

# MULTIPLE ANTIBIOTIC RESISTANCE IN *KLEBSIELLA* SPP. AND OTHER *ENTEROBACTERIACEAE* ISOLATED IN SINGAPORE

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## ABSTRACT

*A common pattern of multiple antibiotic resistance has been noted in bacteria isolated from Singaporean patients. The resistance pattern includes: ampicillin, cefuroxime, ceftazidime and other third generation cephalosporins, aztreonam, gentamicin and other aminoglycosides. The bacterial species implicated are Klebsiellas and other members of the Enterobacteriaceae. Preliminary laboratory investigation with a disk-diffusion augmentation test suggests the presence of extended-spectrum  $\beta$ -lactamases. A retrospective study of laboratory blood culture records shows a rising incidence of resistance in Klebsiella spp. since 1985. Antimicrobial susceptibility results suggest a high degree of co-transfer of aminoglycoside resistance. The high frequency of this type of multiple antibiotic resistance should result in greater caution in the selection of presumptive antibiotic therapy for septicemia, in order to avoid treatment failure and further selection of resistant strains.*

**Keywords:** antimicrobial resistance, extended spectrum beta-lactamases, aminoglycoside resistance

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## INTRODUCTION

Doctors in both hospital and community practice have come to rely on antibiotics as the mainstay in treatment of infectious diseases. The micro-organisms responsible for those diseases have repeatedly demonstrated their ability to evolve strategies for avoiding the lethal or inhibitory effects of antimicrobial chemotherapy. Antibiotic resistance is thus one of the unsought-for consequences of modern medical practice, with new patterns of resistance following the introduction of new antimicrobial agents at ever decreasing intervals.

While there is a certain inevitability about the development of resistance to a newly introduced antibiotic, the clinical setting in which it first appears is less predictable. The appearance of a new type of antibiotic resistance may therefore lead to treatment failure, and a reduced choice of suitable therapeutic agents for specific conditions.

It was recently noted by Kumarasinghe et al that cephalosporin resistance among locally isolated bacteria of the *Enterobacteriaceae* group has been rising for some time<sup>(1)</sup>. There have been recent reports of multiple antibiotic resistance due to extended spectrum  $\beta$ -lactamases from Europe and other parts of the world<sup>(2,3)</sup>. These resistance-conferring enzymes are able to

hydrolyse all cephalosporins, including third generation agents, and aztreonam. One centre reported an association between extended-spectrum  $\beta$ -lactamase mediated resistance and resistance to aminoglycosides<sup>(4)</sup>. There have been no previous reports of this type of antibiotic resistance from the ASEAN region, however extended-spectrum  $\beta$ -lactamase mediated resistance has been documented in India<sup>(5)</sup>.

This study by the Department of Microbiology at the National University Hospital (NUH), Singapore, was prompted by the recognition of a common pattern of multiple antibiotic resistance in *Enterobacteriaceae* isolated from blood cultures. Our objective was to understand the epidemiology of this resistance pattern, and to determine the most likely mechanism of resistance. We therefore investigated bacteria isolated from patients in Singapore hospitals for the production of extended spectrum  $\beta$ -lactamases, and conducted an epidemiological survey using laboratory records.

## METHODS

### Detection of multiple antibiotic resistance

The microbiology laboratory at the National University Hospital (NUH), Singapore, provides a comprehensive diagnostic service to hospital specialists and other medical practitioners. The laboratory is manned by a team of medical and scientific staff. The laboratory runs a rigorous quality control programme involving participation in the scheme run by the College of American Pathologists, and the UK National Quality Assurance Scheme.

During the period of the study all bacteria isolated from blood cultures at the National University Hospital, were identified to species level using standard methods<sup>(6)</sup>. Antibiotic susceptibility testing was performed on Mueller-Hinton agar (OXOID CM 337; Oxoid Australia Pty Ltd, West Heidelberg, Victoria) using the Kirby-Bauer disk diffusion method<sup>(6)</sup>. Facultatively anaerobic, Gram negative bacilli were all tested for susceptibility to a panel of agents including a third generation cephalosporin and an aminoglycoside. Resistance to more than one of the primary panel agents resulted in a further panel of agents being tested. Thus all multiple antibiotic resistant organisms were tested against both the primary and extended panels. All bacteria isolated from blood cultures were then catalogued and stored in glycerol broth at  $-20^{\circ}\text{C}$ .

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### Detection of extended spectrum $\beta$ -lactamase activity

All *Enterobacteriaceae* resistant to third generation cephalosporins (almost all of which were *Klebsiella* spp.) isolated from blood cultures at NUH during the first five months of 1993 were recovered from storage in glycerol broth at  $-20^{\circ}\text{C}$ . Extended spectrum  $\beta$ -lactamases were detected by performing a double disk test, in which three pairs of cefotaxime and augmentin disks were placed at increasing distances on a lawn of the organism to be tested<sup>(7)</sup>. The test was considered positive if there was a zone of increased inhibition between a disk pair after overnight incubation. Isolates from four patients in other Singapore hospitals were also tested.

### Epidemiological survey

The laboratory's computer database was scrutinised for Gram negative blood culture isolates during the first six months of 1993. A full record of identity (to species level), antibiotic susceptibility pattern, specimen date, and patient details was obtained.

Having identified *Klebsiella* as the principal genus involved, the blood culture records were examined from 1985 to the end of 1992, and all instances of first isolation of *Klebsiella* spp. recorded, along with the results of antibiotic susceptibility testing.

### RESULTS

The common pattern of multiple antibiotic resistance involved absent or reduced inhibition zone diameters to all  $\beta$ -lactam agents (ie ampicillin/piperacillin, aztreonam, cephalixin, cefuroxime, ceftriaxone, and ceftazidime) except for imipenem (Table I). There were also reduced/absent zones to aminoglycoside agents such as gentamicin and amikacin. In practice, the only therapeutically useful agents for treatment of septicemia due to these bacteria were the fluoroquinolones (such as ciprofloxacin) and imipenem (Fig 1). One instance was documented of resistance to all agents tested apart from imipenem.

The organisms expressing this pattern of multiple antibiotic resistance in complete or partial form were mainly *Klebsiella* spp., but other members of the *Enterobacteriaceae* were found with the same pattern of resistance (Table II).

All ceftazidime resistant bacteria isolated from blood cultures at NUH during the first three months of 1993 produced an augmentation zone in the double disk test (Fig 2).

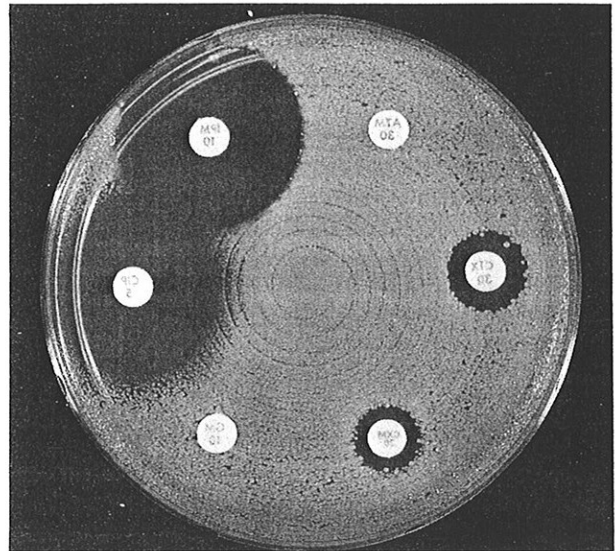
The first third-generation-cephalosporin resistant *Klebsiella* spp. was isolated from a blood culture at NUH in January 1986. Since then, there was a steady rise in the percentage of *Klebsiella* spp. isolated from blood cultures resistant to either third generation cephalosporins alone, or to these agents plus aminoglycosides (Table III). There was no evidence of patient-to-patient spread, or of a predilection for a particular hospital ward or clinical service.

**Table I – Antimicrobial resistance in *Enterobacteriaceae* isolated from blood cultures (NUH, Jan-May 1993)**

genus	Total	Am	Cr	Cxm	Caz	Ipm	Gm	Ak	Cip
<i>Klebsiella</i>	21	21	9	9	9	0	8	4	0
<i>Enterobacter</i>	5	4	4	2	1	0	1	1	1
<i>Citrobacter</i>	3	2	1	1	0	0	0	0	0

Am: Ampicillin  
Cr: Cephaloridine  
Cxm: Cefuroxime  
Caz: Ceftazidime  
Ipm: Imipenem  
Gm: Gentamicin  
Ak: Amikacin  
Cip: Ciprofloxacin

**Fig 1 – Disk diffusion susceptibility test results showing pattern typical of multiple resistant *Klebsiella pneumoniae*: ciprofloxacin (CIP) and imipenem (IPM) sensitive; gentamicin (GM), cefuroxime (CXM), ceftazidime (CAZ) and aztreonam (ATM) resistant.**

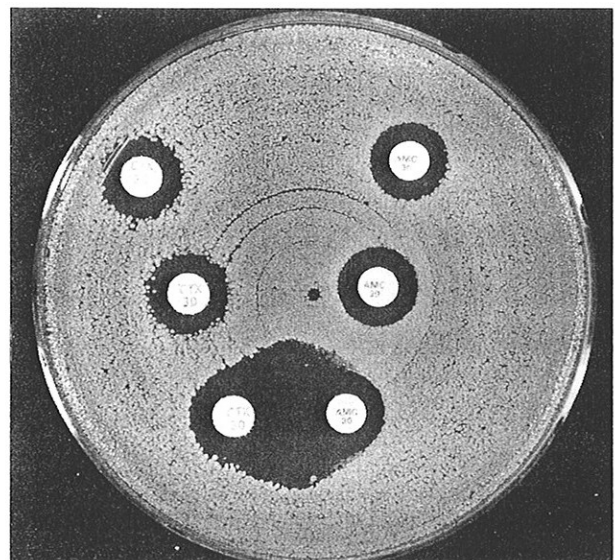


**Table II – Patterns of antimicrobial resistance in *Enterobacteriaceae* isolated from blood cultures (NUH, Jan-May 1993)**

genus	total	Am. only	C/A	C. only	A. only
<i>Klebsiella</i>	21	12	8	1	0
<i>Enterobacter</i>	5	4	1	0	0
<i>Citrobacter</i>	3	2	0	0	0
Total	29	18	9	1	0

Am only: Ampicillin resistance only;  
C/A: resistance to third generation cephalosporins and other  $\beta$ -lactams, and aminoglycosides;  
C only: resistance to third generation cephalosporins and other  $\beta$ -lactams only;  
A only: resistance to aminoglycosides, but not to any cephalosporins.

**Fig 2 – Double disk test with same isolate of *Klebsiella pneumoniae* showing augmentation of cefotaxime (CTX) zone by the calvulanic acid component of augmentin (AMC).**



**Table III – Multiple antibiotic resistance in *Klebsiella* spp. isolated from blood cultures (NUH, 1986-1992)**

Year	Total	3G	(%)	3G/A	(%)
1986	13	1	( 8)	0	
1987*	12	0		0	
1988	47	5	(11)	2	( 4)
1989	74	15	(20)	15	(20)
1990	71	28	(39)	27	(38)
1991	86	26	(30)	16	(19)
1992	78	24	(31)	13	(17)

\*figures available for first six months only; 3G: resistant to third generation cephalosporin but sensitive to aminoglycosides; 3G/A: resistance to third generation cephalosporins and aminoglycosides

## DISCUSSION

Our results suggest that the pattern of multiple antibiotic resistance often seen in Gram negative bacilli isolated from Singaporean patients is due to a combination of extended spectrum  $\beta$ -lactamase and aminoglycoside-modifying enzymes.

The high degree of concurrence of resistance to two distinct families of antibiotics suggests that the mechanism of transmission usually confers resistance to both groups of compounds. Experience in other centres where extended spectrum  $\beta$ -lactamases are present has shown that this form of resistance is usually transmitted on plasmids, although carriage on a transposon has been documented recently<sup>(8)</sup>.

The first local record of *Klebsiella* spp. resistant to third generation cephalosporins was shortly after the opening of the NUH. This form of resistance may have been present at an earlier date in other Singapore hospitals, and the experience of microbiologists in other hospitals locally confirms that multiple antibiotic resistant *Klebsiellas* have been present for several years<sup>(9)</sup>. Extended spectrum  $\beta$ -lactamases were first documented in Europe in the early 1980's<sup>(10)</sup>. Since then, there have been reports of extended spectrum  $\beta$ -lactamase mediated resistance from several centres. One such report documents the results of a three-year multicentre prevalence study<sup>(11)</sup>. The maximum prevalence found was almost 50% in one centre, using the double disk augmentation test to confirm extended-spectrum  $\beta$ -lactamase (ESBL) expression in all isolates tested. In the same study it was found that up to 50% bacterial isolates that produced a positive augmentation test did not have reduced inhibition zones on initial susceptibility testing.

In our study, we have shown an increase in *Klebsiella* spp. with this pattern of multiple antibiotic resistance from 1985 to

the present. Although we did not find any isolate that had been erroneously reported as sensitive to third generation cephalosporins, it remains possible that some cases of resistance may be missed in future if standard disk diffusion testing is to be relied upon as the sole means of susceptibility testing. This is more likely to be the case if the enzyme conferring resistance is not produced in significant quantities until it has been induced by exposure to a suitable antibiotic substrate.

The origin of this type of antibiotic resistance remains obscure, but it appears that extended-spectrum  $\beta$ -lactamases can arise from bacterial strains that produce the classical TEM-1 and TEM-2  $\beta$ -lactamases. It is of note that ceftazidime appears to promote this mutation process at up to ten times the rate of cefotaxime<sup>(12)</sup>.

While the implications for the diagnostic microbiology laboratory are clear, a therapeutic strategy for multiple antibiotic resistant *Enterobacteriaceae* has yet to be worked out. This will depend in part on confirmation of the mechanisms and means of transmission responsible for this resistance pattern. Work to determine the biochemical and genetic features of antibiotic resistance in locally isolated *Klebsiella* spp. is currently under way.

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