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RESISTANCE PATTERN OF DIFFERENT AMINOGLYCOSIDES AGAINST GRAM POSITIVE AND GRAM NEGATIVE CLINICAL ISOLATES OF KARACHI

FAYAIZ AHMED, BAQIR, S. NAQVI, MUHAMMAD HARRIS SHOAIB, KHURHEED HASHMI* AND DILNAWAZ SHAIKH**

Department of Pharmaceutics, Faculty of Pharmacy, University of Karachi
**Department of Pathology, Sind Medical College, Karachi*
***Faculty of Pharmacy, Hamdard Medical University, Karachi*

ABSTRACT

Microbial resistance to majority of the available antimicrobial agent is a serious and global problem. Due to heavy and discriminate usage of antibiotics, high prevalence of drug-resistant bacteria in the indigenous fecal flora, poor standards of sanitation, lack of education and prevalence of malnutrition. This problem is at its extreme in developing countries like Pakistan. For this various Aminoglycosides were tested against different Gram positive and Gram negative isolates.

The results showed that these isolates were resistant against most of these antibiotics with increase in MIC's. In Aminoglycoside group Tobramycin was the most effective agent against *Staph. aureus* and *E. coli* with MIC_{90s} of 1 ug/ml and 2 ug/ml, while against *Klebsiella* and *P. aeruginosa* its activity was moderate to low. Amikacin showed highest activity against *P. aeruginosa, E. coli* and *Klebsiella* species with MIC_{90s} of 4 ug/ml and 8 ug/ml. Kanamycin and Streptomycin were not active against the tested isolates.

INTRODUCTION

Waksman and Coworkers examined a number of soil actinomycets between 1939 and 1943. In 1943, a strain of *Streptomyces griseus* was isolated that elaborate a potent antimicrobial substance. The first public announcement of the discovery of this new antibiotic – Streptomycin – was made by Schataz, Bugie and Waksman early in 1944, and it was soon shown to inhibit the growth of the tubercle bacillus and a number of aerobic Gram positive and Gram negative microorganisms. Resistance in many organisms originally susceptible to the older compounds is now widespread, and resistance to the more recently introduced agents has increased. Marked changes have occurred over the year, for example, resistance to Streptomycin or Kanamycin was fairly common by the mid 1970s in the UK, but resistance to Gentamicin, tobramycin or amikacin was very rare. Since than the incidence of Gentamicin resistance among Gram negative bacilli increased until mid 1980s; thereafter it generally declined. Many strains with plasmid encoded extended spectrum beta lactamases are also aminoglycoside resistant, so large outbreaks of infections with such strains may result in an aminoglycoside resistance rates (Phillips and Shannon, 1997).

Development of surveillance programs at national level is one of the most effective ways to control antibiotic resistance. To accomplish this task seventy clinical isolates of each of *Staphylococcus aureus, Escherichia coli, Klebsiella* species and *Pseudomonas aeruginosa* were collected from different hospitals and pathological laboratories in Karachi. An *in vitro* study of these isolates was carried out by Agar dilution method using eight antimicrobial agents.

EXPERIMENTAL

Agar Dilution Susceptibility Test:

To determine the MIC for one or more bacterial isolates, the study drug may be incorporated into a liquefied agar medium (45-50°C), which is then mixed, poured into petri dishes and allowed to solidify (Barry, 1976 and Snyder *et al.*, 1976). A series of petri plates are prepared with increasing concentration of the drug and with the aid of a multiple inoculum replicator (Steers *et al.*, 1959) as many as 11 different strains can be spot inoculated on to each plate. After overnight incubation, the MIC end point is read as the lowest concentration that completely inhibits growth, disregarding a single colony or faint haze or growth (Barry, 1976; Ericson, 1971 and Washington, 1985).

Preparation of Antimicrobial Plates:

- Dilutions of antimicrobial agents are prepared in sterile double distilled water or other appropriate diluents at a concentration 10 times that desired in the final test (Barry, 1976 and Washington, 1985).
- The Agar medium is then prepared in flask or tubes and allowed to cool in a 50°C water bath.
- Sufficient volumes are prepared to fill each 9 cm petri plates with 20 to 25 ml of Agar.
- The diluted antimicrobial solutions are added to the melted and cooled medium in a ratio of 1 part antimicrobial agent to 9 part medium (2 ml of drug to 18 ml of Agar for each petri plate).
- The medium is then mixed by gently inverting the tube or flask several times. The contents are then poured into the appropriate number of petri plates.
- The plates are then set aside on a flat horizontal surface and allowed to harden undisturbed.
- For reference the Agar plates should be prepared on the same day that the tests are to be performed. However for most other purposes, the antimicrobial plates can be refrigerated in a sealed plastic bag for at least 1 week without a significant loss of antimicrobial activity (Ryan *et al.*, 1970).

Inoculation of Test Plates:

Apply an inoculum (1-2 ml) of each organism to the surface of each antimicrobial plates with the help of a replicating device containing 11 wire loops, one for the standard and 10 for the clinical isolates. The inoculum should be applied as a spot that covers a circle about 5-8 mm in a diameter and each spot should contain about 10^4 viable cells (Ericson, 1971; Barry, 1976 and NCCLS, 1990).

Incubation of Test Plates:

The inoculated plates are allowed to stand undisturbed until the spot of inoculum have absorbed completely. The plates are then inverted and allowed to incubate at 37°C for 16 to 24 hours.

Examine plate for the presence or absence of growth. The lowest concentration of each antimicrobials that inhibit growth (ignore single colony or faint inoculum haze) is considered the MIC (Wentworth, 1987).

RESULT AND DISCUSSION

Bacterial resistance to antimicrobial agents is a continuing serious problem in the treatment of infections. Although this problem was recognized shortly after commercial introduction of antimicrobial agents, it means that resistance is now emerging at a more rapid rate than ever before.

Table 1
Population distribution of MIC aminoglycoside antibiotics
for 70 *S. aureus* isolates

Amikacin

Conc	2	4	8	16	32	64	> 128	% Resistant
Sensitive strains	28	9	15	7	3	5	3	4.28

Gentamicin

Conc	0.25	0.5	1	2	4	8	> 8	% Resistant
Sensitive strains	26	8	11	12	4	4	5	12.86

Tobramycin

Conc	0.125	0.25	0.5	1	2	4	> 4	% Resistant
Sensitive strains	36	11	6	12	4	1	0	1.43

Kanamycin

Conc	1	2	4	8	16	32	> 32	% Resistant
Sensitive strains	24	11	10	10	5	5	5	14.28

Streptomycin

Conc	4	8	16	32	64	128	> 128	% Resistant
Sensitive strains	10	4	4	3	4	4	41	64.28

Population distribution of MIC of aminoglycoside antibiotics
for 70 *E. coli* isolates

Amikacin

Conc	0.5	1	2	4	8	16	> 16	% Resistant
Sensitive strains	36	6	14	5	4	1	1	2.86

Gentamicin

Conc	0.25	0.5	1	2	4	8	> 8	% Resistant
Sensitive strains	34	6	12	10	5	2	1	4.28

Tobramycin

Conc	0.25	0.5	1	2	4	8	> 8	% Resistant
Sensitive strains	35	6	11	11	4	1	2	4.28

Kanamycin

Conc	8	16	32	64	128	256	> 256	% Resistant
Sensitive strains	28	6	6	8	5	6	11	24.28

Streptomycin

Conc	8	16	32	64	128	256	> 256	% Resistant
Sensitive strains	15	4	4	4	6	4	36	57.14

Population distribution of MIC of aminoglycoside antibiotics
for 70 *Klebsiella* isolates

Amikacin

Conc	0.5	1	2	4	8	16	> 16	% Resistant
Sensitive strains	40	8	11	4	4	1	2	4.28

Gentamicin

Conc	0.25	0.5	1	2	4	8	> 8	% Resistant
Sensitive strains	30	6	14	10	5	2	3	7.14

Tobramycin

Conc	0.5	1	2	4	8	16	> 16	% Resistant
Sensitive strains	31	9	12	10	7	1	0	1.43

Kanamycin

Conc	16	32	64	128	256	512	> 512	% Resistant
Sensitive strains	30	5	7	8	10	6	4	14.28

Streptomycin

Conc	4	8	16	32	64	128	> 128	% Resistant
Sensitive strains	7	3	3	5	4	2	46	68.57

Population distribution of MIC of aminoglycoside antibiotics
for 70 *Ps. aeruginosa* isolates

Amikacin

Conc	1	2	4	8	16	32	> 32	% Resistant
Sensitive strains	37	11	12	7	3	0	0	0

Gentamicin

Conc	4	8	16	32	64	128	> 128	% Resistant
Sensitive strains	33	9	10	7	5	5	1	8.57

Tobramycin

Conc	2	4	8	16	32	64	> 64	% Resistant
Sensitive strains	34	12	7	8	5	2	2	5.71

Kanamycin

Conc	16	32	64	128	256	512	> 512	% Resistant
Sensitive strains	5	3	4	6	6	5	41	65.71

Streptomycin

Conc	16	32	64	128	256	512	> 512	% Resistant
Sensitive strains	3	4	8	7	5	5	41	72.86

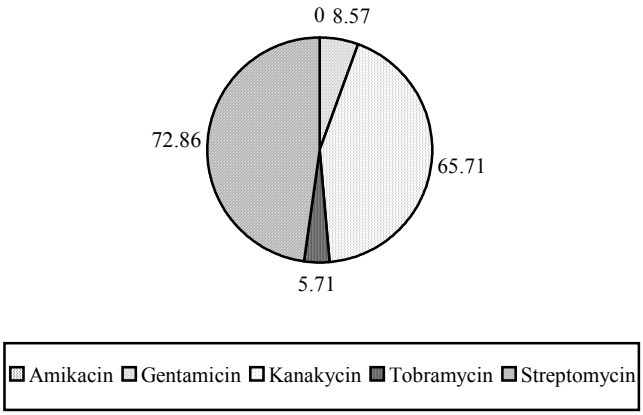
Table 2
Minimum inhibitory concentration (MIC_{50} and MIC_{90}) of Aminoglycoside and Macrolide antibiotics against *S. aureus, E. coli, Klebsiella* spp. and *Ps. aeruginosa*

Name of antibiotics	*S. aureus*		*E. coli*		*Klebsiella* spp.		*Ps. aeruginosa*	
	MIC_{50} (ug/ml)	MIC_{90} (ug/ml)	MIC_{50} (ug/ml)	MIC_{90} (ug/ml)	MIC_{50} (ug/ml)	MIC_{90} (ug/ml)	MIC_{50} (ug/ml)	MIC_{90} (ug/ml)
Amikacin	8	128	<0.5	<8.0	0.5	4	1	8
Gentamicin	1	8	<0.5	<4.0	<0.5	<4.0	<8.0	<64.0
Tobramycin	<0.125	<1.0	0.25	2	<1.0	<8.0	<4.0	<32.0
Kanamycin	2	<32.0	32	<256.0	32	<512.0	>512.0	>512.0
Streptomycin	>128.0	>128.0	>256.0	>256.0	>128.0	>128.0	>512.0	>512.0

Present research work consists of 5 agents of Aminoglycosides group which are tested against 70 isolates of each pathogen i.e. *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella* species and *Pseudomonas aeruginosa.*

Aminoglycosides are highly, potent broad spectrum antibiotics with many desirable properties for the treatment of life-threatening infections (Gilbert, D.N., 1995).

Graphical representation of different aminoglycosides against gram positive and gram negative isolates



Percentage *Pseudomonas aeruginosa* resistance against different aminoglycosides

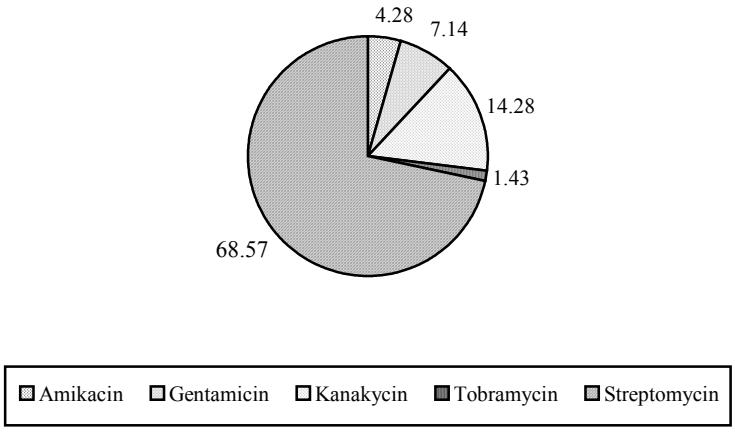
Genatmicin:

It is active *in vitro* against more than 90% strains of *Staphylococcus aureus* (Chambers and Sande, 1995). The present study shows that about 13% strains are resistant to Gentamicin (Table 1). MIC_{50S} and MIC_{90S} are 4.0 ug/ml and <8.0 ug/ml respectively (Table 2). Harnett N. *et al.* in 1991 reported that Gentamicin was active against 58% strains of methicillin resistant *Staphylococcus aureus* with MIC_{90S} of 64.0 ug/ml. Freitas F. I. *et al.* in 1999 showed that 100% strains of methicillin resistant strains were resistant to Gentamicin. Gentamicin is active against nearly all strains of *Escherichia coli* (Alvis Kucers, 1997) with MIC_{90S} 0.5 ug/ml. An increase in MIC is observed in this study from 0.5 ug/ml to 4.0 ug/ml only 4% strains are resistant (Table 2). The MIC_{50S} and MIC_{90S} are <0.5 ug/ml and <4.0 ug/ml . Esuvaranathan K., *et al.* in 1992 observed 89% sensitivity of *E.coli* against Gentamicin. Adji and Bernya in 1997 note 100% sensitivity of *E.coli* against Gentamicin. Gentamicin is also active against *Klebsiella*. 30% resistance among *E. coli* against Gentamicin was reported by Ruczkowaska J. and Dolna I. in 1989. The present work shows 7% resistance among *Klebsiella* spp. with MIC of 4.0 ug/ml (Table 1 and 2). The MIC_{50S} and MIC_{90S}, are <0.5 ug/ml and <4.0 ug/ml (Table 2). *Pseudomonas aeruginosa* is quite sensitive and activity against this organism is one of the important features of Gentamicin (Alvis Kucers, 1997). This research work exhibits that MIC_{90S} of Gentamicin against *Ps. aeruginosa* is increased (Table 1 and 2). The MIC_{50} and MIC_{90} are < 8.0 ug/ml and <64.0 ug/ml (Table 2). Ford, A.S. *et al.* in 1993 observed that 31% strains of *Ps. aeruginosa* were resistant to Gentamicin. Chen H.Y. *et al.* in that only 11.7% strains were resistant with MIC >2ug/ml.

Amikacin:

Amikacin has an *in vitro* antibacterial spectrum which is broader than that of Kanamycin, Gentamicin and Tobramycin (Rise *et al*., 1973 and Meyer, 1981).

Staphylococcus aureus including Penicillin G – resistant strains are usually Amikacin sensitive. Methicillin resistant strains of these organisms may be Amikacin sensitive, but many isolates from hospitals are resistant (Guimaraes *et al.*, 1985). Sowa *et al.* in 1991 observed high (83%) resistance in Methicillin resistant *Staphylococcus aureus* against Amikacin. Kondo, S. *et al.* in 1991 reported 36% resistance in Methicillin resistant *Staphylococcus aureus*. The present study

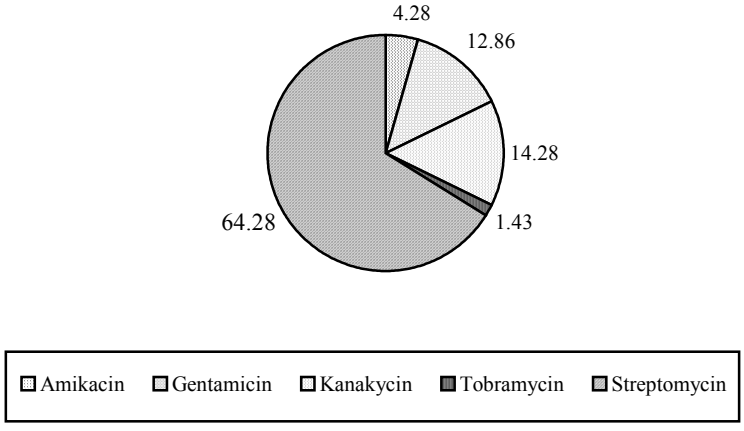


Percentage *Klebsiella pneumoniae* resistance against different aminoglycosides

shows 26% resistance at MIC of 16ug/ml (Table 1 and 2). The MIC_{50S} and MIC_{90S} are <8.0 ug/ml and <128.0 ug/ml respectively (Table 2). Amikacin is highly active against *E.coli* with MIC of 1.0 ug/ml . The present work shows that 97% strains of *E.coli* are inhibited by Amikacin at MIC of 8.0 ug/ml (Table 1 and 2). The MIC_{50S} and MIC_{90S} are <0.5 ug/ml and <8.0 ug/ml (Table 2). Mittermayer *et al.* in 1990 reported 7.5% resistance in *E. coli*. Valdivies, O. *et al.* in 2000 noted that 1.3% *E. coli* where resistant to Amikacin. Amikacin is highly active against *Klebsiella* spp. with MIC 1.0 ug/ml. Fazli *et al.* in 1989 reported that 9% *Klebsiella* were sensitive to Amikacin. Lee *et al.* in 1991 showed that all tested strains of *Klebsiella pneumoniae* were susceptible to Amikacin. During the present study only 4% *Klebsiella* resistance is observed against Amikacin (Table 1 and 2). The MIC_{50S} and MIC_{90S} are >0.5 ug/ml and 4.0 ug/ml (Table 2). Amikacin is active against many strains of *Ps. aeruginosa* which have acquired resistance to Gentamicin and other aminoglycosides. During this study Amikacin is found to be highly active against *Ps. aeruginosa* with 100% sensitivity at MIC 16.0 ug/ml (Table 1 and 2). This MIC_{50S} and MIC_{90S} are >1.0 ug/ml and >8.0 ug/ml (Table 2). Ford, A.S. *et al.* in 1993 noted that 31% strains were resistant to Amikacin. Sader H.S. *et al.* in 1999 observed 86.3% susceptibility in *Ps. aeruginosa* to Amikacin at MIC >32 ug/ml. Lewis *et al.* noted that 77% strains were sensitive to Amikacin.

Tobramycin:

The antimicrobial activity of Tobramycin is very similar to that of Gentamicin (Chambers and Sande, 1995). *Staphylococcus aureus*, including Penicilline and Methicillin resistant strains may be Tobramycin sensitive (Jordan and Hoeprich, 1977). The current research work shows that nearly 2% strains of *Staphylococcus aureus* are resistant to Tobramycin at MIC 2.0 ug/ml (Table 1 and 2). The MIC_{50S} and MIC_{90S} are >0.125 ug/ml and >1.0 ug/ml (Table 2). Ruczkowska, J. and Dolnia, I. in 1989 report 30% resistance to Tobramycin. Mittermayer, H. *et al.* in 1990 observed 26% resistance in *Staphylococcus aureus* against Tobramycin. Tobramycin exhibits excellent activity against *E. coli* (Table 1). Mittermayer H., *et al.* in 1990 observed 11% resistance among *E. coli* to Tobramycin. The present work shows that Tobramycin is highly effective against *E. coli* only 3% strains are resistant at MIC of 8.0 ug/ml (Table 1 and 2). The MIC_{50S} and MIC_{90S} are 0.25 ug/ml and 2.0 ug/ml (Table 2). Tobramycin is also active against *Klebsiella* spp. with MIC of 1.0 ug/ml (Table 1). During this antimicrobial work Tobramycin is found to be quite effective against *E. coli* with only 1.0% resistant strains at MIC 8.0 ug/ml (Table 1 and 2). The MIC_{50S} and MIC_{90S}

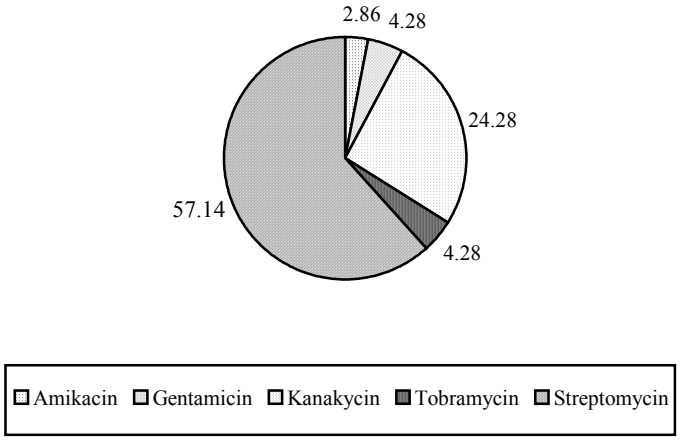


Percentage *Staphylococcus aureus* resistance against different aminoglycosides

are >1.0 ug/ml and >8.0 ug/ml respectively (Table 2). Ruczkowska, J. and Dolna, I. in 1989 noted 30% resistance in *Klebsiella* spp. to Tobramycin. Lee *et al.* in 1991 reported 16.5% resistance in *Klebsiella pneumoniae* against Tobramycin. Tobramycin has supperior activity against *Ps. aeruginosa* than Gentamicin with MIC of 4.0 ug/ml (Table 1). Chamberland, S. *et al.* in 1992 reported that Tobramycin was the most active agent against *Ps. aeruginosa*. Kumamoto *et al.* in 1995 observed a high efficacy of Tobramycin against *Ps. aeruginosa* with MIC_{90S} of 2 ug/ml. Data of the present work shows an increase in the MIC which is 32 ug/ml with about 6% resistance (Table 1 and 2). The MIC_{50S} and MIC_{90S} are <4.0 ug/ml and <32.0 ug/ml (Table 2).

Kanamycin:

The spectrum of activity of Kanamycin is limited as compared with other aminoglycosides (Chambers and Sande, 1995). *Staphylococcus aureus* were Kanamycin sensitive with MIC 2.0 ug/ml. The result of present work shows that there is 14% resistance among *Staph. aureus* to Kanamycin at MIC of 16.0 ug/ml, these results also indicate in increase in the MIC of the drug (Table 1 and 2). The MIC_{50S} and MIC_{90S} are 2.0 ug/ml and <32.0 ug/ml (Table 2). Sowa, S. *et al.* in 1991 reported a 98% Methicillin resistant *Staph. aureus* resistance against Kanamycin. Inouye Y. *et al.* in 1992 observed 92% resistance in Methicillin resistant strains of *Staph. aureus*. Kanamycin was active against *E. coli* (Alvis Kucers 1997). Moellering in 1983 observed that only 15% strains of *E.coli* were Kanamycin resistant. Data of the present antibacterial work exhibits that more than 24% strains of *E.coli* are resistant to Kanamycin at MIC of 128.0 ug/ml (Table 1 and 2). The MIC_{50S} and MIC_{90S} are >32.0 ug/ml and <256.0 ug/ml (Table 2). Kanamycin is not significantly active against *Klebsiella spp*. No significant activity is observed during the present work with very high MIC of 256.0 ug/ml which inhibit 86% *Klebsiella spp*. (Table 1 and 2). The MIC_{50S} and MIC_{90S} are 32.0 ug/ml and >512.0 ug/ml. Lee *et al.* 1991 noted that 26% strains of *Klebsiella pneumoniae* were resistant to Kanamycin. *Ps. aeruginosa* is highly resistant to Kanamycin(Chambers and Sande 1995). Schassan H.H., in 1976 reported that 46.4% strains of *Ps. aeruginosa* were resistant to Kanamycin. Results of the present study shows that there is high level of resistance among *Ps. aeruginosa* to Kanamycin about 66% strains are resistant at MIC of 256.0 ug/ml (Table 1 and 2). The MIC_{50S} and MIC_{90S} are >512.0 ug/ml (Table 2).



Percentage *E. coli* resistance against different aminoglycosides

Streptomycin:

Some strains of *Staphylococcus* are sensitive. Resistance is plasmid mediated but transposon can also be involved (Udo and Grubb, 1991). Kondo S. *et al.* in 1991 reported good activities of Streptomycin (MIC 1.56 – 6.25 ug/ml) against all tested strains of MRSA. The present study shows that 64% strains of *Staph. aureus* are resistant to Streptomycin at MIC of 64.0 ug/ml (Table 1 and 2). The MIC_{50S} and MIC_{90S} are >128.0 ug/ml (Table 2). Streptomycin is not satisfactorily active against *E. coli*. Sabath in 1969 reported that 50% of *E. coli* strains were resistant to Streptomycin. Moellering in 1983 noted a 36% resistance. Results of the current study shows that 57% strains of *E. coli* are resistant to Streptomycin (Table 1 and 2). The MIC_{50S} and MIC_{90S} are >256.0 ug/ml (Table 2) Streptomycin resistant Gram negative bacilli are common for many years (Alvis Kucers 1997). Sabath in 1969 reported that 73% strains of *Klebsiella* were resistant Moellering in 1983 observed a decrease in resistance of *Klebsiella* against Streptomycin, which was 19%. The present research work shows that nearly 69% strains are resistant to Streptomycin at MIC of 64.0 ug/ml (Table 1 and 2). The MIC_{50S} and MIC_{90S} are >128.0 ug/ml (Table 2) Streptomycin is not active against *Ps. aeruginosa* (Table 1). Presently the drug is not in common use (Chambers and Sande 1995). Results of the present antimicrobial work indicate a very high resistance among *Ps. aeruginosa* against Streptomycin, 41% strains are sensitive at MIC of 512.0 ug/ml (Table 1 and 2). The MIC_{50S} and MIC_{90S} are >512.0 ug/ml (Table 2).

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