THE DISTRIBUTION OF URINARY PATHOGEN GRAM NEGATIVE BACILLI AT MARMARA UNIVERSITY HOSPITAL

G. Söyletir, M.D.** / M. Göral, M.D.*** / A. Can, M.D.***
F. Babacan, M.D.** / C. Johansson, Ph.D.*

* *Professor, Department of Microbiology, Faculty of Medicine, Marmara University, Istanbul, Turkey.*
** *Assistant Professor, Department of Microbiology, Faculty of Medicine, Marmara University, Istanbul, Turkey.*
*** *Research Assistant, Department of Microbiology, Faculty of Medicine, Marmara University, Istanbul, Turkey.*

SUMMARY
One hundred ninety five Gram-negative bacilli (94 E. coli, 50 Klebsiella-enterobacter- Serratia group, 30 Pseudomonas spp, 16 Proteus spp and 5 other species) isolated as urinary pathogens were tested for their susceptibility to aminoglycoside antibiotics. The results were evaluated by comparing the MIC values by the breakpoints of the antimicrobial agents. Amikacin were found as the most effective antimicrobial of the group, 94.7 % of bacteria were susceptible to amikacin. The susceptibility to other antimicrobials were ranked as follows: netilmicin (83.6 %), tobramycin (72.1 %), gentamicin (71.0 %) and kanamycin (23.6 %). Significant differences were recorded among the distribution of the bacteria coming from in-patients and out-patients, and among the bacteria coming from different departments ($p < 0.05$). Among the groups of bacteria the Pseudomonas species were the most resistant group and the majority of them were isolated from Urology patients. We concluded that most of the aminoglycoside antimicrobials are still very effective on E.coli strains, while amikacin is very effective for most of the bacteria, including Pseudomonas and Proteus species which are rather hard to eradicate.

Key Words: Urinary tract infections, Gram-negative bacilli, aminoglicosidic antimicrobials.

INTRODUCTION

The discovery of the first effective aminoglycoside was a major milestone in the history of medicine, and subsequent research efforts by industry led to the development of many useful aminoglycosidic antimicrobials. During the course of clinical and pharmacological research it became evident that the activity of these drugs is closely related to their toxicity; therefore, it was virtually impossible to develop a totally non-toxic yet microbiologically active aminoglycoside (1).

Despite this drawback, aminoglycosides have many properties that make them almost ideal antibiotics. They are bactericidal, have low serum protein binding, and are very stable molecules. Over the last decade, the incidence of bacterial resistance to these compounds have been an increasing concern, with the exception of amikacin, which is not effected by most known mechanisms of enzymatic inactivation that foster the development of resistance to the other aminoglycosides.

Clinically, aminoglycosides are used in infections caused by pathogens resistant to other less toxic antibiotics. Although they are not the drug of first choice, aminoglycosidic antimicrobials have been widely used in the treatment of urinary tract infections (UTIs). The family of Enterobacteriaceae and Pseudomonas species form the vast majority of the bacteria isolated from UTIs. (2). In this study we tried to show the distribution of the bacteria isolated from UTIs in Marmara University Hospital (MU Hospital), and the susceptibility of these pathogens to the aminoglycosidic antimicrobials that are available in Turkey.

MATERIAL AND METHOD

We collected a group of the gram-negative bacilli that were isolated as urinary pathogens for a period of 6 months (July 1988 to December 1988) and tested these bacteria for their susceptibility to aminoglycoside group of antimicrobials. The department which the material came from, and whether the patient was an out-patient or in-patient were recorded. The same strains of bacteria that were isolated from the same patient in consecutive days were left out of the investigation.

Specimen collection and processing: The urine specimens were collected as clean-catch mid-stream urine samples. All the specimen were inoculated to blood and McConkey agar plates, and two smears were prepared from each specimen. Smears were stained with methylen blue for evaluation of the cells and with Gram's stain for differentiation of the bacteria. Following over night incubation, the plates were evaluated. Any strain growing in numbers greater than 100.000 CFU/ml was identified and

tested for antimicrobial susceptibility. The strains that grow in numbers greater than 10,000 CFU/ml were evaluated by the help of the smears, and those with significant pyuria were decided as pathogens if the same strain was isolated in the repeated culture.

Identification and Antimicrobial Susceptibility Testing: The bacteria were identified by classical methods (2, 3, 4). Depending on the IMVIC reaction results the gram-negative bacilli were classified into five groups: (1) E. coli group, (2) KES group (Klebsiella spp, Enterobacter spp and Serratia spp), (3) Proteus group, (4) Pseudomonas group, (5) Other bacteria group (4 Citrobacter spp and 1 Acinetobacter spp).

All the bacteria were tested for their susceptibility to amikacin, gentamicin, kanamycin, netilmicin and tobramycin. Bacterial broth dilution method was used to determine the minimal inhibitory concentrations (MIC) of the agents. The protocol given in the National Committee for Clinical Laboratory Standards (NCCLS) publication M7-A were used to perform the tests (6).

The MIC values were compared with the breakpoints of the drugs and a final conclusion of susceptible, intermediate or resistant was made. The breakpoint of an antimicrobial agent is defined as the concentration that can be achieved in the serum with optimal therapy. The lowest concentration of the agent that inhibits growth of the organism is designated the minimum inhibitory concentration (7).

Antimicrobials :Antimicrobial powders were obtained directly from drug manufacturers. The antimicrobial solution were prepared as suggested in NCCLS M7-A standard.

RESULTS

A total of 250 gram-negative bacilli were isolated as urinary pathogens. Among the isolates 103 (41.2 %) were from hospitalized patients and 147 (58.8 %) were from ambulatory patients. E. coli was the most frequently isolated bacteria from out-patients, while KES species were the most common pathogen of in-patients (Figure 1). There is a significant difference in the distribution of bacteria isolated from in-patients and out-patients ($p < 0.05$). Table I shows the distribution of bacteria among the departments.

Among 250 urinary tract pathogens 195 of them were collected to be tested for their MIC values. This group of isolates shared the same distribution properties with the main group ($p > 0.05$). Among the group of bacteria tested E. coli strains formed the most sensitive group. KES group, Proteus species and Pseudomonas species followed E. coli strains by their increasing percentage of resistance. All of the aminoglycosidic antimicrobials, except kanamycin, were very effective on E. coli strains. In fact kanamycin almost had no effect on the gram-negative bacteria tested. While Pseudomonas and Proteus species had the lowest susceptibility ratios to all of the agents, amikacin was very effective even to these groups, with percentages of 86.9 and 93.7 respectively. Tobramycin was effective to 72.1 %, gentamicin to 71.0 % and kanamycin to 23.6 % of the species that were tested for the MIC values. The susceptibility ratios of the bacteria are shown in Table II.

DISCUSSION

UTIs are the most common site of nosocomial infections, accounting for 35 % to 45 % of all cases. The great majority of these infections are caused by gram-negative bacilli (8). The isolation rates of bacteria and the susceptibility patterns of these bacteria to antimicrobial agents are subject to change in time, and from hospital to hospital (9). So it is necessary to show these differences in distribution for each hospital in regular periods.

E. coli strains are the most common isolates of UTIs both in hospitalized patients and ambulatory patients. In hospitalized patients isolation of E. coli strains decreases while isolation rates of the other gram-negative bacteria increase. The reported percentages vary in different studies (2, 9, 10, 11). In MU Hospital, the Pseudomonas species and KES species were isolated more frequently than it was reported in some studies (2, 11). The great majority of the UTIs were from Urology Department with a high frequency of catheterization and high percentage of urinary tract pathologies. In such patients nosocomial infections and isolation of gram-negative bacilli other than E. coli are more frequent (2). An expected distribution of bacteria is seen in out-patients coming from departments other than the Urology Department.

Aminoglicosidic antimicrobials are inactivated by a series of enzymes which show local distribution. This is the reason for the emergence of resistance to these agents and the differences reported in different studies. Among the group, amikacin has a great resistance to these enzymes, which makes amikacin the most effective agent in the group (13). In some surveillance studies it is shown that there is a slight increase in resistance to amikacin, while in some other studies there is a slight decrease in overall aminoglicoside resistance when amikacin is used (9, 13). And in controlled clinical trials, particularly in immunocompromised patients, the overall emergence of resistance to amikacin has been remarkably low and contrasts rather strikingly with what has been observed in some monotherapeutic studies of beta-lactam agents (13).

Our results emphasize that the distribution of gram-negative bacteria in UTIs and their antibiotic susceptibility patterns may show local differences and it can be concluded that the isolation rates of bacteria and their antibiograms must be overviewed each year.

Table I: The Percentage of Bacteria Isolated From Different Departments

DEPARTMENT	Species	Out-Patient %	In-Patient %	Total %
UROLOGY		(101)*	(52)	(153)
	E.coli	54.45	23.07	43.79
	KES	21.78	40.38	28.10
	Proteus spp	6.93	5.76	6.53
	Pseudomonas spp	16.83	28.84	20.91
	Other Bacteria	1.98[a]	1.92[b]	1.95
PEDIATRICS		(17)	(21)	(38)
	E.coli	47.05	42.85	44.73
	KES	23.52	42.85	34.21
	Proteus spp	23.52	14.76	14.28
	Pseudomonas spp	5.88	9.52	7.89
	Other Bacteria	0.0	0.0	0.0
INTERNAL MEDICINE		(12)	(9)	(21)
	E.coli	66.66	77.77	71.42
	KES	25.0	11.11	19.04
	Proteus spp	8.33	11.11	9.52
	Pseudomonas spp	0.0	0.0	0.0
	Other Bacteria	0.0	0.0	0.0
OBSTETRICS AND GYNECOLOGY		(17)	(5)	(22)
	E.coli	70.58	20.00	59.09
	KES	29.41	40.00	31.81
	Proteus spp	0.0	0.0	0.00
	Pseudomonas spp	0.0	40.00	9.09
	Other Bacteria	0.0	0.0	0.0
OTHER DEPARTMENTS**			(16)	(16)
	E.coli		43.75	43.75
	KES		25.00	25.00
	Proteus spp		6.25	6.25
	Pseudomonas spp		12.50	12.50
	Other Bacteria		12.50[c]	12.50

* Numbers in parenthesis show the number of bacteria.
** Intensive Care (6), Neurosurgery (4), Orthopedics (3), Neurology (2), Surgery (1).
a Acinetobacter spp (1) and Citrobacter spp (1)
b Citrobacter spp (1)
c Citrobacter spp (2)

Table II: The Percentage of Bacteria Susceptible of Aminoglycoside Antimicrobials

BACTERIA	Antimicrobial	Out-Patient %	In-Patient %	Total %
E.coli		(17)*	(72)	(89)
	Amikacin	100.0	97.2	97.7
	Gentamicin	100.0	98.6	98.8
	Kanamycin	35.2	33.3	33.9
	Netilmicin	100.0	95.8	96.6
	Tobramycin	88.2	93.0	92.1
KES		(23)	(24)	(47)
	Amikacin	86.9	100.0	93.6
	Gentamicin	47.8	70.8	59.5
	Kanamycin	30.4	25.0	27.6
	Netilmicin	69.5	95.8	82.9
	Tobramycin	47.8	79.1	63.8
Proteus spp		(6)	(10)	(16)
	Amikacin	100.0	90.0	93.7
	Gentamicin	33.3	60.0	50.0
	Kanamycin	16.6	0.0	6.0
	Netilmicin	33.3	60.0	37.5
	Tobramycin	16.6	30.0	25.0
Pseudomonas spp		(17)	(13)	(30)
	Amikacin	88.2	84.0	86.6
	Gentamicin	11.7	15.32	13.3
	Kanamycin	5.8	0.0	3.4
	Netilmicin	58.8	76.9	66.6
	Tobramycin	64.7	46.0	56.6

* The numbers in parenthesis show the number of bacteria.

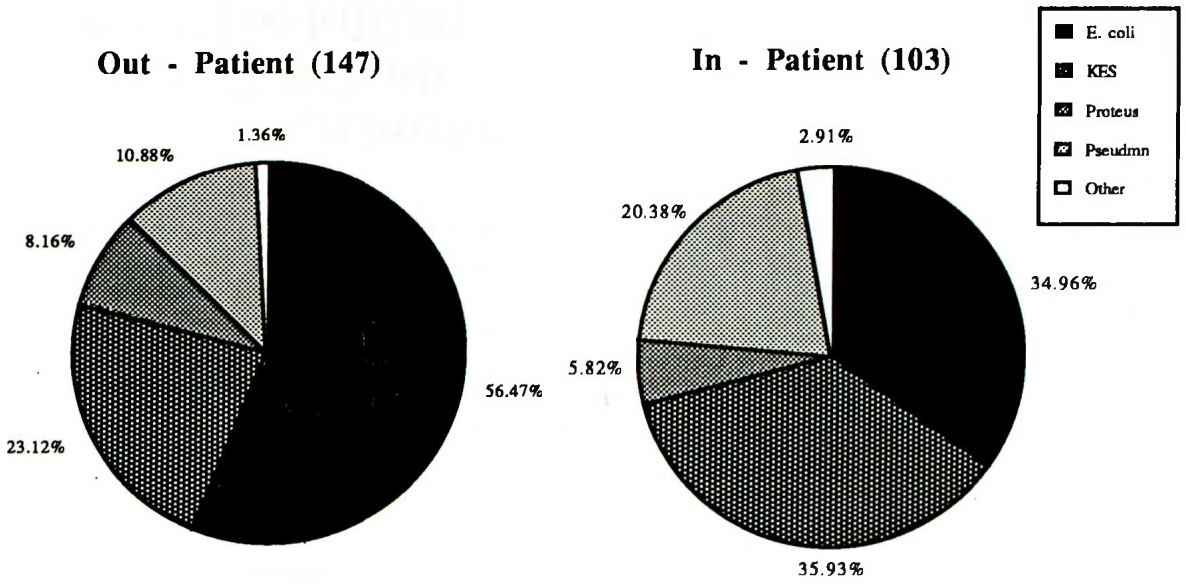

Figure 1. Percentage of gram - negative bacteria isolated from in - patients and out - patients.

ACKNOWLEDGMENT

We would like to thank to Assoc. Prof. Cevat Yakut,M.D. and Serdar Ener, M.D. from Koşuyolu Heart and Research Hospital for their kindness providing their sterilization equipment for the microplates we used.

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