

A Study of Antimicrobial Resistance Among Gram-Negative Bacteria in Patients Hospitalized in the ICU of University Hospitals.

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Patterns of the susceptibility to 16 different antimicrobial agents, and cross resistance of gram-negative bacteria obtained from patients admitted in the Intensive Care Unit (ICU) of three university hospitals, namely, Siriraj Hospital, Chulalongkorn Hospital and Ramathibodi Hospital, were studied during September 1991 and January 1992. Altogether 312 bacterial isolates were obtained from 184 patients. Bacteria commonly found in our study were, in order of frequency, as follows : acinetobacter, *P. aeruginosa*, *K. pneumoniae*, enterobacter, *E. coli*, etc. The study of their resistance based on NCCLS breakpoint revealed that imipenem was less resisted, followed by ciprofloxacin, amikacin, ceftazidime, tobramycin, aztreonam, etc. It was also found that these bacteria often showed cross-resistance among the antimicrobials, aminoglycosides, ureidopenicillins and aztreonam while such cross-resistance occurred less in the group of imipenem and ciprofloxacin. The results of this study indicate that the efficacy of the antimicrobial drugs should be evaluated frequently, and the unnecessary use of more than one antimicrobial drugs should be avoided in order to minimize drug resistance as well as cross-resistance. Moreover, the control and surveillance of spreading of bacterial infections in the hospital wards should be carefully considered, as the hospital patients are prone to infections with severe outcome owing to immunity defects often found in them. (*J Infect Dis Antimicrob Agent* 1994;11:9-16.)

Key words : ICU, antimicrobial resistance, Gram-negative bacterial, *P. aeruginosa*, *K. pneumonia*

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เรื่องย่อ

การศึกษาภาวะการดื้อยาต้านจุลชีพของเชื้อแบคทีเรียแกรมลบในผู้ป่วยในหออภิบาลของโรงพยาบาลมหาวิทยาลัย

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จากการศึกษาแบบแผนความไวของเชื้อแบคทีเรียแกรมลบต่อยาต้านจุลชีพ 16 ชนิดและการดื้อยาร่วมกันของเชื้อที่แยกได้จากตัวอย่างจากหออภิบาลของโรงพยาบาลมหาวิทยาลัย 3 แห่ง คือ ศิริราช, จุฬาฯ และรามาธิบดี ในช่วงเดือนกันยายน 2534 ถึงเดือนมกราคม 2535 พบเชื้อ 312 ตัว จากผู้ป่วย 184 คน เชื้อที่พบบ่อยในผู้ป่วยในหออภิบาล เรียงลำดับจากมากไปน้อย มีดังนี้ *acinetobacter*, *P.aeruginosa*, *K.pneumoniae*, *enterobacter*, *E.coli* และอื่น ๆ ผลการศึกษาภาวะการดื้อยาของเชื้อโดยใช้ NCCLS breakpoint เป็นเกณฑ์ พบว่า เชื้อดื้อยา imipenem น้อยที่สุด ตามด้วย ciprofloxacin, amikacin, ceftazidime, tobramycin, aztreonam และอื่น ๆ นอกจากนี้ยังพบว่าเชื้อเหล่านี้มักมีการดื้อยาร่วมกันระหว่างยาในกลุ่ม cephalosporins, aminoglycosides, ureidopenicillins และ aztreonam ขณะที่ imipenem และ ciprofloxacin เป็นยาที่มีการดื้อยาร่วมกับยากลุ่มอื่นน้อย การศึกษาครั้งนี้บ่งบอกถึงการดื้อยาและการดื้อยาร่วมกันของเชื้อชนิดต่าง ๆ ในหออภิบาล เพื่อช่วยในการควบคุมและเฝ้าระวังการแพร่ระบาดของเชื้อในหออภิบาล เพราะผู้ป่วยในหออภิบาลมักมีความบกพร่องของภูมิคุ้มกันโรค ทำให้ติดเชื้อได้ง่ายและมีอาการรุนแรง (วารสารโรคติดเชื้อและยาต้านจุลชีพ 2537; 11:9-16.)

INTRODUCTION

Infections caused by gram-negative bacteria are known to result in severe consequences with high mortality rate, especially in patients contracting infections in a hospital. Besides, gram-negative bacterial infections often pose problems of treatment due to frequent development of resistance to antimicrobial drugs used. Gram-negative bacteria isolated from hospitals are found to produce higher rate of such resistance than usual. This is due to the frequent use of antimicrobial drugs in hospitals that induced selection of bacteria which can better resist destruction by antimicrobial agents of all groups such as beta-lactams, aminoglycosides, quinolones and others.

Resistant gram-negative bacteria are often found in patients contracting infections in the hospital wards. Such severely ill patients usually have immune defects. Therefore, knowledge of susceptibility pattern to antimicrobial drugs of gram-negative bacteria isolated from hospital wards would indicate the trend of various

drugs sensitivity. Such information is useful for the more efficient selection of effective antimicrobial drugs, and may also be used as a guideline in the control and surveillance of dissemination of resistant bacteria.

The present study of gram-negative bacterial susceptibility to 16 different antimicrobial drugs was conducted by using cross-sectional model of study.

MATERIALS AND METHODS

During August 1991 and January 1992, the specimens of blood, sputum, urine, pus from infected wounds or other infected sites were collected for consecutive isolation of gram-negative bacteria from the patients suspected of bacterial infections admitted to three university hospitals, namely, Siriraj Hospital, Chulalongkorn Hospital and Ramathibodi Hospital. About 100 bacterial specimens were collected from each ICU, including repeated specimen obtained from the same patients who had infections of more than one sites and/or who developed repeated infections.

Table I: Showing microdilution panel used for MIC determination of ICU isolated.
Breakpoints shown are those defining fully susceptible isolates as adopted by NCCLS (1)

Antimicrobial agents	Range (mg/l)		NCCLS Breakpoint (mg/l)	
	Low	High	S	R
Ampicillin	1	32	8	32
Amox/clavul	1/0.5	32/16	8	32
Piperacillin	4	128	16 (64)	128
Imipenem	0.5	16	4	16
Cefazolin	1	32	8	32
Cefuroxime	1	32	8	32
Cefoxitin	1	64	8	32
Cefotaxime	1	64	8	64
Ceftriaxone	1	64	8	64
Ceftazidime	1	32	8	32
Aztreonam	1	32	8	32
Ciprofloxacin	0.25	4	1	4
Gentamicin	1	16	4	16
Tobramycin	1	16	4	16
Amikacin	4	64	16	64
Mezlocillin	4	128	16 (64)	128

* Breakpoints shown in parentheses are used in case of *P. aeruginosa*

All isolated bacteria were examined for minimal inhibitory concentrations (MICs) using microscan MIC plus MIC panel (Baxter-travenol, Hinsdale, Illinois) composed of MIC plus type MK panels, renoculator, inoculation tray, brain heart infusion broth, pluronic-F water and microscan microdilution viewer. The 16 different antimicrobial drugs to be studied were placed in the microtiter panel (Table I). Bacterial susceptibility to antimicrobial drugs was determined using NCCLS guideline (1). *P. aeruginosa* 27853 was used as control while determining the MIC value for its known NCCLS breakpoint. The relationship of the bacterial cross resistance was also analysed.

RESULTS

Cultures of specimens obtained from 184 patients in the ICU yielded altogether 312 gram-negative bacterial isolates. Of these, 24 specimens were repeatedly collected from 20 patients. Out of 184 studied patients, 48 had mixed infections. The collected specimens were obtained from the respiratory tract, skin and soft tissue, urinary tract, gastrointestinal tract, blood and other sites which amounted to 70%, 13%, 5%, 5%, 3% and 4% respectively as shown in Table II.

Table II: Showing derivation of Gram-negative isolates by body site.

Site of isolation	Number of isolates (%)
Respiratory tract	219 (70)
Skin & soft tissue	43 (13)
Urinary tract	14 (5)
Gastrointestinal tract	14 (5)
Blood cultures	10 (3)
Cerebral spinal fluid	3 (1)
Unspecified	9 (3)
Total	312 (100)

Culture of all 312 isolated yielded bacteria as shown, in order of frequency, in Table III as follows :- acinetobacter 26%, *P. aeruginosa* 22%, *K. pneumoniae* 18%, enterobacter 11%, *E. coli* 8% and others 15%. The determination of MIC values based on NCCLS breakpoint revealed that most of the gram-negative isolates showed resistance to multiple antimicrobial drugs. The distribution of drug resistance was shown as follow (Figure 1) :- resistance to imipenem was found in 15%, ciprofloxacin 29%, ceftazidime 43% and

Table III : Percentage susceptibility of Gram-negative isolates to various antimicrobials against Gram-negative bacteria.

Percent of Major Species Susceptible*
Thailand 1992, ICUs from 3 Hospitals**

Species	Imi- penem bpt. (mg/l)	Cef- tazid 8	Aztr- eonam 8	Cefo- taxime 8	Cef- triaxone 8	Cefox- itin 8	Cefu- roxime 8	Cef- azolin 8	Piper acillin 16/64**	Mezio cillin 16/64**	Ampi- cillin 8	Amox. /Clav 8	Genta- micin 4	Tobra- mycin 4	Ami- kacin 16	Cipro- flox. 1	
	No.																
<i>P. aeruginosa</i>	69	81	67	65	10	12	3	3	1	65	57	1	4	39	62	75	80
<i>A. anitratus</i>	68	94	32	10	22	31	1	3	1	24	9	13	41	28	28	29	56
<i>K. pneumoniae</i>	56	95	59	61	50	52	70	43	43	5	4	0	66	54	55	59	86
<i>Enterobacter spp.</i>	25	88	64	68	60	60	24	32	12	44	36	0	12	52	52	80	72
<i>E. coli</i>	24	100	75	75	75	75	88	63	29	17	17	17	38	58	50	71	75
<i>A. calcoaceticus</i>	13	92	54	15	46	54	0	15	0	46	31	31	69	62	69	54	69
<i>Proteus mirabilis</i>	10	70	90	90	90	80	80	70	50	30	30	20	70	90	80	80	90
<i>Enterobacter cloacae</i>	10	90	70	70	70	70	0	30	0	60	60	10	0	70	70	80	100
<i>Pseudomonas spp.</i>	7	57	29	14	14	14	0	0	0	14	29	0	0	14	14	29	43
<i>Non fermentative bacilli</i>	5	40	80	20	0	20	0	0	0	80	60	0	20	20	20	20	20
<i>X. maltophilia</i>	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	20	0
All species	312	85	57	48	37	40	26	22	13	36	29	7	33	45	49	57	71

*%Suscept. at NCCLS breakpoint (bpt), mg/L (**)= NCCLS Higher bpt for PA

**Chulalongkorn, Siriraj & Ramathibodi Hospitals.

Non Susceptible Isolates Distribution

All species, N = 312

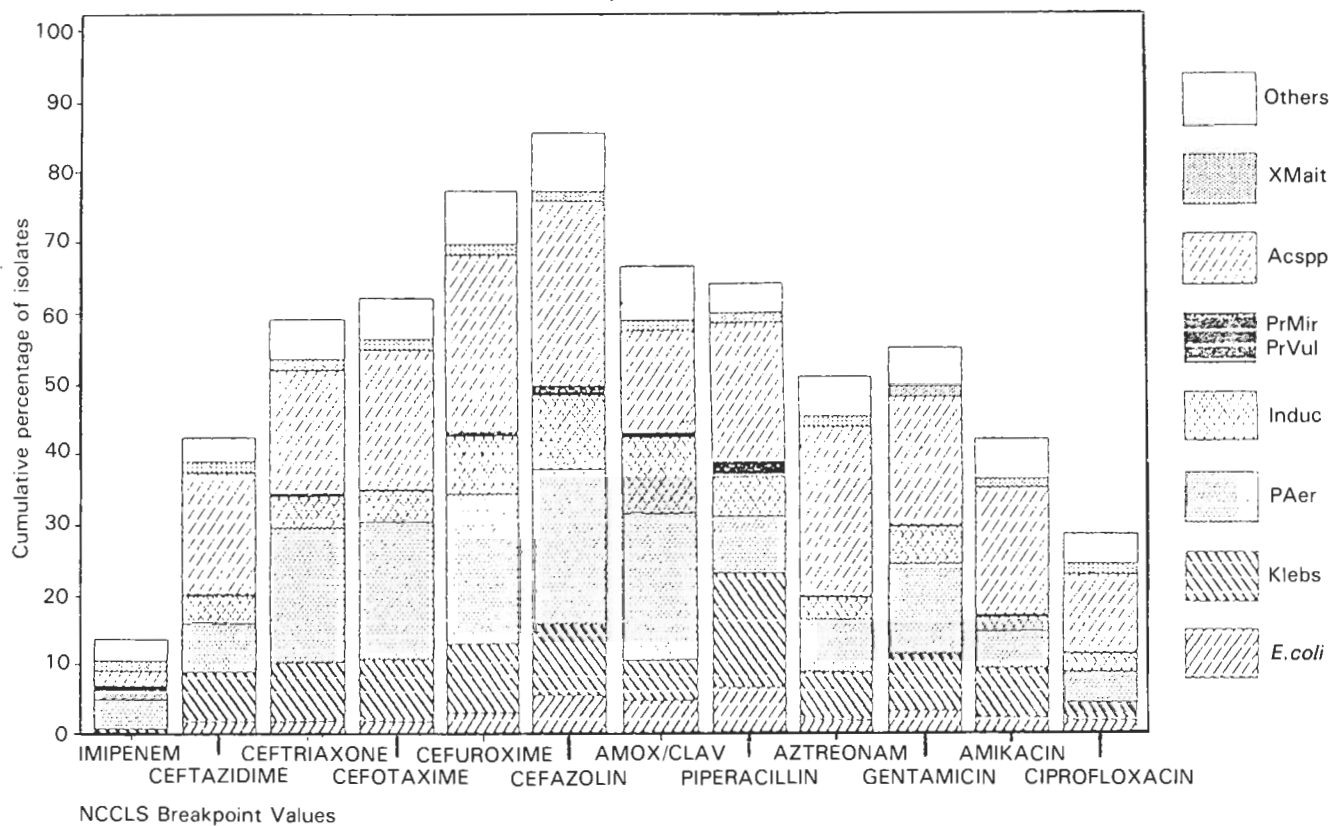


Fig. 1 Showing non-susceptible isolates distribution by major species group.

amikacin 43%. However, their resistance to other antimicrobial drugs such as ceftriaxone, cefotaxime, piperacillin and gentamicin was found to occur in more than 50% of the isolates.

In considering patterns of susceptibility of commonly found bacteria to antimicrobial agents, the susceptibility of gram-negative bacteria, especially the four most commonly encountered, it was found that 91% of acinetobacter was susceptible to imipenem, while 56% to ciprofloxacin and less than 36% to other drugs. *P. aeruginosa* was susceptible to imipenem, ciprofloxacin, amikacin and ceftazidime at the rate of 81%, 75% and 67% respectively. Other bacteria such as *K. pneumoniae*, enterobacter and *E. coli* were also most susceptible to imipenem.

When MIC-correlation between each antimicrobial drug and the bacteria, namely, acinetobacter, *P. aeruginosa* and *K. pneumoniae* was taken into account as shown in Table IV, V and VI, it was found that the multiple cross resistance of each species of bacteria to imipenem occurred least when compared to other drugs. For example, *P. aeruginosa* which is highly resistant to other antimicrobial drugs was most susceptible to imipenem.

DISCUSSION

The limitation of any study on susceptibility of bacteria to antimicrobial drugs was that it may not correlate with the therapeutic efficacy of the same antimicrobial drugs tested. In addition, the isolates were obtained from patients admitted to ICUs of the university hospitals and thus generalization of the findings is also limited. Therefore, the results obtained may be similar or quite different from that obtained from general wards located in other hospitals. Patterns of bacterial susceptibility to the drugs used in various hospitals depends largely patterns of administration of antimicrobial drugs in such hospitals, and also on surveillance on the spreading of bacteria in the hospitals. However, the study helps to reveal the current status of bacterial resistance in the ICU and future trends of resistance development to antimicrobial drugs that may occur in tertiary medical care level such as university hospital.

In our study, more than 70% of the studied specimens were obtained from the sputum. Usually the respiratory tract is the important site of bacterial infections occurring in the hospitals, especially in the elderly patients who often need respiratory support with

Table IV: Showing patterns of associated resistance in 83 isolates of acinetobacter

Population	N	Antibiotics and % resistant isolates				
		Imipenem	Ceftazidime	Amikacin	Ciprofloxacin	Ceftriaxone
All isolates		8	64	67	39	75
Imipenem						
Sensitive	76	—	61	70	39	72
Resistant	7	—	86	100	100	100
Ceftazidime						
Sensitive	30	3	—	23	33	7
Resistant	53	11	—	92	98	66
Amikacin						
Sensitive	27	0	11	—	4	37
Resistant	56	12	89	—	62	93
Ciprofloxacin						
Sensitive	47	2	40	47	—	58
Resistant	30	0	94	97	—	97
Ceftriaxone						
Sensitive	21	0	0	19	5	—
Resistant	62	11	84	84	56	—

Table V: Showing patterns of associated resistance in 69 isolates of *P. aeruginosa*

Population	N	Antibiotics and % resistant isolates					
		Imipenem	Ceftazidime	Aztreonam	Piperacillin	Amikacin	Ciprofloxacin
All isolates		19	33	35	35	25	20
Imipenem							
Sensitive	56	—	27	34	27	20	18
Resistant	13	—	69	54	69	54	46
Ceftazidime							
Sensitive	45	9	—	7	9	5	0
Resistant	24	37	—	88	83	62	58
Aztreonam							
Sensitive	45	13	9	—	13	9	2
Resistant	24	29	83	—	79	62	54
Piperacillin							
Sensitive	45	9	9	11	—	11	2
Resistant	24	37	79	79	—	50	54
Amikacin							
Sensitive	52	12	21	21	23	—	8
Resistant	17	41	88	76	71	—	59
Ciprofloxacin							
Sensitive	55	13	18	18	18	13	—
Resistant	14	43	100	93	93	79	—

Table VI: Showing cross-resistance between β -lactam antibiotics in 56 isolates of *K. pneumoniae*

Population	N	Antibiotics and % resistant isolates				
		Imipenem	Ceftazidime	Cefotaxime	Amikacin	Ciprofloxacin
All isolates		5	41	50	41	14
Imipenem						
Sensitive	53	—	38	47	43	13
Resistant	3	—	100	67	0	33
Ceftazidime						
Sensitive	33	0	—	22	18	6
Resistant	23	13	—	91	74	22
Cefotaxime						
Sensitive	28	4	7	—	4	0
Resistant	28	7	75	—	93	29
Amikacin						
Sensitive	33	9	18	18	—	3
Resistant	23	0	565	90	—	30
Ciprofloxacin						
Sensitive	48	4	35	42	33	—
Resistant	8	12	75	100	87	—

the insertion of endotracheal tube. Not many specimens were obtained from other sites such as wounds of the skin, urine and blood. The specimens were collected consecutively, and more than one specimens might be obtained from the same patients, due to the fact that a patient could have more than one site of infection. The organisms isolated from a specimen may not be causing the current infection, however, they may lead to infection later. For example, bacterial isolates obtained from a specimen of sputum may subsequently cause bronchitis and/or pneumonitis.

Our study showed that the gram-negative bacteria most commonly found were acinetobacter 26%, *P. aeruginosa* 21% and *K. pneumoniae* 18%, which are agreeable with the findings of other studies, indicating that such bacteria were important causes of illness and raised problems of treatment. *P. aeruginosa* was commonly found in the patients in hospital wards, who are immunocompromised. From this study, we found that bacterial susceptibility to the drugs often recommended to the patients, namely, ceftazidime and amikacin amount to only 67% and 75% respectively while imipenem and ciprofloxacin yielded susceptibility of 81% and 80% respectively. When MIC correlation was taken into account, imipenem was found to produce the least drug group cross resistance comparing to other drugs. The low resistance of *P. aeruginosa* to imipenem is unique, it may be explained by the fact that the bacteria have a specific additional porin to let imipenem pass through. Therefore, when other porins are closed, imipenem can still pass though this specific porin while other drugs cannot.

Acinetobacter was found to frequently cause bacteremia and respiratory tract infection which were usually severe and difficult to treat. This organism can grow in simple media and often contaminates hospital equipments and/or water and sinks. Moreover, they were also found to cause super-infection or colonization in patients receiving third-generation cephalosporins and aminoglycosides. Accordingly, the resistant strains were selected as a result of administration of both drugs which were only active against 40% of them or less. Only imipenem showed highest activity against 91% of them.

Another gram-negative bacteria which was also found frequently to cause problems in the hospital wards was *K. pneumoniae* which was susceptible to imipenem and ciprofloxacin at the rate of 95% and 86% respec-

tively. Its susceptibility to other drugs was lower than 70%. At present these bacterial species can produce extended beta-lactamase (2) by changing only 1-3 amino acids in the structure of plasmid. Such enzyme is then capable of destroying various antimicrobial agents belonging to cephalosporins, monobactam and ureidopenicillins, and usually produce resistance to aminoglycosides. Previous studies revealed that the genes governing the production of beta-lactamase also control the production of aminoglycoside modifying enzyme. Moreover, the clinical findings indicated that the resistance pattern of *K. pneumoniae* could spread rapidly in the hospital wards, because it could easily pass the resistant genes to other species that possessed similar hereditary characteristics (3,4), making it difficult to control. This "receptive" bacterial species were frequently found in the hospital environment where the antimicrobial drugs in the group of third-generation cephalosporins were widely used. Fortunately, this bacterial species was still largely susceptible to the carbapenems and cephamecin was still most effective in combating enterobacter and *E. coli*.

The study of susceptibility pattern of gram-negative bacteria in the ICUs indicated that such bacteria were poorly susceptible to third-generation cephalosporins which are widely used today, in contrast to the efficacy results obtained before when this drug group was newly introduced. The study also revealed the rational trend of selecting imipenem and ciprofloxacin to treat patients suffering from super-imposed gram-negative bacterial infections in the ICU. From this study, imipenem was found to be the most active drug for the gram-negative bacteria, while it was also active against anaerobes. Therefore, imipenem may be used alone as an empiric drug to treat patients with infections due to multiple organisms or gram-negative bacilli. However, the use of combined drugs may still be necessary for patients with severe infections in order to attain the highest efficacy and to save the patients' lives, especially the patients in the hospital ICUs or wards.

In conclusion, the resistance of the gram-negative bacteria is still a very important medical problem. Patients admitted to the hospital wards were in high risk of life-threatening for developing nosocomial infection. The spread of resistant bacterial strains to commonly used antibiotics would result in increasing severity of the infections, and at the same time increase mortality. Nowadays patients hospitalized in the tertiary

medical care centers often have immune defects such as AIDS, malignancy, diabetes mellitus and etc. The study in regard to susceptibility patterns of gram-negative bacteria to various antimicrobial drug groups, therefore, can be used as a guideline for the rational administration of effective antimicrobial drugs to the patients suffering from such infections.

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