INT. J. BIOL. BIOTECH., 6 (4): 277-281, 2009.

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, Antibiotoc 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 Jogalakshmi, 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 Nephlometer 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^{o}C for 18-24 hours. After incubation, the turbidity of the culture was compared with 0.5 McFarland Nephlometer Standard to get approximate cell density 150 x 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^{o}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 Ethopia (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	*E. coli*	270	35 (13.0)	46 (17.0)	120 (44.4)	43 (15.9)
2	*K. pneumoniae*	51	5 (9.8)	10 (19.6)	23 (45.1)	13 (25.5)
3	*K. ozaenae*	03	1 (33.3)	1 (33.3)	2 (66.7)	1 (33.3)
4	*P. mirabilis*	05	1 (20.0)	3 (60.0)	3 (60.0)	1 (20.0)
5	*P. aeruginosa*	10	3 (30.0)	7 (70.0)	5 (50.0)	2 (20.0)
6	*S. typhi*	01	0 (0)	0 (0)	0 (0)	0 (0)
7	*S. paratyphi A*	02	0 (0)	0 (0)	1 (50.0)	0 (0)
8	*S. paratyphi B*	01	0 (0)	1 (100)	0 (0)	0 (0)
9	*S. marcescens*	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.

REFERENCES

Ahmad, B.M. and M.T. Kudi (2003). Chronic suppurative otitis media in Gombe, Nigeria. *Nig J Surgical Res.*, 5: 120-123.

Akram, M., M. Shahid and A.U. Khan (2007). Etiology and antibiotic resistance patterns of community-acquired urinary tract infections in JNMC Hospital Aligarh, India. *Annals of Clinical Microbiology and Antimicrobials,* 6: 4-6.

Alos, J.I. (2005). Epidemiology and etiology of urinary tract infections in the community. Antimicrobial susceptibility of the main pathogens and clinical significance of resistance. *Enferm Infect Microbiol Clin.*, 23(4): 3-8.

Anatoliotaki, M., E. Galanakis, A. Schinaki, S. Stefanaki, M. Mavrokosta and A. Tsilimigaki (2007). Antimicrobial resistance of urinary tract pathogens in children in Crete, Greece. *Scand J Infect Dis.*, 39(8): 671-675.

Baron, E.J., L.R. Peterson and S.M. Finegold (1994). Baily & Scott's Diagnostic Microbiology, 9th edition. The C.V. Mosby Company. Pp. 333-351.

Bhowmick, B.K. and H. Rashid (2004). Prevalence and antibiotic susceptibility of *E. coli* isolated from urinary tract infection in Bangladesh. *Pakistan Journal of Biological Sciences,* 7(5): 717-720.

Bousseimi, K., L. Thabet, S. Ouesiat, O.B. Jaber, A. Ouchtati, S. Cherif, S.B. Redjeb and A. Messadi (2005). Epidemiological profile and antibiotic susceptibility of *Pseudomonas aeruginosa* isolates within the hospitalized burned patient. *Critical Care,* 9(1): 10-11.

Bouza, E., F. Gercia-Garrote, E. Cercenado, M. Marin and M.S. Diaz (1999). *Pseudomonas aeruginosa*: a survey of resistance in 136 hospitals in Spain. *Antimicrob Agents and Chemother.,* 43:981-982.

Chah, K.F., C.A. Ezeh and B.N. Oluoha (2003). Frequency and antimicrobial resistance of aerobic bacteria isolated from surgical sites in human and animals in Nsukka, Southeast Nigeria. *Nig Veterinary J.,* 24: 1-9.

Cheesbrough, M. (2000). District Laboratory Practice in Tropical Countries. Part 1. pp. 370-387.

Ferede, D., A. Geyid, S. Lulseged and A. Melaku (2001). Drug susceptibility pattern of bacteria isolates from children with chronic suppurative otitis media. *Ethiopian J Health Development,* 15: 89-96.

Goniugur, U., M.Z. Bakici, L. Ozdemir, I. Akkurt, S. Icagasiogh and F. Gultekin (2003). Retrospective analysis of antibiotic susceptibility patterns of respiratory isolates of *Pseudomonas aeruginosa* in a Turkish University Hospital. *Ann Clin Microbiol Antimicrobials.,* 2: 2-5.

Guidoni, E.B.M., E.N. Berezin, S. Nigro, N.A. Santiago, V. Benini and J. Toporovski (2008). Antibiotic resistance patterns of pediatric community acquired urinary infections. *The Brazilian Journal of Infectious Diseases,* 12(4): 321-323.

Hasan, A.S., D. Nair, J. Kaur, G. Baweja, M. Deb and P. Aggarwal (2007). Resistance patterns of urinary isolates in a tertiary Indian Hospital. *J Ayub Med Coll. Abbotabad,* 19(1): 39-41.

Jombo, G.T.A., P.Jonah and J.A. Ayeni (2008). Multi-drug resistant *Pseudomonas aeruginosa* in contemporary medical practice: findings from urinary isolates at a Nigerian university teaching hospital. *Nigerian Journal of Physiological Sciences,* 23(1-2): 105-109.

Karlowsky, J.A., D.J. Hoban, M.R. Decorby, N.M. Laing, and G.G. Zhanel (2006). Fluoroquinolone-resistant urinary isolates of *Escherichia coli* from outpatients are frequently multi-drug: Results from the North American urinary tract infection collaborative alliance-quinolone resistance study. Antimicrob. Agents Chemother. 50(6): 2251-2254.

Kim, M.E., U.S. Ha and Y.H. Cho (2008). Prevalence of antimicrobial resistance among uropathogens causing acute uncomplicated cystitis in female outpatients in South Korea: a multicenter study in 2006. *Int J Antimicrob.,* 31(1): 15-18.

Kumar, K.A. and S.M. Dass (2004). Antimicrobial resistance patterns of Gram negative bacteria isolated from urine cultures at a general hospital. *Saudi J Kidney Dis. Transpl.,* 15(2): 135-139.

Lavanya, S.V., and D. Jogalakshmi (2002). Asymptomatic bacteriuria in antenatal women. *The New England Journal of Medicine,* 20(2): 105-106.

Magalit, S.L., M.T.S. Gler and T.E. Tupasi (2004). Increasing antimicrobial resistance of community acquired and nosocomial uropathogens in Makati Medical Center. *Phil J Microbiol Infect Dis.,* 33(4): 143-148.

Mashouf, R.Y., H. Babalhavaeji and J. Yousef (2009). Urinary tract infections: Bacteriology and Antibiotic resistance patterns. *Indian Pediatrics,* 15: 1-4.

McNultry, C.A.M., J. Richards, D.M. Liverore, P. Little, A. Charlett, E., Freean, I. Harvey and M. Thomas (2006). Clinical relevance of laboratory-reported antibiotic resistance in acute uncomplicated urinary tract infection in primary care. *J Antimicrob. Chemother.,* 58: 1000-1008.

Mohanty, S., A. Kapil., B.K. Das, and B. Dhawan (2003). Antimicrobial resistance profile of nosocomial pathogens in a tertiary care hospital. *Indian J Med Res.,* 57(4): 148-154.

Nester, E.W., D.G. Anderson, C.E. Roberts, N.N. Pearsall and M.T. Nester (2004). Microbiology: A human perspective, International edition, McGraw Hill. Pp. 633-664, 495-516.

Nicoll, L.E. (2002). Urinary tract infection: Traditional pharmacological therapies. *Dis Mon.,* 49(2): 111-128.

Noor, N., M. Ajaz, S.A. Rasool and Z.A. Pirzada (2004). Urinary tract infections associated with multi-drug-resistant enteric bacilli: characterization and genetic studies. *Pakistan Journal of Pharmaceutical Sciences,* 17(2): 115-123.

Nwanze, P.I., L.M.Nawaza, S. Oranusi, M.U. Dimkpa, M.U. Okwu, VB.B. Babatunde, T.A. Anake, W. Jatto and C.E. Asagwara (2007). Urinary tract infection in Okada village: prevalence and antimicrobial susceptibility pattern. *Scientific Research & Essay,* 2(4): 112-126.

Oni, AA., O.G.B. Nwaorgu, R.A. Bakare, M.O. Ogunkunle and R.A. Toki (2002). The discharging ears in adults in Ibadan, Nigeria: causative agents and antimicrobial susceptibility pattern. *Afr J Clin Exp Microbiol.,* 3: 3-5.

Raufu, A. (2002). Influx of fake drugs to Nigerian worries health experts. *BJM,* 324: 698.

Stratchounski. L.S. and V.V. Rafalski (2006). Antimicrobial susceptibility of pathogens isolated from adult patients with uncomplicated community acquired urinary tract infections in the Russian Federation: two multicenter studies, UTIAP-1 and UTIAP-2. *International Journal of Antimicrobial Agents,* 28(1): 4-9.

Tessema, B., A. Kassu, A. Mulu and G. YTismaw (2007). Predominant isolates of urinary tract pathogens and their antimicrobial susceptibility patterns in Gondar University Teaching Hospital, Northwest Ethiopia. *Ethiop Med.,* 45(1): 61-67.

Vakulenko, S.B. and S. Mobashery (2003). Versatility of aminoglycosides and prospects for their future. *Clinical Microbiology Reviews,* 16(3): 430-450.

Victor, L. (1996). Antibiotics in Laboratory Medicines. Williams & Wilkens Press. pp. 589-590.

Yuksel, S., B. Ozturk, A. Kavaz, Z.B. Ozcakar, B. Acar, H. Guriz, D. Aysev, M. Ekim and F. Yalcinkaya (2006). Antibiotic resistance of urinary tract pathogens and evaluation of empirical treatment in Turkish children in urinary tract infections. *Int J Antimicrob Agents,* 28(5): 413-416.

(*Accepted for publication August 2009*)