

## A STUDY ON PREVALANCE OF MULTI-DRUG-RESISTANT GRAM NEGATIVE BACTERIA

Sabahat Saeed, Asma Naim and Perween Tariq

Department of Microbiology, University of Karachi, Karachi-75270, Pakistan.

---

### ABSTRACT

Antibacterial susceptibility tests against hundred (100) strains, belonging to 11 species of Gram negative bacteria, were carried out by standard disc diffusion method. These comprises *Escherichia coli* (30 strains), *Klebsiella pneumoniae* (25), *Pseudomonas aeruginosa* (15), *Salmonella typhi* (5), *Salmonella paratyphi A* (1), *Salmonella paratyphi B* (1), *Proteus mirabilis* (10), *Proteus vulgaris* (2), *Shigella dysenteriae* (5), *Yersinia enterocolitica* (1), *Enterobacter aerogenes* (5). In the present study, out of 100 isolates, 2% isolates were found resistant to streptomycin, 9% to gentamicin, 38% to ampicillin, 7% to neomycin, 20% to kanamycin, 22% to chloromphenicol, and 40% isolates were found resistant to tetracycline. It was observed that multi-drug-resistant strains were more common as compare to single-drug-resistant strains of Gram negative bacteria i.e. 48% strains were found multi-drug-resistant while single-drug-resistance was found in only 22% strains.

**Key words:** Gram negative bacteria, multi-drug-resistance, tetracycline, antibiotics, disc diffusion technique.

---

### INTRODUCTION

Gram negative bacteria are ubiquitous. They are found in 10-15% of the indigenous bacterial flora. Temperature, moisture and reduction of the normal Gram positive flora favour a rapid establishment of Gram negative bacteria and development of clinical infections (Saeed and Tariq, 2005). Their association with urinary tract infections (Sader *et al.*, 2005; Baysoy *et al.*, 2006), skin infections (Gulay *et al.*, 2006), respiratory infections (Gladstone *et al.*, 2005) and brain abscess (Rau *et al.*, 2002) is well documented. They have also been reported to be associated with blood stream infections (Kang *et al.*, 2005; Loivukene *et al.*, 2006).

A wide variety of antibiotics are commonly used for the treatment of serious infections caused by Gram negative bacteria (Tumah, 2005). In recent years, multiple drug resistance has developed due to indiscriminate use of existing antimicrobial drugs in the treatment of infectious diseases (Saeed *et al.*, 2006). Antibiotic resistance is a threat to human kind because most of the infection causing bacteria has become multi-drug-resistant (Saeed *et al.*, 2005).

Antibiotic resistant bacteria may keep people sick longer, and sometimes people are unable to recover at all. Children, the elderly, and those with weak immune system, including cancer, HIV/AIDS and transplant patients, are particularly vulnerable because their immune system is not as vigorous as those of healthy adults (Plumbi, 2001). The present study was conducted to evaluate the prevalence of antibiotic resistance among Gram negative bacteria isolated from different clinical specimens (pus, blood, faeces and urine).

### MATERIALS AND METHODS

#### Media

Mueller-Hinton agar (MHA) (Merck) was used as antibiotic susceptibility test medium and Mueller-Hinton broth (MHB) (Merck) was used for the preparation of inoculum.

#### Preparation of plates

MHA (20ml) was poured into sterile Petri plates to get a depth of 4-6 mm. All the plates were incubated for 24 hours to check sterility.

#### Preparation of 0.5 McFarland Nephelometer Standard

McFarland tube number 0.5 was prepared by mixing 0.5 ml 1.175% barium chloride solution and 99.5 ml 1% sulphuric acid solution.

#### Standardization of Inoculum

Four to five colonies from pure growth of organisms were transferred to 5 ml MHB. The broth was incubated at 37°C for 18-24 hrs. The turbidity of the culture was compared to 0.5 McFarland turbidity standard. The standardized inoculum was inoculated within 15-20 minutes.

### Inoculation of medium

A sterile cotton swab was immersed into the standardized inoculum, excess broth was drained off by pressing and rotating the swab against the wall of tube and streaked evenly on the surface of the agar plate.

### Disc placement

Antibiotic discs (Dispens-o-disc, DIFCO) were placed on to the surface of inoculated plates by using a sterile forcep. After placement the discs were pressed gently to the agar surface.

### Incubation

The inoculated plates with discs were incubated at 35-37°C for 18-24 hours.

### Interpretation

Inhibition zone diameters were measured in mm and the susceptibility or resistance of the organisms were interpreted on the basis of criteria mentioned in Table 1.

## RESULTS AND DISCUSSION

Increasing multi-drug-resistance in Gram negative bacteria presents a critical problem (Li *et al.*, 2006). Antimicrobial resistance is a natural biological phenomenon exacerbated by the misuse of antibiotics (Saeed and Tariq, 2006). Bacterial resistance to antibiotics is enhanced by antimicrobial selection pressure by antibiotics and the crossed transmission (Donskey, 2006; Ferroni and Zahar, 2006).

Table 1. Criteria for the interpretation of antibiotic resistance/ susceptibility.

Antibiotics	potency ( $\mu$ g)	Inhibition zone diameter in mm		
		Resistant	Intermediate	Susceptible
Ampicillin	10	$\leq 11$	12 – 14	$\geq 15$
Chloramphenicol	30	$\leq 12$	13 – 17	$\geq 18$
Gentamicin	10	$\leq 12$	13 – 14	$\geq 15$
Kanamycin	30	$\leq 13$	14 – 17	$\geq 18$
Neomycin	30	$\leq 12$	13 – 16	$\geq 17$
Streptomycin	10	$\leq 11$	12 – 14	$\geq 15$
Tetracyclin	30	$\leq 14$	15 – 18	$\geq 19$

Table 2. Antibiotic resistance pattern of gram negative bacteria.

Organisms	No. of Isolates	No. of isolates resistant to antibiotics						
		S	G	A	N	K	C	T
<i>E. coli</i>	30	2	0	8	0	4	4	20
<i>K. pneumoniae</i>	25	0	3	10	20	2	2	2
<i>P. aeruginosa</i>	15	0	2	8	4	4	10	6
<i>S. typhi</i>	05	0	0	2	0	4	2	0
<i>S. paratyphi A</i>	01	0	1	1	1	0	0	0
<i>S. paratyphi B</i>	01	0	0	0	0	0	0	0
<i>P. mirabilis</i>	10	0	2	4	0	4	0	8
<i>P. vulgaris</i>	02	0	0	1	0	0	0	0
<i>S. dysenteriae</i>	05	0	0	2	0	0	0	0
<i>Y. enterocolitica</i>	01	0	0	0	0	0	0	0
<i>E. aerogenes</i>	05	0	1	2	0	2	4	4
Total	100	2	9	38	7	20	22	40

S = Streptomycin, G = Gentamicin, A = Ampicillin, N = Neomycin, K = Kanamycin, C = Chloramphenicol, T = Tetracyclin

In the present study, 100 strains belonging to 11 species of Gram negative bacteria viz., *Escherichia coli* (30), *Klebsiella pneumoniae* (25), *Pseudomonas aeruginosa* (15), *Salmonella typhi* (5), *S. typhi A* (1), *S. typhi B* (1),

*Proteus mirabilis* (10), *P. vulgaris* (2), *Shigella dysenteriae* (5), *Yersinia enterocolitica* (1), and *Enterobacter aerogenes* (5), were studied. Most of the species have been reported to be involved in serious infections. For instance, *E. coli* is the major cause of urinary tract infection (Calbo *et al.*, 2006), and diarrhea (Clarke *et al.*, 2002). *P. mirabilis* has also been associated with urinary tract infection (Camps *et al.*, 2000). *K. pneumoniae* and *E. aerogenes* has been involved in respiratory tract infections (Soares *et al.*, 2002; Cuenca *et al.*, 2006). Typhoid fever is caused by different species of *Salmonella* (Ackers *et al.*, 2000). Skin infection with *P. aeruginosa* is a most frequent health problem world wide (Catrine *et al.*, 2001). Besides, bacillary dysentery is caused by *S. dysenteriae* (De-Silva *et al.*, 1992) and *Y. enterocolitica* is also an important cause of enteritis (Brown *et al.*, 1985).

Table 3. Emergence of multi-drug resistance among gram negative bacteria

Organisms	No. of Isolates	No. of isolates resistant to no. of antibiotics							
		None	1	2	3	4	5	6	7
<i>E. coli</i>	30	9	10	4	2	2	3	0	0
<i>K. pneumoniae</i>	25	8	5	4	5	3	0	0	0
<i>P. aeruginosa</i>	15	3	0	6	5	1	0	0	0
<i>S. typhi</i>	05	1	1	1	2	0	0	0	0
<i>S. paratyphi A</i>	01	0	0	0	1	0	0	0	0
<i>S. paratyphi B</i>	01	1	0	0	0	0	0	0	0
<i>P. mirabilis</i>	10	1	3	0	4	2	0	0	0
<i>P. vulgaris</i>	02	1	1	0	0	0	0	0	0
<i>S. dysenteriae</i>	05	3	2	0	0	0	0	0	0
<i>Y. enterocolitica</i>	01	1	0	0	0	0	0	0	0
<i>E. aerogenes</i>	05	2	0	1	0	1	1	0	0
Total	100	30	22	16	19	9	4	0	0

In the present study, 100 Gram negative bacteria were subjected to antibiotic susceptibility testing by disc diffusion method. Out of 100 tested strains, 2% isolates were found resistant to streptomycin, 9% to gentamicin, 38% to ampicillin, 7% to neomycin, 20% to kanamycin, 22% to chloromphenicol, and 40% isolates were found resistant to tetracycline.

The distribution of single-drug-resistant and multi-drug-resistant strains was also noted. It was observed that, out of 100 strains tested, 22% strains were single-drug-resistant, 16% were resistant to 2 antibiotics, 19% to 3 antibiotics, 9% to 4 antibiotics and 4% strains were found resistant to 5 antibiotics thus multi-drug-resistant stains were found to be more common than single-drug-resistant. These results do not match with two previous studies in which it was investigated that single-drug-resistance is more common than multi-drug-resistance in bacteria (Chihara and Someya, 1989; Saeed *et al.*, 2005). It may be due to the differences in the population from where the samples are taken.

It is apparent from the present study that multi-drug-resistant strains are prevailing which may be hazardous to the health particularly in immunocompromized persons. Thus, the increasing problem of multidrug-resistance requires constant monitoring and evaluation of new antimicrobial drugs.

## REFERENCES

- Ackers, M., N.D. Puhr, R.V. Tauxe and F.D. Mintz (2000). Laboratory-based surveillance of *Salmonella* serotype typhi infections the United States. *J.American Medical Association*, 283(20): 2668-2673.
- Baysoy, G., S. Gurel, H. Cakici and A.P. Uyan (2006). Concurrent septic arthritis and urinary tract infection in a patient with nephrocalcinosis and vesicourethral reflux. *The Turkish J.Pediatrics*, 48(3): 275-278
- Brown, R.M., S. Tizipori, G. Gonis, J. Hayes, M. Withers and J.K. Prpic (1985). The pathogenesis of *Yersinia enterocolitica* infection in gonobiotic piglets. *The J.Medical Microbiology*, 19(3): 297-308.
- Calbo, E., V. Romani, A. Xercavins, L. Gomez, C.G. Vidal, S. Quintana, J. Vila and J. Garau (2006). Risk factors for community-onset urinary tract infection due to *Escherichia coli* harbouring extended-spectrum beta-lactamases. *J.Antimicrobial Chemotherapy*, 57(4): 780-783.
- Camps, C.D., R. Bonnet, D. Sirot, C. Chanal and J. Sirot (2000). Clinical relevance of *Proteus mirabilis* in hospital patients: a two year survey. *Journal of Antimicrobial Chemotherapy*, 45: 537-539.

- Chihara, S. and T. Someya (1989). Dynamic aspects of airborne flora over an experimental area in suburb and distribution of resistant strains to antibacterial agents among airborne staphylococci. *Nippon Eiseigaku Zasshi*, 44: 756-762.
- Catrine, A., M.H. Lise and I. Ole-Jan (2001). The impact of *Pseudomonas aeruginosa* serotypes on skin infections in occupational saturation diving system. *Scandinavian J.Infectious diseases*, 33(6): 413-419.
- Clarke, S.C., R.D. Haigh, P.P.E. Freestone and P.H. Williams (2002). Enteropathogenic *Escherichia coli* infection: History and clinical aspects. *British J.Biomedical Sciences*, 1: 1-8.
- Cuenca, F., R.J.M. Martinez, M.L. Martinez and A. Pascual (2006). *In vivo* selection of *Enterobacter aerogenes* with reduced susceptibility to cefepime and carbapenems associated with decreased expression of a 40kDa outer membrane protein and hyperproduction of Amp C beta-lactamase. *Int. J. Antimicrob Agents*, 27(6): 549-542.
- De-Silva, G.H., C.A. Candy, L.N. Mendis, H. Chart and B. Rowe (1992). Serological diagnosis of infection by *Shigella dysenteriae*-1 in patients with bacillary dysentery. *The J. infection*, 25(3): 273-278.
- Donskey, C.J. (2006). Antibiotic regimens and intestinal colonization with antibiotic-resistant Gram negative bacilli. *Clin Infect Dis.*, 43(2): 62-69.
- Ferroni, A. and J.R. Zahar (2006). Infections caused by multi-drug resistance bacteria. *Review Practice*, 56(13): 1397-1404.
- Gladstone, P., P. Rajendran and K.N. Brahmadathan (2005). Incidence of carbapenem resistant non-fermenting Gram negative bacilli from patients with respiratory infections in the intensive care units. *Indian Journal of Medical Microbiology*, 23(3): 189-191.
- Gulay, Y., C. Aysegul, K. Cigdem and D. Riza (2006). Clinical, Microbiological and epidemiological characteristics of *Pseudomonas aeruginosa* infections in a University Hospital, Malatya, Turkey. *American J.Infection Control*, 34(4): 188-192.
- Kang, C., S. Kim, W.B. Park, K. Lee, H. Kim, E. Kim, M. Oh and K. Choe (2005). Blood stream infections caused by antibiotic resistant Gram negative bacilli: Risk factors for mortality and impact of inappropriate initial antimicrobial therapy on outcome. *Antimicrobial Agents and Chemotherapy*, 49(2): 760-766.
- Li, J., R.L. Nation, J.D. Turnidge, R.W. Milne, K. Coulthard, C.R. Rayner and D.L. Paterson (2006). Colistin: the re-emerging antibiotic for multi-drug resistant Gram negative bacterial infection. *Lancet Infect Dis.*, 6(9): 589-601.
- Loivukene, K., K. Kermes, E. Sepp, V. Adamson, P. Mitt, U. Kallandi, K. Otter and P. Naber (2006). Surveillance of antimicrobial resistance of invasive pathogenesis: the Estonian experience. *Euro Surveill*, 11(2): 47-49.
- Plumbi, S. R. (2001). Humans as the world's greatest evolutionary force. *Science*, 293: 1786-1790.
- Rau, C.S., W.N. Chang, Y.C. Lin, C.H. Lu, P.C. Liliang, T.M. Su, Y.D. Tsai, C.J. Chang, P.Y. Lee, M.W. Lin and B.C. Chang (2002). Brain abscess caused by aerobic Gram negative bacilli: clinical features and therapeutic outcomes. *Clinical Neurol Neurosurg.*, 105: 60-65.
- Sader, H.S., D.J. Biedenbach, J.M. Steit and R.N. Jones (2005). Cefdinir activity against contemporary North American isolates from community acquired urinary tract infections. *Int. J. Antimicrob. Agents*, 25: 89-92.
- Saeed, S., N. Masood and P. Tariq (2005). A study on prevalence of antibiotic resistant airborne *Bacillus* species. *Int. J. Biol. Biotech.*, 2(4): 909-912.
- Saeed, S. and P. Tariq (2005). Antibacterial activities of *Mentha piperita*, *Pisum sativum* and *Momordica charantia*. *Pak. J. Bot.*, 37(4): 997-1001.
- Saeed, S., A. Naim and P. Tariq (2006). *In vitro* antibacterial activity of peppermint. *Pak. J. Bot.*, 38(3): 869-872.
- Saeed, S. and P. Tariq (2006). Effects of some seasonal vegetables and fruits on the growth of bacteria. *Pak. J. Biol. Sci.*, 9(8): 1547-1551.
- Soares, A.C., V.S. Pinho, D.G. Souza, T. Shimizu, S. Ishii, J.R. Nicoli and M. Teixeira (2002). Role of platelet aggregation factor (PAF) receptor during pulmonary infection with gram negative bacteria. *British J.Pharmacology*, 137: 621-628.
- Tumah, H. (2005). Fourth-generation cephalosporins: *In vitro* activity against nosocomial gram negative bacilli compared with beta-lactam antibiotics and ciprofloxacin. *Chemotherapy*, 51(2-3): 80-85.

(Accepted for publication October 2006)