Original Article

Distribution of Bacterial Strains and their Antibiotic Sensityvity Profile in an Urban Referral Hospital

M. Bulbul Hasan[1], S Gul Nahar[2], M. Nawshad Ali[3], Mst. Rokeya Khatun[4], Chinmoy Kanti Das[5]

Abstract

Then Present study was done to assess the distribution of bacterial pathogens with their pattern of antibiotic susceptibility in an urban referral hospital in RMCH. A total of 393 bacteria strains were isolated from various specimens over a 10-months period. The majority of the organisms were Escherichia coli (33.33%) followed by Klebsiella species (27.48%), staphylococcus aureus (17.05%), Acinetobcter species (8.14%), Pseudomonas species (7.12%), and others. The third-generation Cephalosporins like Ceftriaxone, Ceftazidime and Cefotaxime were sensitive to Ciprofloxacin of various Enterobacteriaceae was only between 33-40% compared to 52.8-37.9% against Gentamicin. Majority of the Enterobacteriaceae were resistant to Ampicillin, whereas almost all of the Enterobacteriaceae (94-100%) were sensitive to Imipenem. About 97.0% Acinetobacter species were susceptible to Imipenem. Sensitivity of the organism (Acinetobacter) to third-generation Cephalosporins ranged between 50-56%, whereas 40.6% were found sensitive to Ciprofloxacin. The sensitivity to Chloramphenicol, Co-trimixazole, Cephalexin and Ampicillin ranged between 9.3% to 34.3%. About 93.0% of Pseudomonas species were sensitive to Imipenem. The rate of susceptibility to Gentamicin and Netilmicin was higher than those of the Ciprofloxacin and Ceftriaxone (37.8% and 53.5% vc. 39.2%). About 70% of isolated S. aureus were resistane of Oxacillin but all were sensitive to Vancomycin. The result of this study would help the physicians to make a judicious choice of antibiotics for therapeutic purposes.

Key words: distribution, bacterial strains, antibiotic sensitivity profile, urban referral hospital, Enterobacteriaceae

Introduction

Infectious diseases still remain a major cause of morbidity and mortality in third-world countries including Bangladesh. Bacterial pathogens resistant commonly used antimicrobials are now creating a challenge to the clinicians and researchers. The multi-drug-resistant organisms are a serious medical problem that has significantly affected the treatment of infectious diseases[1,2] and has become a major clinical concern globally[3,4]. Bacterial resistance pattern to antimicrobial agents can differ significantly from one country to another and within a country as evidenced by several recent surveillance studies[5,6,7,8].

Introduction of newer antimicrobial agents is usually followed sooner or later by emergence of bacterial resistance of these drugs for many

[1] Associate Professor, Department of Microbiology, Rajshahi Medical College, Rajshahi.
[2] Assistant Professor, Department of Microbiology, Rajshahi Medical College, Rajshahi.
[3] Associate Professor, Department of Pediatric Surgery, Rajshahi Medical College, Rajshahi.
[4] Assistant Professor, Department of Gynae and Obstetrics, Rajshahi Medical College, Rajshahi.
[8] Associate Professor, Department of Community Medicine, Rajshahi Medical College, Rajshahi.

reasons[9]. Development of multi-drug resistance in clinical isolates like Salmonella typhi, Pseudomonas species and Klebsiella species has been reported in Bangladesh[10,11,12]. The study on antibiotic susceptibility pattern is particularly important is developing countries that do not control antibiotic usage and maintain adequate epidemiological surveillance.

Therefore, the present study has been designed to find out the antibiotic resistance patterns of medically important bacteria in an urban hospital. The outcome of this study might enable to determine the trend of drug resistance prevailing in Bangladesh.

Methods

This was a prospective study carried out in RMCH. Samples were collected over a 10-months period during January to October, 2005 from both sexes and different age groups. A total of 393 clinical isolates were tested and the specimens included were Urine, Pus, Sputum, Vaginal swab, Throat swab and Conjunctival swab.

All samples were routinely cultured on MacConkey and Blood agar plates. In addition to these plates, Chocolate agar media were used for culturing Pus, Vaginal swab and Conjunctival swab specimens. After overnight incubation, plates were checked for the presence of suspected pathogens. All the suspected colonies were identified by colony characteristics, motility, Gram staining results, and biochemical reactions[13].

Antimicrobial susceptibility test of the isolated organisms was done by disk diffusion method using the Kirby-Bauer technique[14] and as per recommendations of the national Committee for clinical Laborratory Standards[6] Antimicrobial agents used for determining antibiogram of isolated organisms were Penicillin (10 units), Ampicillin (10 μg), Cufuroxime (30 μg), Ceftazidime (30 μg), Cefotaxime (30 μg), Co-trimoxazole (25 μg), Imipenem (10 μg), Gentamicin (10 μg), Tetracycline (30 μg), Ciprofloxacin (5 μg), Vancomycin (30 μg), Chloramphenical (30 μg), Netilmicin (30 μg),

Oxacillin (1 μg), Erthromycin (15 μg), Rifampicin (5 μg), Fusidic acid (10 μg), Cephalexin (30 μg), and Ceftriaxone (30 μg), All disks were obtained from Oxoid Ltd, Basinstoke, Hampsire, UK.

In order to monitor the quality of the test result, on each day of testing the reference ATCC stains Escherichia coli ATCC 25922 and staphylococcus aureus ATCC 292213 were included. The zone of inhibition was compared with recommended standard values[15].

Results

A Total of 393 strains of bacteria were isolated from various specimens. The specimens wee Urine (189, 48.09%), Sputum (88, 22.39%), Pus (71, 18.07%), Throat swab (20, 5.09%) and others (25, 6.36%), including Vaginal and Conjunctival swabs.

Out of 393 organisms isolated, majority were Escherichia coli (131, 33.33%) followed by Klebsiella species (108, 27.48%), other isolates included Staphylococus aureus (67, 17.05%), Acinetobacter species (32, 8.14%), Pseudomonas species (28,7.12%) and Provindencia species (11,2.80%). (Table 1)

Table 1: Pattern of organism isolated from various samples

Table 1: Pattern of organism isolated from various samples

Serial	Name of organisms	Number	Percentage
1.	Escherichia coli	131	33.33
2.	Klebsiella species	108	27.48
3.	Staphylococcus aureus	67	17.05
4.	Acinetobacter species	32	8.14
5.	Pseudomonas species	28	7.12
6.	Providencia species	11	2.80
7.	Proteus species	09	2.29
8.	Enterobacter species	01	0.25
9.	Others*	06	1.53
	Total	393	100

Others indicate S. saprophyticus (n=3), S. epidermidis (n=2) and beta hemolytic streptococci (n=1)

Among the total bacterial isolates, 260 (66.6%) were members of the Enterobacteriaceae family including E. coli, Klebsiella species, Providencia species, Proteus species, and Enterobacter species. Considering susceptibility pattern with different antimicrobials, it is found that almost all of the Enterobacteriaceae were sensitive Imipenem (94-100%). The third-generation Cephalosporins like Cefriaxone, Ceftazidime and Cefotazime were sensitive against 45-66% isolated Enterobacteriaceae was only between 33-40%. Sensitivity to Gentamicin ranged between 52.8-67.9%. (Table II).

Table II: Sensitivity of the isolated Enterobacteriaceae (n=260)

Organisms	Number (%) sensitive to-												
	Amp	Cepl	CXM	CAZ	CRO Gen	CTX		Tet	Cip	Chl	Cot	Imp	Net
E.coli (n=131)	10 (7.6)	28 (21.3)	49 (37.4)	74 (56.4)	76 (58.0)	80 (61.1)	43 (32.8)	89 (67.9)	53 (40.4)	83 (63.3)	31 (23.6)	129 (98.4)	78 (59.5)
Klebsiella spp (n=108)	6 (5.5)	19 (17.5)	38 (35.1)	60 (55.5)	56 (51.8)	62 (57.4)	44 (40.7)	57 (52.8)	39 (36.1)	56 (51.8)	33 (30.5)	102 (94.4)	67 (62.0)
Providencia spp (n=11)	01 (9.0)	02 (18.1)	05 (45.4)	06 (54.5)	06 (54.5)	05 (45.4)	06 (54.5)	07 (63.6)	04 (36.3)	05 (45.4)	04 (36.3)	11 (100)	08 (72.7)
Proteus spp (n=9)	01 (11.1)	02 (22.2)	04 (44.4)	05 (55.5)	05 (55.5)	06 (66.6)	04 (44.4)	06 (66.6)	03 (33.3)	04 (44.4)	02 (22.2)	09 (100)	05 (55.5)
Emterobacter spp (n=1)	00	00	00	00	00	00	00	00	00	00	00	00	00

Amp = Ampicillin, cepl= Cephalexin, CFM= Cefixime, CAZ= Ceftazidime, CRO= Ceftriaxone, CTX= Cefotaxime, Tet= Tetracycline, Gen= Gentamycin, Cip= Ciprofloxacin, Chl= Chloramphenicol, Cot= Co-trimoxazole, Imp= Imipenem, Net= Netilmycin

Almost all of Acinetobacter species (31, 96.8%) were susceptible to Imipenem, While 23 (71.8%) and 21 (65.6%) of the isolates were sensitive to Tetracycline and Gentamicin respectively. The sensitively to third-generation Cephalosporins ranged between 50-56%, whereas 40.6% were found sensitive to Ciprofloxacin. The sensitive of the organism to Chloramphenicol, Co-trimoxazole, Cephalexin and Ampicillin were 34.3%, 31.2%, 15.6% and 9.3% respectively.

Out of 28 isolates of Pseudomonas species, 26 (92.8%) were sensitive to Imipenem. Only 01 (3.5%) isolate was susceptible to Ampicillin, while 12(42.8%) were sensitive to Carbenicillin and Cefotaxime. The sensitivity of the organism to Ciprofloxacin and Ceftriaxone was 39.2% each, while the rate of susceptibility to Gentamicin and Netilmicin was higher than the formers (67.8 % and 53.5%). Susceptibility to Co-trimoxazole, Chloramphenicol and Tetracycline were between 17.8%-32.1% respectively. (Table III)

Table III: Antibiogram of isolated Acinetobacter species and Pseudomonas species by disk diffusion method

Organisms	Number (%) sensitive to-												
	Amp	Cepl	CXM	CAZ	CRO Gen	CTX		Tet	Cip	Chl	Cot	Imp	Net
Acinetobacter (n=32)	03 (9.3)	05 (15.3)	15 (46.8)	17 (53.1)	18 (56.2)	16 (50.0)	23 (71.8)	21 (65.6)	13 (40.6)	11 (34.3)	10 (31.2)	31 (96.8)	18 (56.2)
Pseudomonas spp (n=28)*	01 (3.5)	-	-	13 (46.4)	11 (39.2)	12 (42.8)	09 (32.1)	19 (67.8)	11 (39.2)	08 (28.5)	05 (17.8)	26 (92.8)	15 (53.5)

* Carbenicillin was tested in addition and 12 (42.8%) Pseudomonas were sensitive

Considering sensitivity of Staphylococcus aureus, It was found that only 05 (7.4%) and 08 (11.9%) of the isolates were sensitive to Penicillin and Ampicillin respectively. Only 20 (29.8%) isolates were sensitive to 1 µg Oxacillin showing a calculated 70.2% of the isolates being Methicillin

resistant or MRSA. Only 23 (34.3%) of the isolates were sensitive to Ciprofloxacin. However, all of them were sensitive to Vancomycin and the sensitivity to Rifampicin and Fusidic acid ranged between 85-88%. (Table IV)

Table IV: Antibiogram of isolated S. aureus by disk diffusion method

Organisms	Number (%) sensitive to-												
	Pen	Amp	Ox	Cepi	Cip	Ery	Gen	Tet	Chl	Cot	Net	Van	Rif
S. aureus	05	08	20	25	23	15	24	39	44	18	53	67	57
(n=67)	(7.43)	(11.9)	(29.5)	(37.31)	(34.3)	(22.3)	(35.8)	(58.2)	(65.6)	(26.8)	(79.1)	(100)	(85.0)

Amp =Ampicillin, Cepl= Cephalexin, CXM= Cefuroxime, CAZ= Ceftazidime, CRO= Ceftriaxone, CTX= Cefotaxime, Tet= Tetracycline, Gen= Gentamicin, Cip= Ciprofloxacillin, Chl= Chloramphenicol, Cot= Co-trimoxazole, Imp= Imipenem, Net= Netilmicin, Pen= Penicillin, Ox= Oxacillin, Ery= Erythromycin, Rif=Rifampicin, Van= Vancomycin and Fus= Fusidic acid

Discussion

Antibiotic resistance is a common phenomenon in developing countries where drugs are available freely without prescription. Now-a-days, antibiotics have been extensively and newer antibiotics are continuously being added for the treatment of various infections. Proper use of antibiotics is very important in reducing unnecessary expenses, development of resistance to useful and life-saving antibiotics as well as to minimize many side effects. The resistance pattern varies from one country to another.

In the present study, most of the Enterobacteriaceae were sensitive to Imipenem ranging between 94-100%. This high level of sensitivity to Imipenem could be due to its restricted and limited use in the clinical practice. The drug has only recently been introduced in Bangladesh and is very expensive which has further restricted its widespread use. Similar effectiveness of Imipenem has also been reported from other countries[15,7,16].

The third-generation cephalosporins like Ceftriaxone, Ceftazidime and Cefotaxime were sensitive against 45-66% isolated Enterobacteriaceas. The first-and second-generation Cephalosporins were less effective. In the United States, the frequency of resistance to Ceftazidime has increased from 1.5% to 3.6% from 1991 to 1997 as reported by the National Nosocomial Infections Surveillance system. A surveillance trial involving 102 medical centres in the United States detected 10.3% and 23.8% Ceftazidime-resistant E.coli and K. pneumoniae respectively[16].

However, the sensitivity of various Enterobacteriaceae to Ciprofloxacin in the present study was only between 33-40%. This low-level of sensitivity to Quinolones and Cephalosporins was the result of very extensive use of these antibiotics in clinical practice. A large majority of patients were found prescribed by these drugs on their first contact with physicians[16].

All the staphylococcus anreus isolated in this study were sensitive to Vancomycin whereas 70% were Methicillin resistant (MRSA). The prevalence of MRSA differs strongly between countries[17]. In the present study, very high isolation rate of MRSA was found as detected by 1 μg Oxacillin disk. Another study with wound specimens from diabetic patients in Bangladesh in 1994, reported an isolation rate of 37.2% and 21.6% MRSA amongst the hospitalized and non-hospitalized diabetics respectively[18]. The present findings show that the prevalence of MRSA in hospitalized patients has increased significantly over time.

This study would help the physicians to make judicious choice of antibiotics and would be helpful for formulation of an antibiotic policy.

References

1. Berger-Bachi B. Resistance mechanisms of gram-positive bacteria. Int J Med Microbiol 2002; 292:27-35.

2. Poole K. overcoming multidrug resistance in gram-negative bacteria. Curr Opin investig Drugs 2003;4:128-139

3. Jones R.N, Marshall SA. Antimicrobial activity of cefepime tested against Bush group I beta-

lactamase producing strains resistant to ceftazidime. Diagn Microbiol infects Dis 1994; 19: 33-38

4. Mocellering RC. Emerging resistance with Gram-positive acrobic infections. Where do we go from here? Introduction: Problems with antimicrobial resistance in Gram-positive cocci. Clin infectDis 1998; 26:1177-1178

5. Jones RN, Salazar JC, Pfaller MA, Doern GV. Colombian Antimicrobial Resistance Study (CARS)Group. Multi center evaluation of the antimicrobial activity for six broad-spectrum beta-lactams in Colombia using the E-test method. Diagn Microbiol infect Dis 1997;29:265-272

6. National Committee for Clinical Laboratory Standards. Tentative stabdard M2-A6: Performance Standards for Antimicrobial Disk Susceptibility Tests. NCCLS, villanova, Pa; 1997.

7. Yamaguchi K, Mathai D, Biedenbach DJ, Lewis M,Gales AC, Jones RN, Japan Antimicrobial Resistance Study (JARS) Group. Evaluation of the in vitro activity of six broad-spectrum beta-lactam antimicrobial agents tested against over 2000 clinical isolates from 22 medical centers in Japan. Diagn Microbiol Infect Dis 1999; 34:123-134.

8. Sader HS, Mimica I, Rossi F, et al. Evaluation of the in vitro activity of cefepime compared to other broad spectrum cephalosporins against clinical isolates from eighteen Brazilian hospitals by using the E-test. Diagn Microbiol Infect Dis 1997; 28:87-92.

9. Shanahan PMA, Thospson CJ, Amyes SGB. The global of antibiotic-resistant bacteria: their sources and reservoirs. Rev Med Microbiol 1994; 5:174-182.

10. Asna SMZ, Haq JA. Decrease of antibiotic resistance in Salmonella typhi isolated from patients attending hospitals of Dhaka City over a 3-year period. Intl J Antimicrob Agents 2000; 3:249-251

11. Bequi AAMA, Rahman KM. Transferable drug resistance in Pseudomonas aeruginosa, Bangladesh Med Res Council Bull 1987; 2:61-68

12. Ahmad AAA, Sriniwas. Patterns of antibiotic resistance of Klebsiella pneumoniae. Bangladesh Med Rev 1987; 13:9-14.

13. Baron EJ, Peterson LR, Finegold SM. Enterbacteriaceae. In Bailey and Scotts Diagnostic Microbiology, 9th edition. St. Louis: Mosby; 1994: pp.374-379

14. Bauer AW, Kirby WMM, Sherris JC, Truck M. Antibiotic susceptibility testing by a standard single disk method. Am J Clin Pathol 1966; 45: 493-496.

15. National Committee for Clinical Laboratory Standards. Tentative standard M100-S8: Performance Standards for Antimicrobial Susceptibility Resting. NSSLS, Villanova, Pa; 1998.

16. Johnson DM, Biedenbach DJ, Jones RN, The Philippines Antimicrobial Resistance Study (PARS) Group. In vitro evaluation of broad-spectrum beta-lactams in the Philippines medical centers: role of fourth-generation cephalosporins. Diagn Microbiol Infect Dis 1999; 35:291-297

17. Christina MJE, Vandenbroucke G.Management of Methicillin resistant Staphylococus aureus in the Netherlands. Rev Med Microbiology 1998; 9:109-116

18. Jinnah F, Chowdhury K, Begum J, et al. Multidrug resistant Staphylococcus aures isolated from the wound of Diabetic patients. J Infect Dis Antimicrob Agents 1998; 15:15-18.

All corresponds to
Bulbul Hasan
Associate Professor
Department of Microbiology
Rajshahi Medical College
Rajshahi, Bangladesh
E-mail: bulbul010163@yahoo.com