

RESISTANCE PATTERN OF GRAM NEGATIVE UROPATHOGENS AGAINST AMINOGLYCOSIDES

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ABSTRACT

The antibiotic resistance pattern of 345 Gram negative bacilli, isolated from urinary tract infections in females, was determined against aminoglycosides viz., neomycin, streptomycin, gentamicin and kanamycin. The highest rate of resistance was recorded against neomycin (44.6%). The rates of resistance to other antibiotics were observed as kanamycin 9.7%, streptomycin 17.4% and gentamicin 13%. The susceptibility pattern of uropathogens varied among the genera.

Key words: Neomycin, kanamycin, streptomycin, gentamicin, Gram negative bacilli, Antibiotic resistance.

INTRODUCTION

Urinary tract infections (UTIs) are common both in community and hospital settings especially in females and cause significant morbidity (Karlowsky *et al.*, 2006). The common Gram negative organisms causing UTIs are *Escherichia coli* (Guidoni *et al.*, 2008), *Klebsiella pneumoniae* (Lavanya and Jogonalakshmi, 2002), *Pseudomonas aeruginosa* (Jombo *et al.*, 2008), *Proteus mirabilis* and *Serratia marcescens* (Mohanty *et al.*, 2003). Antimicrobial therapy is usually indicated for symptomatic infection (Nicoll, 2002). There are numerous reports on the incidence of growing resistance of urinary isolates to available antibiotics (Alos, 2005; McNultry *et al.*, 2006; Tessema *et al.*, 2007). Multiple drug resistance among microorganisms has been a well-recognized problem with UTIs. Resistance has been observed in multiple genera including *Escherichia*, *Enterobacter*, *Klebsiella*, *Proteus*, *Salmonella*, *Serratia*, *Pseudomonas* and *Staphylococcus* (Noor *et al.*, 2004). Instead of using narrow spectrum antibiotics, the use of broad spectrum antibiotics is a reason for resistance of bacteria to antibiotics (Akram *et al.*, 2007). The rate of bacterial resistance to antibiotic in the management of UTIs are also due to the use of fake and substandard antibiotics (Raufu, 2002).

Uropathogens have shown a slow but steady increase in resistance to several antibiotics over the last decade. Now-a-day, development of resistance to antibiotics has become one of the major concerns of public health. It is obvious from literature that resistance to aminoglycosides for uropathogens has been on increase (Bhowmick and Rashid, 2004; Stratchounski and Rafalski, 2006; Anatoliotaki *et al.*, 2007; Kim *et al.*, 2008). Keeping this in view, the present study aimed to examine the susceptibility patterns of various Gram negative uropathogens against aminoglycosides.

MATERIALS AND METHODS

Bacterial isolates:

A total of 345 Gram negative uropathogens isolated from community acquired UTIs in females were used to determine the antimicrobial resistant pattern against aminoglycosides (gentamicin, neomycin, kanamycin and streptomycin). These strains comprised *Escherichia coli* (270), *Klebsiella pneumoniae* (51), *K. ozaenae* (03), *Proteus mirabilis* (05), *Pseudomonas aeruginosa* (10), *Salmonella typhi* (01), *S. paratyphi A* (02), *S. paratyphi B* (01) and *Serratia marcescens* (02).

Determination of antimicrobial susceptibility:

Antimicrobial susceptibility testing was performed using disc diffusion method as described by National Committee for Clinical Laboratory Standards (Presently called as Clinical Laboratory Standards Institute) (Cheesbrough, 2000).

Media:

Mueller Hinton Agar (MHA) (Merck) was employed for determination of antimicrobial susceptibility test and Mueller Hinton Broth (MHB) (Merck) was used for preparation of inoculum.

Preparation of turbidity standard:

McFarland Nephelometer Standard tuber number 0.5 was used to standardize the turbidity of test inoculum (Baron *et al.*, 1994).

Preparation of inoculum:

A loopful of pure culture of organisms was transferred to 5ml MHB. The broth was incubated at 35-37°C for 18-24 hours. After incubation, the turbidity of the culture was compared with 0.5 McFarland Nephelometer Standard to get approximate cell density 150×10^6 CFU/ml. The standardized inoculum was inoculated within 15-20 minutes.

Inoculation of medium:

A sterile cotton swab was immersed into the standardized inoculum suspension. Excess broth was drained by pressing and rotating the swab against the inside of a suspension tube. Then it was streaked evenly on the surface of MHA plate.

Disc placement:

Antibiotic disc (6mm diameter) (Table 1) were placed on the surface of inoculated MHA plates by using a sterile forcep.

Incubation:

Plates were incubated at 35-37°C for 18-24 hours.

Interpretation:

Inhibition zone diameters (including diameter of disc) were measured with a ruler. The susceptibility or resistance was interpreted on the basis of criteria mentioned in Table 1.

RESULTS AND DISCUSSION

Aminoglycosides are used extensively in clinical practice and have broad activity against aerobic Gram negative bacteria including *Enterobacteriaceae* and non-fermentative Gram negative bacilli. Therefore, they have had a major impact to treat bacterial infections caused by Gram negative bacteria for the past half century (Vakulenko and Mobashery, 2003). However, aminoglycosides are not effective against anaerobes, *Enterococci* and *Streptococci* because they are actively transported into bacterial cells by a process that requires respiratory metabolism (Nester *et al.*, 2004). Aminoglycosides work by inhibiting protein synthesis and also disrupt normal permeability of cell wall of Gram negative bacteria (Victor, 1996). To extend the spectrum of activity, aminoglycosides are sometimes used with combination of a beta-lactam drug. The beta-lactam drugs interfere with cell wall synthesis and allow the aminoglycosides to enter the cell more easily (Nester *et al.*, 2004).

The antibiotic resistance patterns observed in the present study indicated that the resistance rates of *E. coli* against aminoglycosides were high (gentamicin 13%, kanamycin 17%, neomycin 44.4% and streptomycin 15.9%). Gentamicin was observed as more effective antibiotic against *E. coli* as compared to other aminoglycosides (Table 2). These results are in harmony with the findings of Kumar and Dass (2004) who had also reported 13% resistant isolates of *E. coli* to gentamicin. However, there is great variation in the resistance rates of *E. coli* to gentamicin in different studies such as 19.5% (Kim *et al.*, 2008), 18.8% (Mashouf *et al.*, 2009), 8.1% (Bhowmick and Rashid, 2004), 4.9% (Anatoliotaki *et al.*, 2007) and <3% (Stratchounski and Rafalski, 2006) isolates of *E. coli* were found resistant to gentamicin.

The antibiogram patterns of *K. pneumoniae* and *K. ozaenae* exhibited considerable resistance rates against antibiotics used in the study (Table 2). It was observed that aminoglycosides displayed higher resistance rates for *K. pneumoniae* and *K. ozaenae* which were noted as streptomycin 25.5% and 33.3%, gentamicin 9.8% and 33.3%, kanamycin 19.6% and 33.3% and neomycin 45.1% and 66.7%, respectively. These results are not comparable with the findings of a previous study, Nwanze *et al.* (2007) reported resistance rate of *K. pneumoniae* to gentamicin as 41%. While Magalit *et al.* (2004), observed gentamicin resistance rates for *K. pneumoniae* isolated from community acquired and hospital acquired UTIs as 13% and 14%, respectively. In another study, 57.1% *Klebsiella* species were gentamicin-resistant (Hasan *et al.*, 2007). Furthermore, in a recent study, the gentamicin-resistance rate of *Klebsiella pneumoniae* was recorded as 43.3% (Mashouf *et al.*, 2009).

In the present study, 70% isolates of *P. aeruginosa* were found resistant to kanamycin, 50% to neomycin, 30% to gentamicin and 20% to streptomycin. In a previous study (Bouza *et al.*, 1999) resistance rates to aminoglycosides for *P. aeruginosa* were noted as high as 72% to gentamicin, 69.2% to tobramycin and 40% to amikacin. In several other studies *P. aeruginosa* has been shown resistant to aminoglycosides. In a study carried out in Tunisia

(Bousseimi *et al.*, 2005), 80% isolates of *P. aeruginosa* were resistant to gentamicin. In a similar study carried out in Turkey (Goniugur *et al.*, 2003), resistance of *P. aeruginosa* to gentamicin was found to be 70%. While a study carried out in Nigeria (Chah *et al.*, 2003) also showed same rate of gentamicin-resistance i.e. 70%. In another study in Gombe (Ahmed and Kudi, 2003), 85.5% isolates of *P. aeruginosa* were found sensitive to gentamicin. In Ibadin (Oni *et al.*, 2002), >90% isolates of *P. aeruginosa* were found to be susceptible to gentamicin. In Ethiopia (Ferede *et al.*, 2001), *Pseudomonas* species were found to be sensitive to kanamycin (72%) and gentamicin (88%). While in a recent study, 60% isolates of *P. aeruginosa* were found to be gentamicin and tobramycin-resistant (Mashouf *et al.*, 2009).

There are considerable geographic variations in bacterial patterns and resistance properties depending on local antimicrobial prescription practices (Yuksel *et al.*, 2006). The susceptibility pattern of uropathogens to antibiotics varies with time and place (Magalit *et al.*, 2004).

The emergence of antibiotic resistance in the management of UTIs is an important public health issue. Therefore, it requires regular monitoring of resistance patterns in uropathogens.

Table 1. Criteria for the potency of the antibiotics and the degree of resistance in Uropathogens.

Antibiotics	Disc code	Potency (µg)	Inhibition zone diameter (mm)		
			Resistant	Intermediate	Sensitive
Gentamicin	GM	10	≤ 12	13-14	≥ 15
Kanamycin	K	30	≤ 13	14-17	≥ 18
Neomycin	N	30	≤ 12	13-16	≥ 17
Streptomycin	S	10	≤ 11	12-14	≥ 15

Table 2. Antibiotic resistance patterns of Gram negative bacteria.

S. No.	Organisms	No. of Isolates	Number of isolates resistant to antibiotics			
			GM	K	N	S
1	<i>E. coli</i>	270	35 (13.0)	46 (17.0)	120 (44.4)	43 (15.9)
2	<i>K. pneumoniae</i>	51	5 (9.8)	10 (19.6)	23 (45.1)	13 (25.5)
3	<i>K. ozaenae</i>	03	1 (33.3)	1 (33.3)	2 (66.7)	1 (33.3)
4	<i>P. mirabilis</i>	05	1 (20.0)	3 (60.0)	3 (60.0)	1 (20.0)
5	<i>P. aeruginosa</i>	10	3 (30.0)	7 (70.0)	5 (50.0)	2 (20.0)
6	<i>S. typhi</i>	01	0 (0)	0 (0)	0 (0)	0 (0)
7	<i>S. paratyphi A</i>	02	0 (0)	0 (0)	1 (50.0)	0 (0)
8	<i>S. paratyphi B</i>	01	0 (0)	1 (100)	0 (0)	0 (0)
9	<i>S. marcescens</i>	02	0 (0)	0 (0)	0 (0)	0 (0)
Total		345	45 (13.0)	68 (19.7)	154 (44.6)	60 (17.4)

Figures in parentheses are the percentages of resistant isolates. GM = gentamicin, K = kanamycin, N = neomycin, S = streptomycin.

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