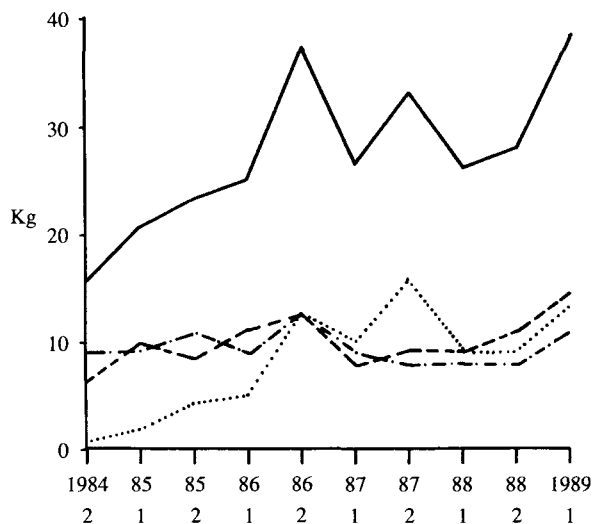
Fig. 1. Six-monthly (1 = Jan–June; 2 = July–Dec.) cephalosporin usage at Sir Charles Gairdner Hospital 1984–9. Total cephalosporins (——); 1st generation (—·—); 2nd generation (— —); 3rd generation (······).

The degree to which the selection of stably derepressed mutants contribute to therapeutic failures during treatment with cephalosporins is a cause for concern. The frequency with which this type of resistance emerges was reported to be from 14 to 56 % of patients infected with these organisms. Of these patients, clinical failure or relapse occurred in 25–75 % of cases [4].

A factor which may contribute to the overall problem of cephalosporin resistance, but has hitherto received little attention, is the increasing use of cephalosporins. The aim of this study was to determine what effect, if any, increasing use of cephalosporins may have on the ecology of these organisms in the hospital environment.

METHODS

The setting

The Sir Charles Gairdner Hospital located in Perth, Western Australia, is a 670-bed teaching hospital, supporting a wide range of surgical and medical specialities.

Bacterial species and isolation rates

The survey analysed a total of 12 883 clinical isolates from 115 373 selected specimens received from in-patients during the period from July 1984 to June 1989. From 1984 to 1986 all isolates were identified by API Systems (Montalieu Vercieu, France) and subsequently by Vitek AMS (Hazelwood, Missouri, USA).

Isolation rates were determined for the various members of the Entero-bacteriaceae family and for *P. aeruginosa* for each 6-month period of the survey. Isolates were placed in one of two groups dependent upon the recognized capacity of the species, but not individual strains, to produce a chromosomally-mediated β-lactamase of the Richmond and Sykes Class 1 type [4]. Since testing of individual strains for stable derepression mutation or β-lactamase induction was not performed for the total period of the survey, the following groupings of genera

were adopted. Group 1 included *Enterobacter* species, *Citrobacter* species, *Serratia marcescens*, *A. calcoaceticus*, indole-positive *Proteus* species, *M. morganii* and *P. aeruginosa*, while *Escherichia coli*, *Proteus mirabilis* and *Klebsiella* species were included in group 2. Only isolates deemed to be significant on clinical grounds were included. Specimens collected in the hospital's admission centre and isolates from those specimens were identified by our data-collection system and excluded from the study. Community-acquired strains may contribute significantly to the ecology of hospital bacteria especially when resistant strains are introduced into an environment with existing selective pressures. However, since these strains have formed part of the total ecological picture only momentarily and a large proportion of them are cephalosporin susceptible, they may mask trends in the established population of hospital strains.

Isolation rates for each 6-month period were expressed as a percentage of two different denominators. The first denominator used was the total number of selected clinical specimens received by the laboratory. The selected specimens included urine, sputum, peritoneal fluid, wound drainage fluid, wound swabs and blood cultures. The second denominator examined was 100 patient admissions [14, 15].

Use of antimicrobial agents

The hospital pharmacy provided figures for the total amount, in grams, of cephalosporins purchased for each 6-monthly period of the survey. This was considered to adequately reflect usage throughout the survey. Cephalosporins were divided into 1st generation (cephalothin and cephalexin), 2nd generation (cephamandole and cefoxitin) and 3rd generation (cefotaxime, ceftriaxone and ceftazidime).

Statistical methods

Usage rates for the three generations of cephalosporins and total usage were correlated with isolation rates for the two groupings of bacteria. Poisson regression analysis was performed using the GLIM (version 3.77), Royal Statistical Society London, computerised statistical programme. Plots of overlapping (moving) averages were constructed to demonstrate trends in isolation rates. The percentage difference between high and low points on the trend plots gave a clear indication of relative isolation rate changes between the two groups. Poisson regression coefficients, standard errors and P values were calculated. A negative regression coefficient indicates a negative relationship between antimicrobial usage and bacterial isolation rate per 100 specimens. Positive regression coefficients, more than twice the standard error, and P values < 0.001, suggest a strong positive or causal relationship [16] between usage of the antimicrobial group and isolation rates.

RESULTS

The usage of cephalosporins throughout the 5-year study period is presented in Fig. 1. Of the total, cephalexin usage for urinary tract infections dominated the 1st generation group while cephamandole use in surgical prophylaxis accounted for the 2nd generation almost exclusively. Use of cephamandole has remained

Table 1. *Results of Poisson regression analysis of cephalosporin usage and isolations of grouped bacterial species (corrected for specimen numbers)*

	Poisson regression coefficient/standard error (P value)		
Cephalosporin group	Chromosomal β-lactamase producers	*Pseudomonas aeruginosa*	Non-chromosomal β-lactamase producers
C1G	0·2474/0·1402 (0·0775)	0·2926/0·0858 (< 0·001)	0·0797/0·0584 (0·365)
C2G	−1·061/0·2103 (< 0·001)	−0·4837/0·1235 (< 0·001)	0·0399/0·0815 (0·754)
C3G	0·1330/0·0309 (< 0·001)	0·1461/0·019 (< 0·001)	0·011/0·012 (0·172)
Total cephabsporin	0·3854/0·1220 (< 0·001)	0·4506/0·0748 (< 0·001)	0·101/0·054 (0·625)

relatively constant throughout the period surveyed in the presence of increasing admission numbers. Nevertheless, cephamandole contributed to one third to one half of the total cephalosporins used and may have been expected to contribute significantly to any selective pressures. The most significant changes occurred with the 3rd generation group which also constituted one third to one half of the total usage since 1986.

The results of statistical correlation analyses between usage rates of 1st, 2nd and 3rd generation cephalosporins and the total usage, and isolations numbers, corrected for selected specimen numbers, are presented in Table 1. For the chromosomal β-lactamase-producing Enterobacteriaceae and *P. aeruginosa* there were significant causal relationships with the 3rd generation cephalosporins but not the 2nd generation. The 1st generation cephalosporins showed a strong relationship with the Enterobacteriaceae but not with *P. aeruginosa*. In contrast, no evidence of any cephalosporin-mediated selective pressure upon non-chromosomal β-lactamase-producing strains was found. Minimal selective pressures by cephalosporins on non-chromosomal β-lactamase-producing strains are indicated by the lack of correlation with all cephalosporin groupings. This finding is supported by the fact that cephalosporin resistance is low in the Sir Charles Gairdner Hospital. The percentage susceptible ranges for *E. coli, P. mirabilis* and *Klebsiella* species combined were 85–90 % for oral cephalosporins, 90–97 % for cephamandole and > 99 % for 3rd generation cephalosporins, over the period surveyed.

Isolation numbers, specimen numbers and isolation rates expressed as a percentage of specimen numbers are presented in Table 2. Graphical representations of this data for the three groups of organisms (Figs. 3–5), include plots of moving averages to show trends. The trend plots reveal significant increases in the isolation rates of all the chromosomal β-lactamase producers including *P. aeruginosa*. Variations between low and high points on the trend plots are + 60·8 % for the former and + 64 % for the latter. The isolation rate for the *E. coli, Klebsiella* sp, *P. mirabilis* group increased by only 15 %.

Total numbers of selected specimens per 6-monthly period are presented graphically in Fig. 2 for comparison with isolation rate trends derived using various denominators (Figs 3–5, 7).

Isolation rates, expressed as a percentage of patient admissions, are presented in Table 3. Graphical representations of this data (Fig. 7) showed a moderate negative variation of − 37 % (trend plot not shown) for the non-chromosomal β-lactamase-producing strains.

Table 2. *Isolation numbers and rates percent of specimens for three groupings of bacterial species*

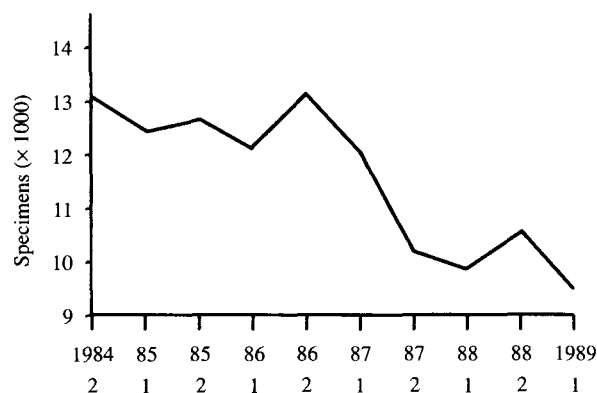|					No. of isolations (% of specimens)					
Strains	1984	1985	1985	1986	1986	1987	1987	1988	1988	1989
	2	1	2	1	2	1	2	1	2	1
Chromosomal β-lactamase producers	142 (1·1)	126 (0·9)	111 (0·9)	132 (1·1)	175 (1·3)	125 (1·0)	124 (1·2)	155 (1·6)	143 (1·4)	166 (1·8)
Pseudomonas aeruginosa	127 (2·0)	300 (2·4)	295 (2·3)	404 (3·3)	499 (3·8)	374 (3·1)	367 (3·6)	380 (3·8)	370 (3·6)	365 (3·9)
Non-chromosomal β-lactamase producers	818 (6·4)	911 (7·3)	828 (6·6)	824 (6·8)	832 (6·3)	941 (7·7)	625 (6·1)	730 (7·4)	743 (7·2)	751 (7·9)
No specimens	12844	12361	12563	12177	13203	12247	10219	9887	10384	9488

Fig. 2. Total selected specimens from in-patients of Sir Charles Gairdner Hospital for ten 6-monthly periods (1 = Jan.–June; 2 = July–Dec.).
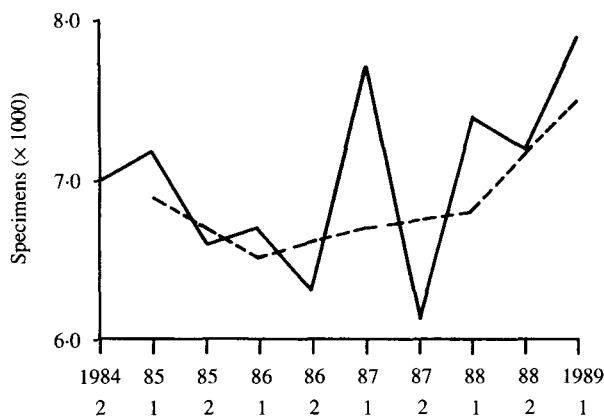

Fig. 3. Isolations of non-chromosomal β-lactamase producers including *E. coli*, *P. mirabilis* and *Klebsiellae* sp. as percentage of specimens (——). Trend (———) (1 = Jan.–June; 2 = July–Dec.).
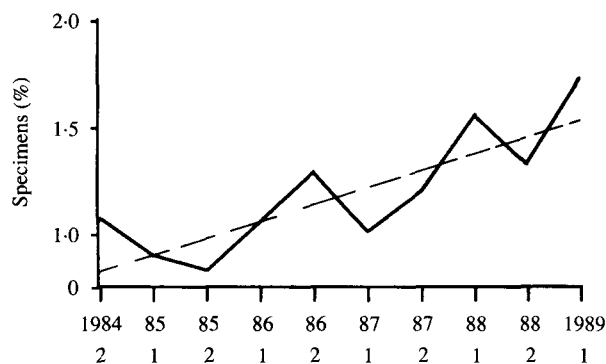

Fig. 4. Isolations of chromosomal β-lactamase producers as a percentage of specimens (——). Trend (———) (1 = Jan.–June; 2 = July–Dec.).
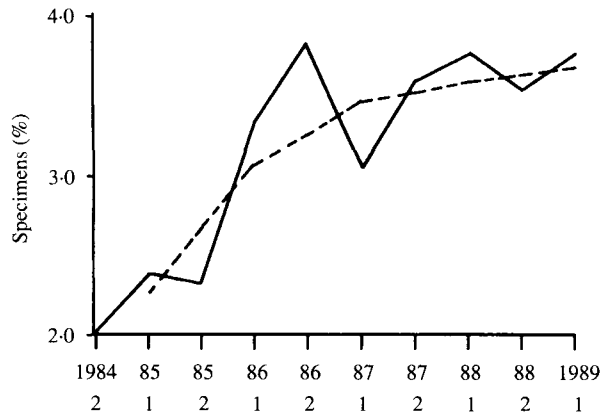
Fig. 5. *P. aeruginosa* isolations as a percentage of specimens (———). Trend (— — —)
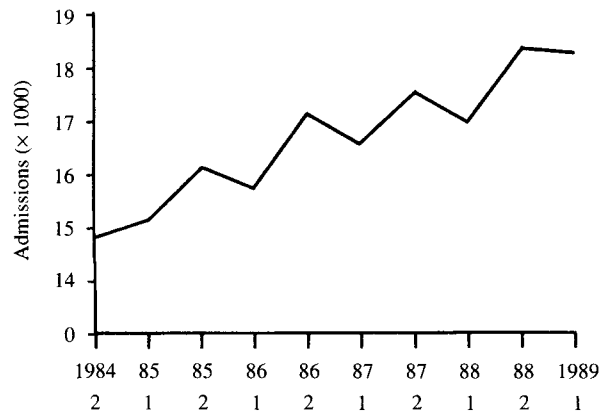(1 = Jan.–June; 2 = July–Dec.).

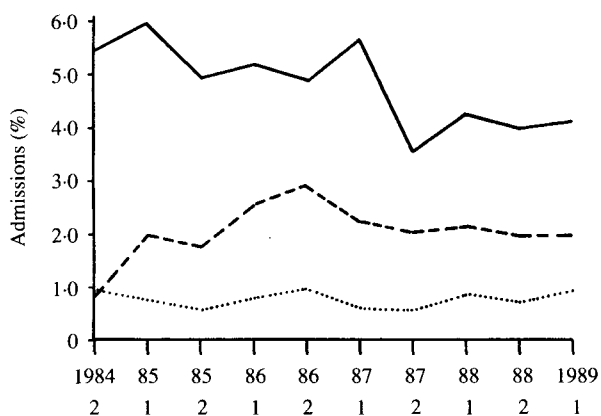Fig. 6. Admission rates to Sir Charles Gairdner Hospital 1984–9 (1 = Jan.–June;
2 = July–Dec.).

Fig. 7. Isolation rates (percentage of admissions) for non-chromosomal (———),
chromosomal (— — —) β-lactamase producers and *P. aeruginosa* (······).

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Table 3. *Isolation numbers and rates percent of admissions for three groupings of bacterial species*

Strains	1984	1985	1985	1986	1986	1987	1987	1988	1988	1989
	2	1	2	1	2	1	2	1	2	1
				No. of isolations (% of admissions)						
Chromosomal β-lactamase producers	142 (1·0)	126 (0·8)	111 (0·7)	132 (0·8)	175 (1·0)	125 (0·8)	124 (0·7)	155 (0·9)	143 (0·8)	166 (0·9)
Pseudomonas aeruginosa	127 (0·9)	300 (2·0)	295 (1·8)	404 (2·6)	499 (2·9)	374 (2·3)	367 (2·1)	380 (2·2)	370 (2·0)	365 (2·0)
Non-chromosomal β-lactamase producers	818 (5·5)	911 (6·0)	828 (5·0)	824 (5·2)	832 (4·9)	941 (5·7)	625 (3·6)	730 (4·3)	743 (4·0)	751 (4·1)
No. of admissions	14823	15147	16719	15785	17043	16525	17552	16929	18379	18211

The variation for *P. aeruginosa* was $+57\cdot8\%$ and that for the remaining chromosomal β-lactamase-producing species was $+10\cdot1\%$. It should be noted that admission numbers increased steadily, by a total of 20 %, over the survey period (Fig. 6).

DISCUSSION

This study examines the relationships between total cephalosporin usage and increasing isolation rates for chromosomal β-lactamase-producing Gram-negative bacilli in a large general hospital. In a similar study [17], a correlation between increased isolations of *S. marcescens*, *Acinetobacter* species and *P. aeruginosa*, and cephalosporin usage, was noted. This finding was related to β-lactamase production in *A. calcoaceticus*. However, the simultaneous marked decrease in susceptibility to aminoglycosides in this species over a period when aminoglycoside usage greatly increased, made a selective role for cephalosporins difficult to establish. In the present study there was no increase in aminoglycoside resistance over the 5-year study period and increased isolations of chromosomal β-lactamase-producing bacteria could be related directly to increased cephalosporin usage. The advent of new cephalosporins over the years has ensured that cephalosporin use continues to rise.

A highly significant correlation between increasing isolations of resistant species and use of 3rd generation cephalosporins was found. This group of cephalosporins was used almost exclusively for specific therapy in hospital in-patients. This contrasts with cephamandole usage which was almost exclusively for surgical prophylaxis. Significantly, no correlation was demonstrated between usage of this antimicrobial and isolation rates for any group of organisms. The significant negative relationship between *P. aeruginosa* isolations and cephamandole use may be explained by the fact that infections with these organisms occurred mainly in the respiratory and oncology wards of the hospital. These wards are well demarcated from the surgical areas where cephamandole use is concentrated. Since much of the cephalexin usage was for the empirical treatment of urinary tract infections at admission these patients, their specimens and bacterial isolates were excluded from the study, a lack of correlation between chromosomal β-lactamase producers and 1st-generation cephalosporins was not surprising.

There was a 60–64 % increase in isolation rate for those species most able to defend themselves in an environment subject to increasing exposure to cephalosporins. Isolation rates for chromosomal β-lactamase-producing species, excluding *P. aeruginosa*, increased by $60\cdot8\%$, as calculated from the trend plot (Fig. 4). Similarly, *P. aeruginosa* isolation rates increased by 64 %, over the period of the survey (Fig. 5). In contrast, the non-chromosomal β-lactamase-producing species, showed an isolation rate increase of only 15 % (Fig. 3).

A National Nosocomial Infection Surveillance Study (Centres for Disease Control, Atlanta, Georgia) analysed isolation frequency data from 90 United States hospitals [18]. Isolation rates for *Pseudomonas* and *Enterobacter* species ranked first and third (up from fourth in 1984) respectively as the most frequent cause of nosocomial specticaemia. Sanders and colleagues [4] found that the selective process appeared to be responsible for steadily increasing isolation rates in some centres. The importance of denominator choice in identifying bacterial

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population shifts cannot be over-emphasized. In discussions published with the work of Sanders and co-workers [4], percentage isolation rates were reported from several hospitals but the use of a variety of denominators precluded direct comparison of results. The denominators included total numbers of specimens, 1000 admissions and total numbers of Gram-negative bacilli.

Two denominators were examined in this study. Selected specimen numbers, from in-patients only, provided a useful denominator that was easily obtained. Only those specimen types that regularly yielded the organisms of interest were included. Piddock [19], used total, apparently unselected specimen numbers, in a similar study. The use of denominators containing subsets of units which are incapable of contributing to the populations of relevant organisms can be misleading, particularly when the subsets are not constant. The trends revealed in Figs. 3–5, using specimens as the denominator, were effectively masked when patient admission numbers were utilized (Table 3). Admission numbers for some hospitals may contain significant but unstable proportions of patients with minimal potential for infection. Similarly, specimen numbers should not include those from patients with community-acquired infections. Although it is accepted that community reservoirs of resistant organisms are important [3], they are unlikely to assist in reflecting inappropriate therapy or overuse with cephalosporins and the attendant changes to the microbial ecology in all or part of a hospital.

Comparison of the isolation rate trends for non-chromosomal β-lactamase producers, when expressed as a percentage of specimens and of admissions, indicated another important effect of denominator selection. The former produced a trend variation in isolation rate of $+15\%$ while the latter showed a declining variation of -37%, both inversely proportional to the trends in the respective denominators (Figs. 2 and 6). Such an effect is anticipated when no antibiotic or other selective effects are operating. Conversely, the same changes in denominator trend may mask selective effects by producing flat trends in isolation rate plots as is the tendency in Fig. 7. Here, the isolation rate has risen against the dilution effect of increasing admission numbers. Such effects may at least partly explain the divergence of results alluded to by Sanders [20] when data from several similarly sized institutions were examined for evidence of a selective process favouring chromosomal β-lactamase producing species. Another denominator which has been used to express isolation rates is the total number of Gram-negative bacilli [16]. This denominator was considered inappropriate since changes in isolation numbers may be masked by changes in the totals. The use of this denominator revealed only the relative proportions of the species for the period of counting. Trends in isolation rates could not be determined using this denominator.

In planning to use flexible computerized data collection systems in epidemiological studies, early attention to such parameters as isolation rate denominators becomes important. In order to evaluate the effects of various hospital practises and policies on isolation rates a common approach to this parameter is essential.

Attention has been drawn to some of the problems of identifying changes in the ecology of hospital bacteria due to antibiotic-mediated selective pressures. In hospitals where increasing isolations of chromosomal β-lactamase-producing

species are evident, the clinical usefulness of the cephalosporin group of antibiotics will continue to be eroded. Remaining to be determined at institutional level are acceptable levels of erosion and levels of constraint and restraint that need to be applied.

From the laboratory point of view, standardized disk and micro-broth dilution (automated and manual) susceptibility tests are poor predictors of β-lactam resistance among chromosomal β-lactamase-producing species [21, 22]. In many instances the frequency of derepressed mutants, in an otherwise susceptible population of bacterial cells, is too low to be sampled by the rigidly standardized inocula demanded by the test procedures. These mutants are responsible for therapeutic failures [4]. Unless specific laboratory tests are undertaken to detect resistant mutants [23, 24], therapy for chromosomal β-lactamase-producing organisms using cephalosporins is not without risk. The risk is to the patient in the first instance and ultimately to adversely affect the ecology of hospital bacteria.

Over the survey period, several policy changes have occurred at Sir Charles Gairdner Hospital in respect of β-lactam antibiotic susceptibility testing and reporting for chromosomal β-lactamase producing Enterobacteriaceae. Testing and reporting was not selective prior to 1986. From 1986 to 1988 all strains continued to be tested by the NCCLS agar dilution method with the inclusion of an agar dilution β-lactamase induction test [25]. β-lactam antibiotics were not reported for 'inducible' strains identified by this method. Our current policy is not to report any β-lactam result for those genera capable of elaborating chromosomal β-lactamase by stable derepression. Specific testing for the presence of mutants is not performed routinely. This approach highlights the importance of the accurate identification of Gram-negative bacilli so that clinically relevant interpretation of susceptibility data may be made.

Insidious changes in the ecology of bacterial organisms isolated at the Sir Charles Gairdner Hospital, Perth, Western Australia have been identified. Correlations between usage rates of cephalosporins and isolation rates of chromosomal β-lactamase-producing species and other species provides clear warnings of the nature of resistance problems which may be anticipated in the future, should current antibiotic prescribing habits persist.

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