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Original Article

Evaluation of Antimicrobial Resistance Among Gram-Negative Isolates Collected from Intensive Care Units and Reliability of Routine Disc Susceptibility Tests at a Teaching Hospital in Tehran

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Abstract

Antibiotic resistance of Intensive Care Unit (ICU) born aerobic gram-negative bacteria was evaluated during a six months period at a teaching hospital in Tehran, and determination of the validity of the results obtained from disc diffusion tests, using discs manufactured in Iran.

Disc susceptibility tests using Iranian and standard discs (diffusion discs available in international markets) were performed on 108 aerobic gram-negative isolates obtained from the clinical samples of patients with at least 72 hours of stay in the ICU. The Minimum Inhibitory Concentrations (MIC) was subsequently determined by collaborators not involved with the disc testing evaluation.

Acinetobacter was the most frequently isolated gram negative species (26%). High resistance rates were obtained for all antibiotics studied except for imipenem (98% sensitive). Results of disc diffusion tests performed by the Iranian discs were in moderate to strong agreement with those obtained from the standard discs. When comparing disc results with the MIC results, it was noted that the total number of very major, and minor discrepancies were approximately the same with both sets of discs. The total number of major discrepancies was higher for the Iranian discs (more false positive results). The total number of very major discrepancy rates was more than the acceptable 1.5% limits for each antibiotic tested, using both Iranian and standard discs.

High rates of resistance in aerobic gram-negative isolates studied, leaves imipenem as the only reliable agent for the empirical treatment of ICU infections. The high rates of very major discrepancies with both sets of discs show that physicians cannot rely on disc diffusion tests only, in order to guide therapy for the treatment of very serious infections in the ICUs, even if standard discs are used instead of the Iranian discs, and there is great need to establish a fast and easy way to determine the MIC values. Although it is better to repeat the study with a much larger sample size in order to make good judgment.

Keywords: disc diffusion; MIC; gram-negative; ICU; antimicrobial resistance.

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Introduction

Patients hospitalized in Intensive Care Units (ICUs) are 5 to 10 times more likely to acquire nosocomial infections than other hospitalized patients (1). This will result in consumption of nearly 10 times the antimicrobial agents used in general wards (2). Based on these reports ICUs are considered epicenters of antibiotic resistance and the principal sources of multi-resistant bacterial outbreaks (3). This increase in bacterial resistance will result in increased morbidity and mortality, and inflation of health care costs (4-8). Therefore optimizing the treatment of infectious diseases in the ICUs is crucial and requires the following:

1) *To be aware of the antimicrobial resistance pattern in the ICU, in order to guide the clinician in the choice of an optimal empiric antibiotic regimen*. In fact, updated unit-specific antibiograms should be provided to the clinicians at least once a year to ensure that the data are current and useful (4). This will help physicians to devise empiric regimens that have a greater likelihood of covering the organisms posing the greatest risk, and at the same time reduce the unnecessary administration of broad spectrum antibiotics. (4, 8, 9)

2) *To be insured of the validity of the results of in vitro antibiotic susceptibility testing.* There are various *in vitro* antibiotic susceptibility tests that will assist the clinician in the choice of an appropriate antibiotic for the treatment of infected patients. The majority of laboratories in Iran use the disc diffusion technique. The antibiotic discs used are manufactured in Iran, based on the National Committee for Clinical Laboratory Standards (NCCLS) guidelines. Many physicians have lack of confidence in the results of antibiogram tests using these discs, because of the inconfidence in their quality control process. As far as we know there has been no published study to evaluate Iranian-made antibiogram discs.

The aim of this study was to 1. determine the frequency of antibiotic resistance of ICU-born gram-negative bacteria during a six months period (Oct.2003-April 2004) at a teaching hospital (Shariati Hospital) in Tehran. The results of this could guide ICU physicians in the empiric therapy of nosocomial infections. 2. Compare the results of antibiogram tests using Iranian-made discs with those using the standard discs, on clinical isolates separated from ICU patients. 3. Determine the validity of the disc diffusion methods by measuring the minimum inhibitory concentration of the antibiotics used against the gram-negative bacteria.

Experimental

Samples were collected from the general ICU, Neurosurgery ICU, and the Open Heart ICU of Shariati Hospital in Tehran, and sent to the hospital's microbiology department during a six months period from Oct.2003 to Apr.2004. All aerobic gram-negative isolates that were collected from patients with at least 72 hours of stay in the ICUs were entered into the study. Repeated cultures of the same microorganism derived from the same site of the same patient was not included. After identification and gram staining, Isolates were cultured and antibiogram sensitivity tests were performed using Iranian-made antibiotic discs (Patanteb, Iran) and the National Committee for Clinical Laboratory Standards (NCCLS) guidelines (10). These isolates were sub cultured in Trypticase Soy Agar (TSA), and antibiogram sensitivity tests were repeated at the end of each week using standard antibiotic discs (antibiotic discs available in international market). The standard antibiotic discs used in this study were ceftriaxone and ceftazidime 30µg discs from Difco, Becton Dickinson and company, 1 Beccton Drive Franklin Lakes, NJ USA; ciprofloxacin 5µg and imipenem10µg from BBL, Becton Dickinson and company, 1 Beccton Drive Franklin Lakes, NJ USA; gentamicin10µg and amikacin30µg from Mast Diagnostics, Mast group Ltd, Merseyside, UK.

After performing the antibiogram tests, MIC determination via serial microdilution test following the NCCLS guidelines, (11) were performed on slopes of all isolates sent to the Ministry of Health Reference Laboratory. Since most commercial MIC systems do not test concentrations of the drugs in the range that is obtained in urine, the results of these MIC tests for treating urinary tract infections

in practice cannot be relied on. On the other hand, the MIC determination via the serial microdilution method is a costly, time consuming procedure. Therefore, MIC determinations for urine samples were not performed in this study. The antibiotics used for MIC evaluation were amikacin (COMPLANT IMP. & EXP. CO., LTD Add: 12/F, Zhongshan Mansion, 93 East Zhongshan Rd., Ningbo 315000 China.), gentamicin (Fujian Fukang Pharmaceutical Co., Ltd (Fukang) NO.138, Xiangban Road, Fuzhou, China), ceftriaxone (Hanmi Fine Chemical Co., Ltd**.** 1248-8, Chongwang-Dong, Shihung-City, Kyonggi-Do, Korea), ceftazidime (Hanmi, Korea) and ciprofloxacin (USP, USA). All antibiotic powders were Quality Controlled (QC) certified. Control strains used in this study were *E-coli* ATCC 25922, and *Pseudomonas aeruginosa* ATCC 27853.

Results of the disc diffusion tests using the Iranian (Ir) and the standard (St) discs were compared and their levels of agreement were determined using the kappa measure of agreement (K). Kappa values were determined considering all intermediate (I) isolates as resistance (R), and $P<0.05$ was considered significant. Kappa value ranges of 0.8-1, 0.6-0.79, 0.2-0.59, and < 0.2 indicate strong, substantial, moderate, and poor agreements respectively (12).

To determine the discrepancies between the results of disc diffusion and broth microdilution tests, MIC zone diameter scattergrams for each of the 5 drugs tested were prepared with zone diameters on the x-axis and MIC on the y-axis; with horizontal and vertical lines showing the proposed interpretive breakpoints (13). According to NCCLS guidelines, because of the ±1 dilution variation of MICs and the ± 3 to 4 mm variation of zone diameters that are intrinsic to the testing systems, it is appropriate to allow greater discrepancy rates for strains with MICs at the intermediate MIC ± 1 twofold concentration (I+1 to I-1), and more limited discrepancy rates for strains with MICs with ≥ 2 twofold concentrations above (≥I+2) or below (≤I-2) the intermediate MIC. Discrepancy rates are categorized as very major (resistant MIC and susceptible zone diameter), major (susceptible MIC and resistant zone diameter), and minor (intermediate by one method and resistant or susceptible by the other). Acceptable discrepancy rates for the three above-mentioned MIC groups have been proposed as follows: For I+1 to I-1, <10% major, <10% very major, and <40% minor discrepancies; for ≥ I+2, <2% very major, and <5% minor discrepancies; and for ≤ I-2, <2% major, and <5% minor discrepancies. For assessing already established criteria with routine clinical isolates, <1.5% very major and <3% major discrepancies using the total population as the denominator is ideal. No limits on minor discrepancies are provided (13, 14).

Result and Discussion

A total of 108 aerobic gram-negative bacterial isolates were obtained From 43 patients who met the nosocomial infection criteria. Of these, 70 were single isolates and 17 were derived from poly-microbial growths of the same sample. Organisms were isolated mainly from the urinary tract (n=43; 39.8%), respiratory tract (n=27; 25%), and blood (n=22; 20.4%). The remaining 16 isolates (14.8%) were from wounds,

Table 1. Susceptibility (percent) of isolated microorganisms (results of disc diffusion using Iranian discs)

Organism	Antibiotic						
	n[a]	%	AMK	GEN	CRO	CAZ	CIP
Acinetobacter spp.	28	25.9	17.9	17.9	0	7.1	3.6
Enterobacter spp.	17	15.7	47.1	23.5	0	0	70.6
E.coli	17	15.7	76.5	29.4	29.4	23.5	41.2
Klebsiella spp.	18	16.7	38.9	11.1	5.6	0	27.8
Pseudomonas spp.	25	23	76	60	12	40	68
Others	3	2.8	66.7	66.7	33.3	33.3	66.7
Total	108	100	50	30.6	9.3	15.7	40.7

AMK, amikacin; GEN, gentamicin; CRO, ceftriaxone; CAZ, ceftazidime; CIP, ciprofloxacin.
a. number of microorganisms that had undergone disc diffusion test with Iranian discs

Table 2. Susceptibility (percent) of isolated microorganisms (results of disc diffusion using Standard discs)

Organism	Antibiotic							
	n[a]	%	AMK	GEN	CRO	CAZ	CIP	IPM
Acinetobacter spp.	27	26.2	33.3	18.5	0	3.7	3.7	92.6
Enterobacter spp.	17	16.5	82.4	29.4	0	0	82.4	100
E.coli	16	15.5	81.3	31.3	31.3	37.5	43.8	100
Klebsiella spp.	18[b]	17.5	61.1	16.7	11.8	17.6	33.3	100
Pseudomonas spp.	22	21.4	81.8	59.1	9.1	50	68.2	100
Others	3	2.9	100	66.7	100	66.7	66.7	100
Total	103	100.0	66	32	11.8	22.5	43.7	98.1

AMK, amikacin; GEN, gentamicin; CRO, ceftriaxone; CAZ, ceftazidime; CIP, ciprofloxacin; IPM, Imipenem.
a. number of microorganisms that had undergone disc diffusion test with the standard discs. Of the total 108 isolates, 103 were tested with the AMK,GEN, and CIP, and 102 with CRO and CAZ discs. This was due to the unavailability of the same standard discs at the end of the study.
b. 17 out of 18 Klebsiella isolates were tested with CRO and CAZ St discs.Therefore overall 102 isolates were tested with these two antibiotic discs.

peritoneal fluid, chest tube, CSF, eye, and ear. *Acinetobacter* spp were the most frequently isolated gram negative species (n=28; 26%), followed by *Pseudomonas* spp (n=25; 23%), *Klebsiella* spp (n=18; 16.7%), *Enterobacter* spp (n=17; 15.7%) and *E.coli* (n=17; 15.7%). The remaining 2.8% (n=3) of the isolates were *Proteous vulgaris* and *Morganella morganii*. The most frequently obtained microorganism from the urine, respiratory tract, and blood were *E-coli* (n=12; 28%), *Acinetobacter* spp. (n=10; 37%), and *Klebsiella* spp. (n=6; 27.3) respectively.

Tables 1 and 2 show the sensitivity to the antibiotics tested for all aerobic gram negative bacterial (GNB) isolates using disc diffusion tests with the Ir and St discs respectively. Discrepancies between the results obtained from the two sets of antibiograms are noticed for sensitivities of *Acinetobacter, Enterobacter*, and *Klebsiella* spp to amikacin, and *Klebsiella* spp. to ceftazidime. All other results are comparable between the two tests. According to the results obtained from disc diffusion tests via Ir discs (Table1), ciprofloxacin was the most effective antibiotic for *Enterobacter* spp, while amikacin was the most effective for all other gram negative bacteria tested. The least effective antibiotic for all GNB isolates were the third generation cephalosporines. Iranian imipenem discs were not manufactured at the time of this investigation. However disc diffusion test with standard imipenem discs from BBL, USA were performed for 104 of the isolates (Table2). Results obtained from disc diffusion tests using the St discs (Table2) show that after imipenem, amikacin was the most effective antibiotic for all GNB tested. For *Enterobacter* species ciprofloxacin was equally effective (82.4% sensitive with both amikacin and ciprofloxacin, n=17). The third generation cephalosporines were the least effective drug.

MIC values were determined for only 55 of the GNB isolates. Sensitivity values according to MIC results, are shown in Table 3. Ciprofloxacin was the most effective antibiotic

Table 3. Susceptibility (percent) of isolated microorganisms (results of MIC tests)

Organism	Antibiotic						
	n[a]	%	AMK	GEN	CRO	CAZ	CIP
Acinetobacter spp.	18	32.7	5.6	0	0	0	5.6
Enterobacter spp.	9	16.4	77.8	11.1	0	0	44.4
E.coli	5	9.1	60	0	0	40	20
Klebsiella spp.	11	20.0	36.4	0	0	0	27.3
Pseudomonas spp.	10	18.2	60	50	20	50	80
Others	2	3.6	100	50	50	50	50
Total	55	100	41.8	12.7	5.5	14.5	32.7

AMK, amikacin; GEN, gentamicin; CRO, ceftriaxone; CAZ, ceftazidime; CIP, ciprofloxacin.
a. number of microorganisms that had undergone MIC test

Table 4. Comparison of disc diffusion test results with Iranian (Ir) and standard discs [a]

Antibiotic (disc content)/species		Susceptibility determined by standard discs: sensitive – Ir disc result			Intermediate – Ir disc result			Resistant – Ir disc result				
	n [d]	S	I	R	S	I	R	S	I	R	kappa [b]	Approx.[c] Sig. (P)
All aerobic GNB	108											
Amikacin (30µg)	103	48	11	9	0	4	9	3	1	18	0.555	0.000*
Gentamicin (10µg)	103	27	2	4	1	1	6	1	2	59	0.816	0.000*
Ceftriaxone (30µg)	102	6	2	4	2	3	6	0	0	79	0.558	0.000*
Ciprofloxacin (5µg)	103	40	4	1	0	4	1	0	1	52	0.900	0.000*
Ceftazidime (30µg)	102	13	4	6	0	0	9	1	0	69	0.649	0.000*
Amikacin (30µg)												
Acinetobacter spp.	27	4	2	3	0	0	2	1	1	14	0.438	0.014*
Enterobacter spp.	17	8	3	3	0	0	3	0	0	0	0.320	0.072
E.coli	16	11	2	0	0	2	0	1	0	0	0.455	0.064
Klebsiella spp.	18	6	3	2	0	2	4	1	0	0	0.365	0.088
Pseudomonas spp.	22	17	1	0	0	0	0	0	0	4	0.861	0.000*
Proteous spp.	2	1	0	1	0	0	0	0	0	0	NA	NA
Morganella morganii	1	1	0	0	0	0	0	0	0	0	NA	NA
Total	103											
Gentamicin (10µg)												
Acinetobacter spp.	27	4	0	1	0	0	2	1	1	18	0.755	0.000*
Enterobacter spp.	17	4	0	1	0	0	1	0	0	11	0.850	0.000*
E.coli	16	4	1	0	0	0	0	0	0	11	0.846	0.001*
Klebsiella spp.	18	1	0	2	1	1	3	0	1	9	0.308	0.180
Pseudomonas spp.	22	12	1	0	0	0	0	0	0	9	0.908	0.000*
Proteous spp.	2	2	0	0	0	0	0	0	0	0	NA	NA
Morganella morganii	1	0	0	0	0	0	0	0	0	1	NA	NA
Total	103											
Ceftriaxone (30µg)												
Acinetobacter spp.	27	0	0	0	0	0	0	0	0	27	NA	NA
Enterobacter spp.	17	0	0	0	0	0	1	0	0	16	NA	NA
E.coli	16	4	1	0	0	0	0	0	0	11	0.846	0.001*
Klebsiella spp.	17	1	0	1	0	0	3	0	0	12	0.638	0.005*
Pseudomonas spp.	22	0	1	1	2	3	2	0	0	13	-0.100	0.639
Proteous spp.	2	1	0	1	0	0	0	0	0	0	NA	NA
Morganella morganii	1	0	0	1	0	0	0	0	0	0	NA	NA
Total	102											
Ceftazidime (30µg)												
Acinetobacter spp.	27	1	0	0	0	0	3	1	0	22	0.649	0.000*
Enterobacter spp.	17	0	0	0	0	0	1	0	0	16	NA	NA
E.coli	16	3	2	1	0	0	4	0	0	6	0.556	0.013*
Klebsiella spp.	17	0	0	3	0	0	1	0	0	13	NA	NA
Pseudomonas spp.	22	8	2	1	0	0	0	0	0	11	0.727	0.000*
Proteous spp.	2	1	0	1	0	0	0	0	0	0	NA	NA
Morganella morganii	1	0	0	0	0	0	0	0	0	1	NA	NA
Total	102											
Ciprofloxacin (5µg)												
Acinetobacter spp.	27	1	0	0	0	2	1	0	1	22	1.000	0.000*
Enterobacter spp.	17	12	2	0	0	0	0	0	0	3	0.679	0.003*
E.coli	16	6	0	1	0	0	0	0	0	9	0.871	0.000*
Klebsiella spp.	18	5	1	0	0	0	0	0	0	12	0.870	0.000*
Pseudomonas spp.	22	14	1	0	0	1	0	0	0	6	0.899	0.000*
Proteous spp.	2	1	0	0	0	1	0	0	0	0	1.000	0.157
Morganella morganii	1	1	0	0	0	0	0	0	0	0	NA	NA
Total	103											

a. S,Sensitive: for amikacin, zone diameter ≥17mm; for gentamicin zone diameter ≥15mm; for ceftriaxone and ciprofloxacin, zone diameter ≥21; and for ceftazidime, zone diameter ≥18mm. I, Intermediate: for amikacin, zone diameter 15-16mm; for gentamicin zone diameter 13-14mm; for ceftriaxone, zone diameter 14-20; for ceftazidime, zone diameter 15-17, and for ciprofloxacin, zone diameter 16-20. R,Resistant: for amikacin and ceftazidime, zone diameter ≤14mm; for gentamicin zone diameter ≤12mm; for ceftriaxone, zone diameter ≤13; and for ciprofloxacin, zone diameter ≤15.

b. kappa values calculated considering all Intermediates as Resistant

c. NA, not applicable

d. Of the total 108 isolates tested with the iranian discs, 103 were tested with the amikacin, gentamicin, and ciprofloxacin, and 102 with ceftriaxone and ceftazidime standard discs. This was due to the unavailability of the same standard discs at the end of the study.

* P<0.05, considered statistically significant

Table 5. MIC zone diameter discrepancy rates for five antibiotics with gram negative bacterial isolates, using Ir discs (Left), and St discs (Right).

Antimicrobial agent and MIC range	No.	No. of discrepancies (discrepancy rate [%]) [a]			No.	No. of discrepancies (discrepancy rate [%]) [a]		
		Very Major	Major	Minor		Very Major	Major	Minor
Ciprofloxacin								
>=I+2	32	2 (6.25)	NA	2 (6.25)	32	2 (6.25)	NA	2 (6.25)
I+1 to I-1	5	0	0	3 (60)	5	0	0	3 (60)
<=I-2	18	NA	1 (5.5)	2 (11)	17	NA	0	1 (5.9)
Total	55	2 (3.63)	1 (1.8)	7 (12.7)	54	2 (3.7)	0	6 (11)
Ceftriaxone								
>=I+2	46	0	NA	3 (6.5)	45	0	NA	6 (11)
I+1 to I-1	7	0	1 (14.3)	1 (14.3)	7	0	0	3 (42.8)
<=I-2	2	NA	0	0	1	NA	0	0
Total	55	0	1 (1.8)	4 (7.3)	53	0	0	9 (17)
Ceftazidime								
>=I+2	43	3 (6.9)	NA	0	42	2 (4.7)	NA	2 (4.7)
I+1 to I-1	6	1 (16.6)	2 (33.3)	1 (16.6)	6	1 (16.6)	1 (16.6)	0
<=I-2	6	NA	0	2 (33.3)	5	NA	0	0
Total	55	4 (7.3)	2 (3.6)	3 (5.4)	53	3 (5.6)	1 (1.8)	2 (3.7)
Gentamicin								
>=I+2	38	3 (7.9)	NA	2 (5.2)	38	3 (7.9)	NA	4 (10.5)
I+1 to I-1	11	3 (27.3)	0	4 (36.3)	11	3 (27.3)	0	3 (27)
<=I-2	6	NA	0	1 (16.6)	5	NA	0	0
Total	55	6 (10.9)	0	7 (12.7)	54	6 (11)	0	7 (13)
Amikacin								
>=I+2	12	3 (25)	NA	0	12	2 (16.6)	NA	0
I+1 to I-1	29	4 (13.8)	3 (10.3)	12 (41)	29	7 (24)	1 (3.4)	12 (41)
<=I-2	14	NA	1 (7)	2 (14.3)	13	NA	0	0
Total	55	7 (12.7)	4 (7.3)	14 (25.4)	54	9 (16.6)	1 (1.8)	12 (22)

a. NA, not applicable

against *Pseudomonas* spp. based on the MIC values. Amikacin was the most effective for all other GNB isolates, while the third generation cephalosporines were the least effective drug.

Comparison of standard (St) discs with Iranian (Ir) discs

Disc diffusion tests with the Iranian discs were performed for all (n=108) isolates, whereas 103/108 isolates were tested with the standard amikacin, gentamicin, and ciprofloxacin discs, and 102/108 with the ceftriaxone and ceftazidime St discs.

Susceptibility data of all gram negative isolates tested by both sets of discs, together with the K and P values are shown in Table 4. Agreements between the two sets of tests are categorized as strong, substantial, moderate, and poor, according to the kappa measure of agreements. Kappa value was determined considering all intermediate isolates as resistant.

Considering all gram negative isolates together, according to K the results of Ir ciprofloxacin and gentamicin discs had strong agreement with the results of the St discs. There was substantial agreement for ceftazidime and moderate agreement for ceftriaxone and amikacin. All of the above were significant (P<0.5).

Considering each microorganism separately, for the antibiotics in which the overall agreements between the results of both tests were considered moderate (amikacin and ceftriaxone), it can be notified that the results of Ir amikacin discs were in strong agreement with St discs for *Pseudomonas* spp. (P<0.5), but in moderate agreement for all other microorganisms listed in the Table. Kappa was significant for *Acinetobacter* but not significant for *Enterobacter, E.coli* and *Klebsiella*. As for the ceftriaxone discs, the agreement between the two discs were considered strong and substantial for *E.coli* and *Klebsiella* spp., respectively (P<0.5). Kappa could not be measured for *Acinetobacter* and *Enterobacter* species since all isolates were resistant with both discs. There

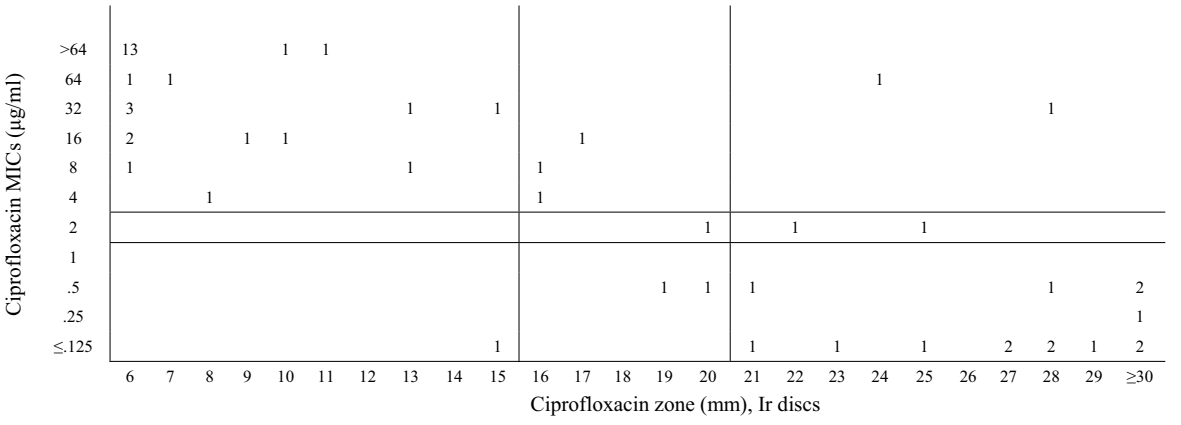

Figure 1A. Ciprofloxacin zone diameters obtained from Iranian discs versus their corresponding MICs with 55 GNB isolates. Horizontal lines represent established MIC breakpoints.

was a tendency towards disagreement between the results of the two kinds of ceftriaxone discs for Pseudomonas isolates according to kappa although this disagreement was not significant.

Discrepancies between disc results with MIC results

Out of 108 isolates, 43 were obtained from urine which were not sent for MIC determination. Of the 65 isolates left, 10 were unrecovered for MIC testing when slopes were received by the reference laboratory. Therefore the total number of isolates that had undergone the MIC test were 55. MIC zone diameter scattergrams for each of the 5 antibiotics tested are provided in Figures 1A-5A and 1B-5B, and discrepancy rates are listed in Tables 5.

The total numbers of very major and minor discrepancies were almost the same with both kinds of discs considering each antibiotic tested, but the major discrepancies were lower with the standard discs.

We found that *Acinetobacter* spp. are the most frequently isolated GNB (26%), followed by *Pseudomonas* spp.(23%) in the ICUs. *Klebsiella, Enterobacter* and *Ecoli* are also commonly isolated. There are previous reports indicating *Pseudomonas* spp. as the most frequent GNB of the ICUs (6, 15-17). Previous studies have identified various risk factors for *Acinetobacter* infection or colonization; factors related to host, period of hospitalization, intubation, catheter lines, previous antibiotic therapy (cephalosporines/ fluoroquinolones), etc (17, 18). The majority of patients in this study had most of these risk factors, therefore reporting *Acinetobacter* spp. as the predominant pathogen is not surprising.

Data reported from 112 medical ICUs in US between 1992 and 1997 indicated that UTIs were the most common nosocomial infection (31%) followed by pneumonia (27%), and bloodstream

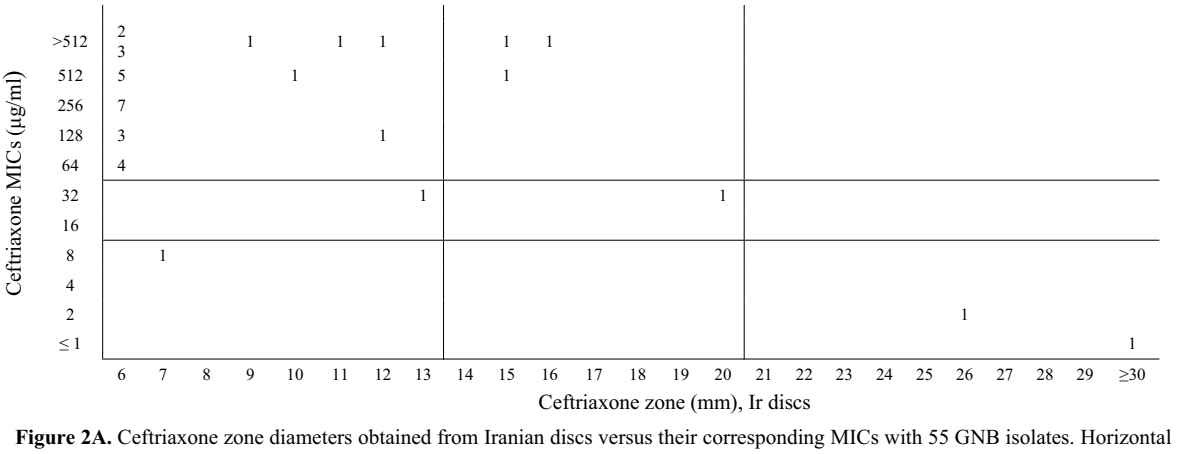

Figure 2A. Ceftriaxone zone diameters obtained from Iranian discs versus their corresponding MICs with 55 GNB isolates. Horizontal and vertical lines represent established MIC and zone diameter breakpoints, respectively.

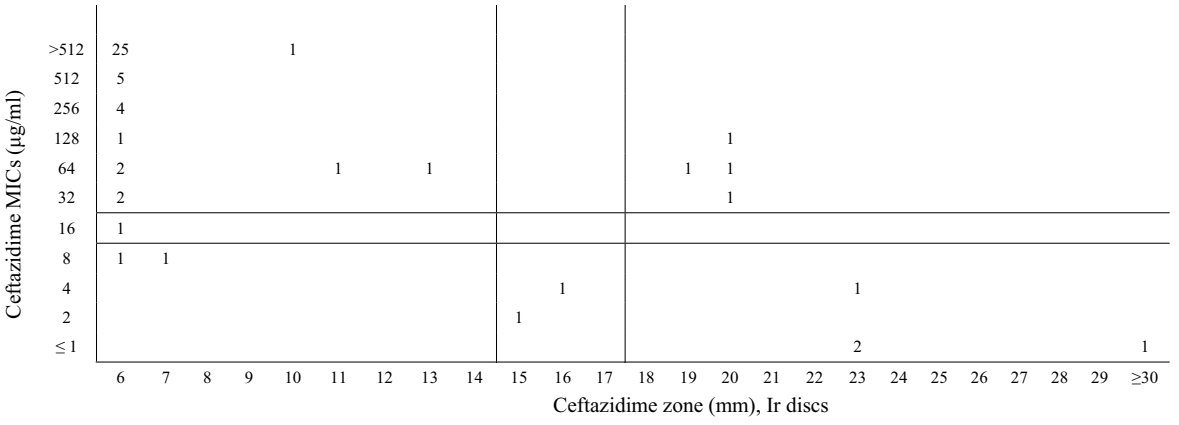

Figure 3A. Ceftazidime zone diameters obtained from Iranian (Ir) discs versus their corresponding MICs with 55 GNB isolates. Horizontal and vertical lines represent established MIC and zone diameter breakpoints, respectively

infections (19%) (19). In accordance with these data most of the isolates in this study were obtained from urine (39.8%), respiratory tract (25%), and blood (20.4%). This could also be a good reason for the predominance of *Acinetobacter* species, because studies on *Acinetobacter* in various countries have shown a predominance of isolation from urine (21-27%) and tracheobronchial secretions (24.8- 48.8%) (18).

High resistance rates were observed for all antibiotics studied except for imipenem which was the most active agent against all GNB isolated. Ciprofloxacin and amikacin were relatively effective but still 40-60% of all isolates were resistant to these antibiotics. Imipenem, amikacin, and ciprofloxacin were the most active agents against Pseudomonas species followed by ceftazidime with susceptibility rates of 40-50%. For the *Enterobacter* species, imipenem, amikacin, and ciprofloxacin had the greatest susceptibility rates. Similar results were obtained from a multicenter study in Turkey during 1997 (15).

Klebsiella spp. were consistently susceptible to imipenem, but in contrast to the multicenter study in Turkey ciprofloxacin was not an active agent, and around 70% of *Klebsiella* species were resistant to ciprofloxacin. Unlike the study performed in Turkey in which *Ecoli* was generally susceptible to most antibiotics studied, results obtained from our ICUs showed that except for imipenem and amikacin which had good activity against the *Ecoli* strains, all other antibiotics had high resistancy rates to this species (around 60-70% or even more). *Acinetobacter* spp. obtained from our ICUs were more susceptible to imipenem compared with those from the ICUs of Turkey (susceptibility rates were 92.6% and 55.5% respectively). In fact imipenem was the only antibiotic effective against *Acinetobacter* spp. in our study, and the sensitivity rates of the other antibiotics against this species were much

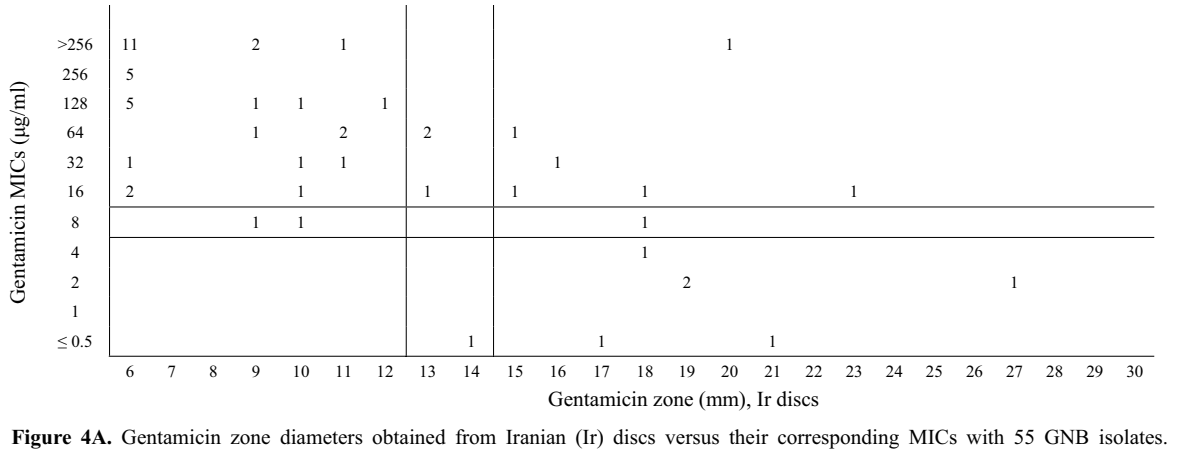

Figure 4A. Gentamicin zone diameters obtained from Iranian (Ir) discs versus their corresponding MICs with 55 GNB isolates. Horizontal and vertical lines represent established MIC and zone diameter breakpoints, respectively

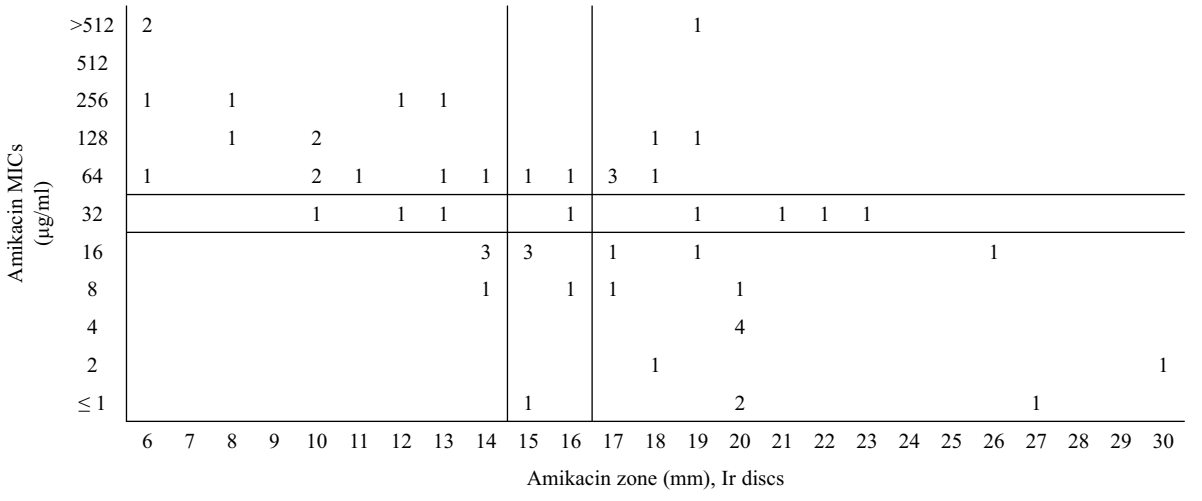

Figure 5A. Amikacin zone diameters obtained from Iranian (Ir) discs versus their corresponding MICs with 55 GNB isolates. Horizontal and vertical lines represent established MIC and zone diameter breakpoints, respectively

lower than those of the same antibiotics against *Acinetobacters* obtained from the Turkish ICUs (15). One probable reason for the high sensitivity of imipenem to all isolates studied, is recent introduction of this drug to the Iranian drug market (1999). Also due to its limited availability and high price it was not used extensively until 2001, when the availability increased, and the price decreased (20).

The weight of evidence suggests that empirical use of third generation cephalosporines as workhorse empirical antibiotic therapy should be avoided. In many ICUs these drugs maybe unable to provide adequate empirical coverage, and in addition, they have been implicated in outbreaks of multidrug resistant organisms of a variety of species (21). Data from our ICUs also show that this class of drugs has very low susceptibility rates to the GNB isolated (30% or less), attributable to the excess use of these drugs. These data suggest that the use of 3rd generation cephalosporines should be limited in our ICUs as the empiric treatment of nosocomial infections. Results of the disc diffusion tests show that results of disc diffusions performed by Ir gentamicin, ceftazidime, and ciprofloxacin discs are in strong agreement with those obtained by the standard discs according to kappa values. The agreement was considered moderate for amikacin and ceftriaxone discs. Higher sensitivity rates were obtained using the St discs especially with amikacin and ceftriaxone. These differences confirm the fact that despite standardization of the inoculum size, test media composition, disc content of antibiotic, incubation condition, and interpretive criteria by the NCCLS, minor variations, particularly of inoculum size, may affect the disc diffusion test result (22).

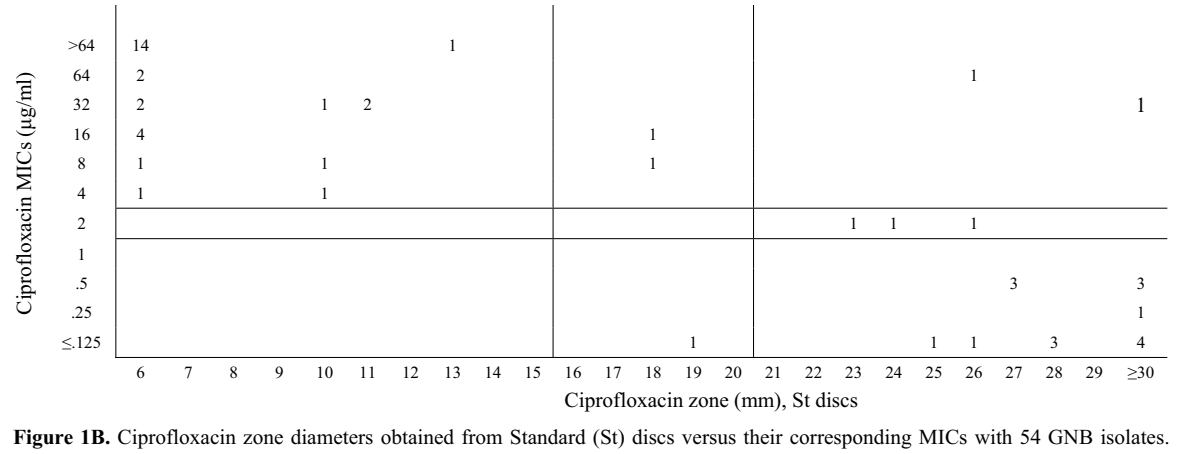

Figure 1B. Ciprofloxacin zone diameters obtained from Standard (St) discs versus their corresponding MICs with 54 GNB isolates. Horizontal lines represent established MIC breakpoints.

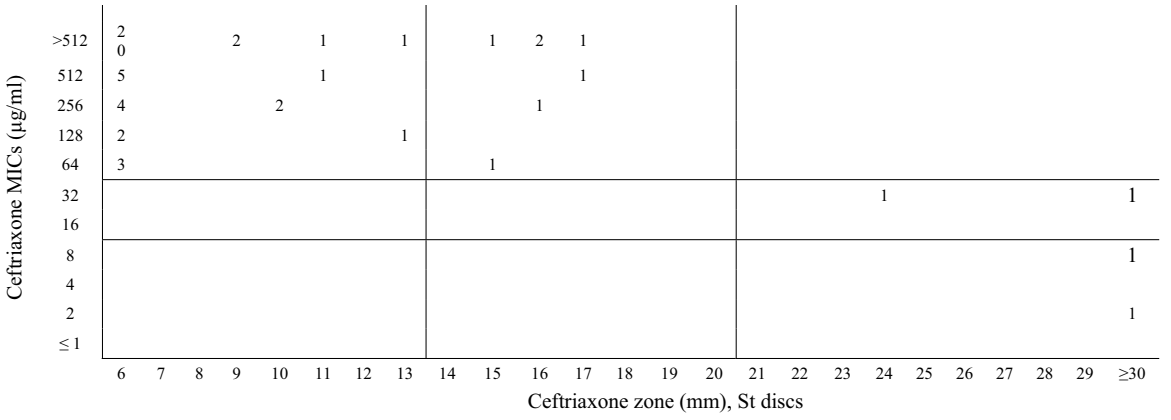

Figure 2B. Ceftriaxone zone diameters obtained from Standard (St) discs versus their corresponding MICs with 53 GNB isolates. Horizontal and vertical lines represent established MIC and zone diameter breakpoints, respectively

On the other hand, when comparing disc results with the MIC results (Tables 5), it is noted that the total number of very major and minor discrepancies are approximately the same for these two sets of discs, but the total major discrepancies are higher for the Ir discs. This means that with disc diffusion performed with the Ir discs more false resistant results are noted compared with the St discs. According to NCCLS the total number of major discrepancies should be <3% using the total population as the denominator (13, 14). Therefore from our data the major discrepancy rates (DRs) were acceptable for all antibiotic discs tested except those obtained from Ir amikacin discs (major DR of 7.3%). The total number of very major discrepancies should be less than 1.5% for each antibiotic tested in order to be acceptable according to NCCLS. (13, 14) It is important to keep the number of very major discrepancies to a minimum, because these discrepancies mislead the clinician by reporting a resistant organism as sensitive and therefore may adversely affect patient outcome. In our study the very major DRs were much more than 1.5% for most of the antibiotics tested with both kinds of discs. According to NCCLS, (13) the discrepancy limits should be calculated with at least 500 unselected clinical isolates. Therefore it is best to repeat the study with a larger population of isolates, not limited in the ICU, but much evenly distributed, meaning that equal amounts of susceptible and resistant isolates are present. In this way the quality of the Ir discs could be evaluated more precisely. Disc diffusion performed with the amikacin discs had the largest very major DRs as shown in Table 5 (16.6 for the St and nearly 13% for the Ir discs). In fact our data shows that only 56% of the species reported as sensitive by the disc diffusion tests (both Ir and St discs) were confirmed as susceptible by the serial micro dilution tests. That is, in 44% of cases the

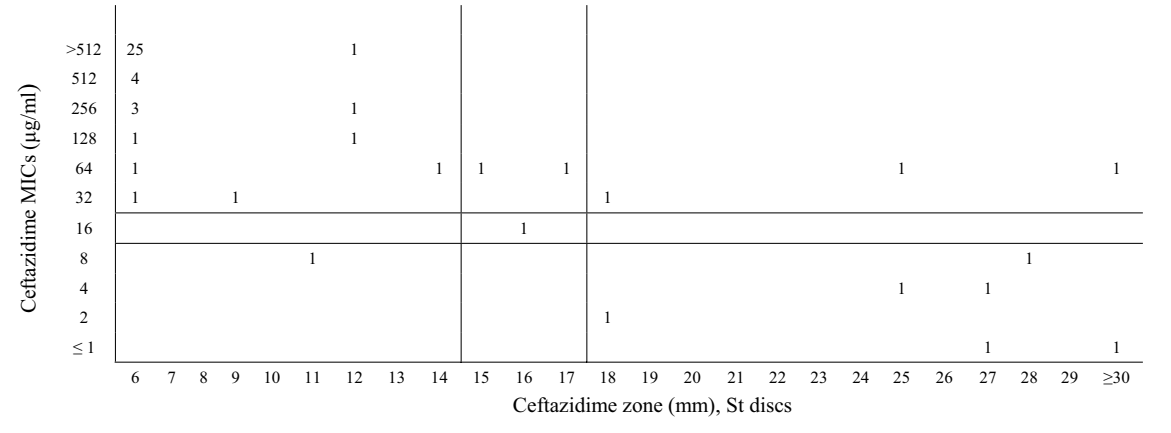

Figure 3B. Ceftazidime zone diameters obtained from Standard (St) discs versus their corresponding MICs with 53 GNB isolates. Horizontal and vertical lines represent established MIC and zone diameter breakpoints, respectively.

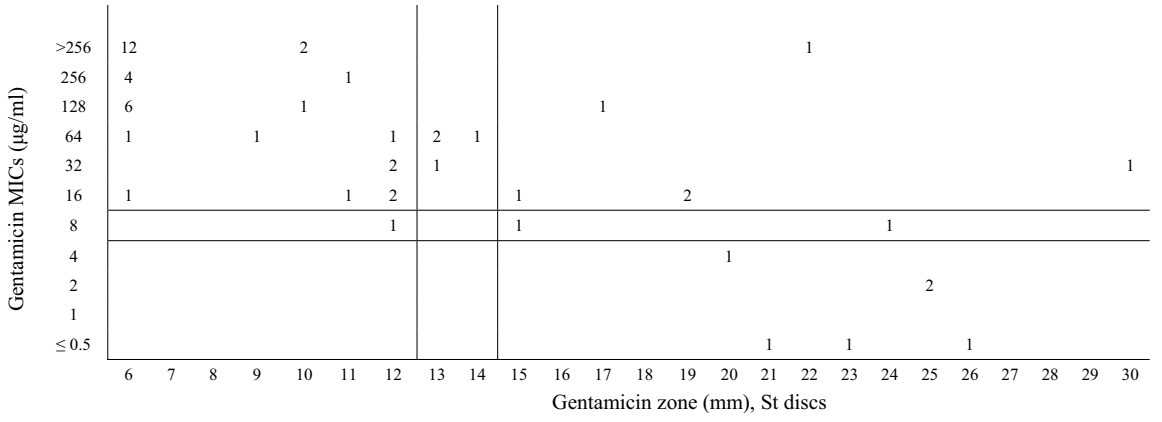

Figure 4B. Gentamicin zone diameters obtained from Standard (St) discs versus their corresponding MICs with 54 GNB isolates. Horizontal and vertical lines represent established MIC and zone diameter breakpoints, respectively.

isolate was actually resistant to the drug. These DRs are too large to be neglected. To understand this large very major DRs for amikacin, it can be said that since DRs are directly proportional to the percentage of isolates with antibiotic MICs in the range of one two-fold concentration above and one two-fold concentration below the intermediate MIC (I+1 to I-1), (13) and since most isolates tested had Amikacin MICs in this range (53% of the isolates); it is not unusual to obtain this large very major error for this antibiotic compared with the other drugs tested in which only 10-20% of the MICs were in this range. In order to definitely evaluate the amikacin discs this study should be repeated using isolates with more evenly distributed MIC values.

This study has shown that there are high rates of resistance in aerobic gram-negative isolates from the ICUs of Shariati Hospital. Overall resistance rates were highest with the third generation cephalosporines and lowest with imipenem. Therefore 3rd generation cephalosporine use should be limited and other antibiotics substituted for the empiric treatment of infections in these ICUs. Our data shows that the very major discrepancies between the results of disc diffusion tests by both Ir and St discs and the MIC results were almost the same. Although these discrepancy rates were above the acceptable range of NCCLS, we cannot make good judgment due to the small number of isolates tested. Overall the results show that physicians cannot rely on the disc diffusion tests only, in order to choose the best antibiotic for the treatment of patients with very serious infections like blood stream infections and pneumonia, even if St discs are used instead of Ir discs. Actually there is great need for establishing a fast and easy way to determine the MIC values

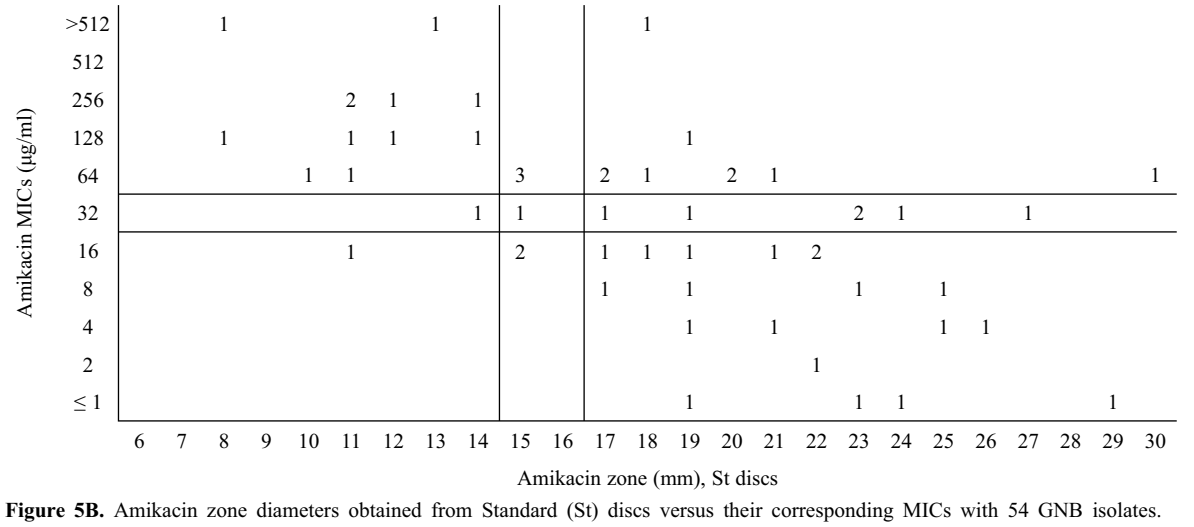

Figure 5B. Amikacin zone diameters obtained from Standard (St) discs versus their corresponding MICs with 54 GNB isolates. Horizontal and vertical lines represent established MIC and zone diameter breakpoints, respectively.

(i.e. E-test), (23) in our hospital, and to perform this test at least for clinical isolates derived from the ICUs where the most serious infections occur.

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