INT. J. BIOL. BIOTECH., 4(1): 71-74, 2007.

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

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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 abcess (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^{o}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^{o}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**	**Inhibition zone diameter in mm**		
	(μg)	**Resistant**	**Intermediate**	**Susceptible**
Ampicillin	10	≤ 11	12 – 14	≥ 15
Chloramphenicol	30	≤ 12	13 – 17	≥ 18
Gentamicin	10	≤ 12	13 – 14	≥ 15
Kanamycin	30	≤ 13	14 – 17	≥ 18
Neomycin	30	≤ 12	13 – 16	≥ 17
Streptomycin	10	≤ 11	12 – 14	≥ 15
Tetracyclin	30	≤ 14	15 – 18	≥ 19

Table 2. Antibiotic resistance pattern of gram negative bacteria**.**

Organisms	**No. of**	**No. of isolates resistant to antibiotics**						
	Isolates	**S**	**G**	**A**	**N**	**K**	**C**	**T**
E. coli	30	2	0	8	0	4	4	20
K. pneumoniae	25	0	3	10	20	2	2	2
P. aeruginosa	15	0	2	8	4	4	10	6
S. typhi	05	0	0	2	0	4	2	0
S. paratyphi A	01	0	1	1	1	0	0	0
S. paratyphi B	01	0	0	0	0	0	0	0
P. mirabilis	10	0	2	4	0	4	0	8
P. vulgaris	02	0	0	1	0	0	0	0
S. dysenteriae	05	0	0	2	0	0	0	0
Y. enterocolitica	01	0	0	0	0	0	0	0
E. aerogenes	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 = Chroramphenicol, 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**	**No. of isolates resistant to no. of antibiotics**						
	Isolates	**None**	**1**	**2**	**3**	**4**	**5**	**6**	**7**
E. coli	30	9	10	4	2	2	3	0	0
K. pneumoniae	25	8	5	4	5	3	0	0	0
P. aeruginosa	15	3	0	6	5	1	0	0	0
S. typhi	05	1	1	1	2	0	0	0	0
S. paratyphi A	01	0	0	0	1	0	0	0	0
S. paratyphi B	01	1	0	0	0	0	0	0	0
P. mirabilis	10	1	3	0	4	2	0	0	0
P. vulgaris	02	1	1	0	0	0	0	0	0
S. dysenteriae	05	3	2	0	0	0	0	0	0
Y. enterocolitica	01	1	0	0	0	0	0	0	0
E. aerogenes	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.

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(*Accepted for publication October 2006*)