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Antimicrobial Susceptibility of Bacteria Isolated in 1985

– With Special Reference to Prevalence of Methicillin-Resistant *Staphylococcus aureus* and Activities of Cefazolin, Cefotaxime and Piperacillin –

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Antimicrobial susceptibility of nine species and one group of bacteria isolated from patients at the hospitals of Seoul National University, Severance, Hanyang University, and Kyungpuk University were tested by agar dilution method. S. aureus *was most susceptible to cefazolin, methicillin and cotrimoxazole, and enterococci to ampicillin. Isolates of Enterobacteriaceae were most frequently susceptible to aminoglycosides and cefotaxime. Cefazolin susceptibility was markedly different from species to species. Aminoglycosides and piperacillin were more active than others against* P. aeruginosa, *and amikachin against* A. anitratus. *A large proportion of strains of several different species were conditionally susceptible to either tetracycline, ampicillin, cefazolin or cotrimoxazole suggesting the usefulness of these drugs for treatment of urinary tract infection. Activity of cefotaxime was highest against* E. coli, *and* K. pneumoniae, *while lowest against* A. anitratus *and* P. aeruginosa. *Decrease in the proportion of susceptible isolate was noted in* E. coli *and* K. pneumoniae *to cefazolin,* K. pneumoniae, E. cloacae *and* S. marcescens *to cotrimoxazole, and* P. aeruginosa *to tobramycin and gentamicin.*

Key Words: *Antimicrobial susceptibility; cefazolin; cefotaxime; piperacillin; methicillin-resistant S. aureus.*

INTRODUCTION

Disk diffusion antimicrobial susceptbility test, which is used widely in clinical bacteriology, is very useful for screening activities of many antimicrobial agents, but it does not reveal susceptibility in quantitative terms. Studies have been supported by the Korean Medical Association to monitor quantitive susceptibility of clinical isolates of bacteria since 1981 (Park et al., 1982; Lee et al., 1983; Suk et al., 1985). The studies were undertaken by a few hospitals located in Seoul, Chunju and Taegu.

In the present study the participating laboratories were hospitals of Seoul National University, Yonsei University Severance, Han Yang University and Kyungpuk National University. Altogether nine species and one group of bacteria isolated at these hospitals in 1985 were tested. In this study, *Salmonella* was not included because it remains to be

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very susceptible (Suk et al., 1985). *Shigella* was not included because their frequent resistance to ampicillin, chloramphenicol, tetracycline and co-trimoxazole and frequent susceptibility to cephalothin and aminoglycoside had not changed markedly in the previous studies (Park et al., 1982; Lee et al., 1983; Suk et al., 1985). Chloramphenicol was not included as it was rarely used except for salmonellosis. Cefazolin was included instead of cephalothin, because cefazolin was listed as a new class antimicrobial agent (NCCLS, 1984). In 1983 moxalactam was tested, but in this study cefotaxime, another 3rd generation cephalosporin, was included. Methicillin-resistant *S. aureus* became a problem in large medical centers in Korea (Chong et al., 1985) and therefore methicillin was included to determine the prevalence of methicillin-resistant *S. aureus*. Piperacillin replaced carbenicillin because of decreased activity of the latter (Suk et al., 1985).

MATERIALS AND METHODS

Antimicrobial susceptibility was tested according to the NCCLS method (NCCLS, 1983). Briefly, antimicrobial agents were dissolved in solvents indicated and were added to Müller-Hinton medium (Oxoid) which was sterilized and cooled to around 45-50°C. Plates were made and used within a week.

A total of 961 isolates from various clinical materials were stored at −20°C or below until used for the test. The stored strains were subcultured before the test.

The test organisms were inoculated into Tryptic soy broth and adjusted to the turbidity of McFarland nephelometer no. ½. The suspensions were further diluted 1:20 with saline.

Bacterial suspensions were inoculated using Steers inoculator or similar devices delivering approximately 1-2 μl. Inoculated plates were incubated at 35°C and the results were read after 16-18 hours. The MICs were interpreted using the breakpoints (NCCLS, 1983). For the quality control, *Staphylococcus aureus* ATCC 25923, *Escherichia coli* 25922 and *Pseudomonas aeruginosa* ATCC 27853 were tested simultaneously.

Table 1. Antimicrobial susceptibility of clinical isolates of bacteria

Bacteria (no. tested)	Antimicrobial agent	MIC (μg/ml) Range	50%	90%
S. aureus (137)	Methicillin	0.25->128	2	128
	Penicillin G	0.015-128	0.5	32
	Cefazolin	0.25->128	0.5	128
	Tetracycline	≤0.12->128	32	128
	Cotrimoxazole	0.12->128	2	32
Enterococcus (60)	Ampicillin	0.06-32	0.25	0.5
	Tetracycline	0.12->128	64	>128
E. coli (120)	Ampicillin	0.5->128	128	>128
	Cefazolin	0.5-64	2	8
	Cefotaxime	0.008-2	0.12	0.12
	Amikacin	0.25-4	1	2
	Gentamicin	0.12-64	0.5	4
	Tobramycin	0.12-32	0.5	4
	Tetracycline	0.5->128	128	>128
	Cotrimoxazole	≤0.06->128	32	>128
K. pneumoniae (120)	Ampicillin	2->128	128	>128
	Cefazolin	0.5->128	2	128
	Cefotaxime	0.008-128	0.12	0.5
	Amikacin	0.5->128	1	8
	Gentamicin	≤0.12->128	0.5	64
	Tobramycin	0.25->128	0.5	64

(continued)

Table 1 – Continued

Bacteria (no. tested)	Antimicrobial agent	MIC (μg/ml) Range	50%	90%
K. pneumoniae	Tetracycline	0.5->128	128	>128
	Cotrimoxazole	0.5->128	8	>128
E. cloacae (110)	Ampicillin	1->128	128	>128
	Cefazolin	1->128	>128	>128
	Cefotaxime	≤0.008-128	0.5	64
	Amikacin	0.25->128	2	8
	Gentamicin	0.06->128	1	32
	Tobramycin	0.12->128	1	64
	Tetracycline	1->128	4	>128
	Cotrimoxazole	0.25->128	8	>128
S. marcescens (119)	Ampicillin	16->128	>128	>128
	Cefazolin	8->128	>128	>128
	Cefotaxime	0.12->128	1	64
	Amikacin	0.5->128	8	128
	Gentamicin	0.25->128	8	>128
	Tobramycin	0.5->128	8	>128
	Tetracycline	8->128	128	>128
	Cotrimoxazole	1->128	64	>128
Indole positive *Proteus* and *Providencia* (27)	Ampicillin	16->128	>128	>128
	Cefazolin	2->128	128	>128
	Cefotaxime	≤0.008-4	0.03	0.12
	Amikacin	1-32	2	16
	Gentamicin	0.25-128	0.5	32
	Tobramycin	≤0.12-128	1	16
	Tetracycline	2->128	128	>128
	Cotrimoxazole	2->128	8	64
P. mirabilis (29)	Ampicillin	≤0.12->128	16	64
	Cefazolin	≤0.12-16	4	8
	Cefotaxime	0.015-0.06	0.015	0.03
	Amikacin	≤0.12-8	2	4
	Gentamicin	≤0.12-16	1	16
	Tobramycin	≤0.12-8	1	4
	Tetracycline	≤0.12-128	64	128
	Cotrimoxazole	1->128	16	>128
P. aeruginosa (119)	Piperacillin	1->128	8	>128
	Cefotaxime	0.5->128	16	64
	Amikacin	1->128	8	32
	Gentamicin	0.5->128	8	>128
	Tobramycin	0.25->128	8	128
A. anitratus (120)	Ampicillin	1->128	128	>128
	Cefazolin	4->128	>128	>128
	Cefotaxime	0.25->128	16	126
	Amikacin	≤0.12->128	4	>128
	Gentamicin	0.12>128	4	>128
	Tobramycin	0.25->128	8	128
	Tetracycline	0.25->128	8	>128
	Cotrimoxazole	0.5->128	16	>128

Table 2. Interpretation of minimum inhibitory concentration

Organism (no. tested)	Antimicrobial agent	% of isolates			
		Susceptible	Moderately susceptible	Resistant	Conditionally susceptible
S. aureus (137)	Methicillin	84	0	16	–
	Penicillin G	10	0	90	18
	Cefazolin	83	1	16	≥9
	Tetracycline	30	2	68	65
	Cotrimoxazole	83	4	13	≥3
Enterococcus (60)	Ampicillin	93	5	2	2
	Tetracycline	17	0	83	72
E. coli (120)	Ampicillin	7	23	70	15
	Cefazolin	90	8	2	2
	Cefotaxime	100	0	0	0
	Amikacin	100	0	0	0
	Gentamicin	90	5	5	2
	Tobramycin	90	6	4	3
	Tetracycline	15	14	71	44
	Cotrimoxazole	36	22	42	≥13
K. pneumoniae (120)	Ampicillin	0	10	90	49
	Cefazolin	77	6	17	≥11
	Cefotaxime	94	3	3	3
	Amikacin	93	1	6	6
	Gentamicin	73	3	24	6
	Tobramycin	70	12	18	6
	Tetracycline	41	16	43	17
	Cotrimoxazole	54	23	23	≥9
E. cloacae (110)	Ampicillin	1	6	93	46
	Cefazolin	6	1	93	≥29
	Cefotaxime	77	8	15	≥13
	Amikacin	95	1	4	0
	Gentamicin	66	12	22	7
	Tobramycin	56	6	38	14
	Tetracycline	7	48	45	23
	Cotrimoxazole	59	8	33	≥19
S. marcescens (119)	Ampicillin	0	0	100	46
	Cefazolin	1	0	99	≥25
	Cefotaxime	70	17	13	13
	Amikacin	74	9	17	5
	Gentamicin	49	13	38	3
	Tobramycin	27	25	48	12
	Tetracycline	0	5	95	52
	Cotrimoxazole	27	23	50	≥35
Indole + *Proteus* and *Providencia* (27)	Ampicillin	0	4	96	26
	Cefazolin	0	0	100	≥19
	Cefotaxime	100	0	0	0

(continued)

Table 2 – Continued

Organism (no. tested)	Antimicrobial agent	% of isolates			
		Susceptible	Moderately susceptible	Resistant	Conditionally susceptible
Indole + *Proteus* and *Providencia* (27)	Amikacin	96	4	0	0
	Gentamicin	78	4	18	4
	Tobramycin	85	0	15	7
	Tetracycline	0	11	89	74
	Cotrimoxazole	52	26	22	≥19
P. mirabilis (29)	Ampicillin	28	24	48	41
	Cefazolin	97	3	0	0
	Cefotaxime	100	0	0	0
	Amikacin	100	0	0	0
	Gentamicin	83	0	17	17
	Tobramycin	97	3	0	0
	Tetracycline	7	0	93	93
	Cotrimoxazole	45	41	14	0
P. aeruginosa (119)	Piperacillin	66	14	20	8
	Cefotaxime	37	50	13	≥11
	Amikacin	89	3	8	2
	Gentamicin	48	2	50	3
	Tobramycin	50	2	48	12
A. anitratus (120)	Ampicillin	1	28	71	32
	Cefazolin	1	3	96	≥32
	Cefotaxime	48	27	25	≥22
	Amikacin	66	4	30	8
	Gentamicin	50	1	49	13
	Tobramycin	49	8	42	18
	Tetracycline	13	37	50	28
	Cotrimoxazole	48	13	38	≥18

RESULTS

Activities of antimicrobial agents (Table 1) against *S. aureus* showed that MICs were different greatly from strain to strain. Cefazolin showed the lowest MIC_{50}, i.e., ≤0.5 μg/ml, while cotrimoxazole showed the lowest MIC_{90}, ≤32 μg/ml.

Amplicillin was more active than tetracycline against enterococci. MIC_{90} of ampicillin was ≤0.5 μg/ml, while MIC_{50} of tetracycline was 64 μg/ml (Table 2).

Cefotaxome was the most active drug against *E. coli* strains with very low MIC range of 0.008-2 μg/ml, and both MIC_{50} and MIC_{90} of 0.12 μg/ml (Table 1, Fig. 1). Amikacin, gentamicin and tobramycin showed relatively low MIC_{50} and MIC_{90}, but the range was wide indicating the presence of strains inhibited only at high concentrations. MIC_{50} of cefazolin was low, 2 μg/ml, while that of ampicillin, tetracycline and cotrimoxazole were high, 128 μg/ml, 128 μg/ml and 32 μg/ml respectively.

Against *Klebsiella pneumoniae,* cefotaxime, amikacin, gentamicin and tobramycin were more active than others. However, some strains were inhibited only at ≥128 μg/ml of these agents. MIC_{90} of cefazolin, 128 μg/ml, was higher than the above mentioned drugs, but lower than those of the rest.

Cefazolin was much less active against *Enterobacter cloacae* than against *K. pneumoniae* MIC_{50} was > 128 μg/ml. The MICs of other agents were similar to those against *K. pneumoniae.*

Against *Serratia marcescens,* cefotaxime was the most active, showing MIC_{50} of 1 μg/ml, while amikacin, gentamicin and tobramycin were slightly

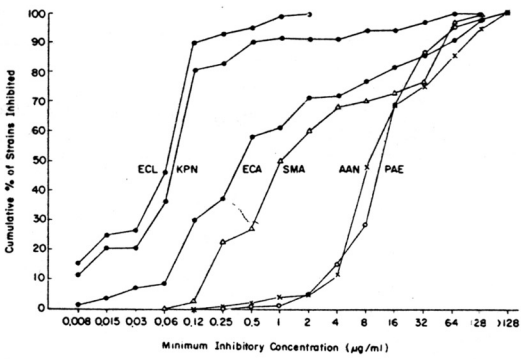

Fig. 1. Antimicrobial activity of cefotaxime against *E. coli* (ECL), *K. penumoniae* (KPN), *E. cloacae* (ECA), *S. marcescens* (SMA), *A. anitratus* (AAN) and *P. aeruginosa* (PAE).

less active, showing MIC_{50} of 8 μg/ml. Ampicillin and cefazolin were the least active ones with MIC ranges of 16−≥128 μg/ml and 8−≥128 μg/ml, respectively.

Cefotaxime was very active against indole-positive *Proteus* and *Providencia* group, i.e., the MIC range was ≤0.008-4 μg/ml. Activities of other agents varied greatly depending on the test strains. Cefazolin ampicillin and tetracycline were equally inactive.

MIC range of cefotaxime was 0.015-0.06 μg/ml against *P. mirabilis*, while that of aminoglycosides and cefazolin was ≤0.12-16 μg/ml. Activities of ampicillin, tetracycline and cotrimoxazole varied greatly by the strains.

Amikacin showed the highest activity against *P. aeruginosa*, but its MIC was ≥128 μg/ml for some strains. MICs of the rest of the antimicrobial agents were different markedly by strains. MIC_{50} of piperacillin and aminoglycosides were 8 μg/ml and that of cefotaxime 16 μg/ml.

None of the antimicrobial agents showed high activity against *Acintetobacter anitratus*, but MIC_{50} were comparatively low with amikacin and gentamicin, i.e., 4 μg/ml, tobramycin and tetracycline, 8 μg/ml, and cefotaxime and cotrimoxazole, 16 μg/ml.

Prevalence of resistant isolates

The breakpoints were used to interprete MICs (Table 2). Ninety percent of *S. aureus* isolates were resistant to penicillin G and 68% to tetracycline. The proportions of methicillin- and coctimoxazole-resistant strains were similar, i.e., 16% and 13%, respectively. To ampicillin, only 2% of *Enterococcus* were resistant, while to tetracycline 83% wer resistant.

All of the *E. coli* isolates were susceptible cefotaxime and amikacin, while 90% of them wer susceptible to cefazolin, gentamicin and tobramci Ampicillin and tetracycline-susceptible isolates a counted for only 7% and 15% respectively.

94% and 93% of *K. pneumoniae* isolates we susceptible to cefotaxime and amikacin, respe tively and around 70% wer susceptible to cefazoli gentamicin and tobramycin. While 41% and 54% the isolates were susceptible to tetracycline ar cotrimoxazole respectively, none were susceptib to ampicillin.

Among the *E. cloacae* strains 77% were susce tible to cefotaxime and 95% to amikacin. Only 6 of the strains were susceptible to cefazolin and 7 to tetracycline. It was noteworthy that 59% of th isolates were susceptible to cotrimoxazole.

Among the *S. marcescens* isolates, 74% we susceptible to amikacin and 70% to cefotaxim Because of the large proportion of moderately su ceptible strains, only 13% were resistant cefotaxime and 17% to amikacin. Strains suscep ble to gentamicin were 49% and to tobramycin ar cotrimoxazole 27%, while none of the strains we susceptible to either ampicillin or tetracycline.

All of the indole-positive *Proteus* and *Providenc* isolates were susceptible to cefotaxime, but r sistant to ampicillin, cefazolin and tetracycline. amikacin, 96% were susceptible, while to gentan cin and tobramycin, 78% and 85% respective were susceptible. All of the *P. mirabilis* isolat were susceptible to both cefotaxime and amikac while 97% to cefazolin and tobramycin.

Among the *P. aeruginosa* strains, 89% were su ceptible to amikacin, and 66% to piperacillin, wh only around 50% were to gentamicin and tobrar cin As 50% of the strains were moderately su ceptible to cefotaxime, only 13% were resistant.

Among the *A. anitratus* isolates 66% were su ceptible to amikacin and around 50% cefotaxime, gentamicin, tobramycin and c rimoxazole. Only 13% were susceptible to t racycline and almost none to ampicillin a cefazolin.

Trend of susceptibility

When the present data were compared to tho of 1981, slight decrease in cefazolin-susceptil strains of *S. aureus* (87% vs 83%), *E. coli* (98% 90%) and *K. pneumoniae* (88% vs 77%) w

noted. When the results from in 1982 and 1985 were compared cotrimoxazole-susceptible *K. pneumoniae* (72% vs 54%) and *S. marcescnes* (61% vs 27%) strains, decreased but *A. anitratus* (30% vs 48%) strains increased slightly.

Proportion of amikacin-susceptible *P. aeruginosa* strains remained high, but decrease in gentamicin- (76% vs 48%) and tobramycin-susceptible strains (73% vs 56%) was noted.

DISCUSSION

Frequent resistance of bacteria to antimicrobial agents became a major problem in the treatment of bacterial infections. Certain species of bacteria acquire resistance more easily. Strains isolated from inpatients are more frequently resistant.

It has been known that resistant bacteria are more prevalent in certain countries such as France (O'Brien et al., 1978) and South Africa (International Surveillance of Antibiotic Resistance Group, 1979). Korea is also one of such countries where resistant bacteria are very prevalent (Chung, 1985). On the contrary, most of the clinical isolates in the United States were reported to remain susceptible, i.e., 90% of *S. aureus* to tetracycline, 77% of *E. coli* to ampicillin and 81% of *K. pneumoniae* to chloramphenicol (Atkinson and Lorian, 1984). Susceptibility of Japanese isolates is difficult to be compared with others, because they use higher breakpoints and different disk diffusion methods in Japan (Goto and Kaneko, 1983).

S. aureus is the most frequently isolated pathogen among gram-positive cocci (Hong et al., 1984; Kim et al., 1985). MICs of antimicrobial agents were markedly different depending on the *S. aureus* isolates. MIC_{50} of methicillin, cefazolin and cotrimoxazole was relatively low. Although MIC_{50} of penicillin G was also low, only 10% of the isolates were interpreted as susceptible, because the breakpoint was very low, i.e., 0.12 μg/ml. Infections due to methicillin-resistant *S. aureus* became a problem in some hospitals (Kim et al., 1983; Yang et al., 1983; Hong et al., 1984). Detection of methicillin resistance by disk diffusion test is difficult because of technical reason, but from this and other studies (Yang et al., 1983; Chong et al., 1985) it may be safely said that methicillin-resistant *S. aureus* is around 15-25% in large hospitals. Methicillin-resistant *S. aureus* may produce severe infection. Vancomycin and fusidic acid may be effective for the treatment of such infection (Chong et al., 1985).

Recent increase in enterococcal infections may possibly be due to the use of cephalosporins instead of others active against this organism (Gombert et al., 1983). MIC of ampicillin against majority of enterococci remains low. Since ampicillin-resistant severe enterococcal infections are known, (Gombert et al., 1983), close monitoring of the susceptibility may still be necessary.

Most frequently isolated bacteria from clinical materials belong to Enterobacteriaceae (Hong et al., 1984; Kim et al., 1985). They are known to be very frequently resistant to antimicrobial agents. Ampicillin, once most widely used drug, was almost completely inactive against *K. pneumoniae, E. cloacae, S. marcescens* and indole-positive *Proteus*. Aminoglycosides retained good activity, e.g., amikacin was active against 74-100% of the species of Enterobacteriaceae. Cefazolin, listed as a new class disk (NCCLS, 1984), was active against most of the *E. coli, K. pneumoniae* and *P. mirabilis,* but inactive against other species. This drug seemed slightly more active than cephalothin against gram-negative bacilli (Kim et al., 1982; Chong and Lee, 1983). Cefotaxime was very active against isolates of Enterobacteriaceae, although there were some isolates of *K. pneumoniae, E. cloacae* and *S. marcescens* which were not inhibited at 128 μg/ml. All of the *E. coli* and *Proteus* were susceptible to this drug. Cotrimoxazole-susceptible strains accounted for 27% to 59% of Enterobacteriaceae depending on the species.

Among the glucose nonfermenting gram-negative bacilli, *P. aeruginosa* and *A. anitratus* are the most frequently isolated species (Ahn and Lee, 1983; Hogn et al., 1984). Most of the *P. aeruginosa* isolates were inhibited by relatively low concentrations of aminoglycosides and piperacillin. Cefotaxime was slightly less active and 37% of the isolates were susceptible to this drug. Piperacillin seemed to be more active than carbenicillin (Chong and Lee 1985; Suk et al., 1985). Only 66% of *A. anitratus* isolates were susceptible to amikacin which were the most active drug againt this organism.

Recently, "conditionally susceptible" category was added to the agar dilution interpretation (NCCLS, 1983). This category means that the infecting organism is susceptible if infection occurs in tissues where antimicrobial concentrations considerably exceed those in blood. Such an example is urinary tract infection. In this study many isolates fell to this category, i.e., to tetracycline 65% of

S. aureus, 72% of enterococci, 44% of *E. coli*, 52% of *S. marcescens*, 74% of indole-positive *Proteus*, 93% of *P. mirabilis*; to ampicillin 49% of *K. pneumoniae*, 46% of *E. cloacae* and *S. marcescens*, 41% of *P. mirabilis* and 32% of *A. anitratus*. To cefazolin and contrimoxazole quite a proportion of gram-negative bacilli were conditionally susceptible. Therefore, in urinary tract infections, most of the antimicrobial agents might be effective.

From this study, it was obvious that susceptibility changes unpredictably. Therefore, identification of the species and determination of their susceptibility become increasingly important for the proper selection of therapeutic agents. At the same time, we wish to emphasize the importance of continuous monitoring of the resistance of local strains by dilution susceptibility tests.

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