Bangladesh J Med Microbiol 2013; 07 (01): 15-19
Bangladesh Society of Medical Microbiologists

Original Article

Antimicrobial Resistance Patterns of Bacterial Isolates in a Medical College Hospital of Bangladesh

Nurun Nahar Mawla[1], KM Shahidul Islam[2], Afzalunnessa Binte Lutfor[3], Shahin Sultana[1]

[1]Department of Microbiology, Delta Medical College, [2]Dhaka Medical College, [3]Ad-Din Medical College, Dhaka, Bangladesh

Abstract

The present study have been undertaken to determine the antibiotic susceptibility patterns of isolated bacterial strains to guide the clinician in selecting the best antimicrobial agent for an individual patient and to accumulate epidemiological information for public health importance. Isolates were identified by conventional methods from different samples of urine, blood, pus, wound swab, stool, high vaginal swab and pleural fluid from patients attending to Sir Salimullah Medical College and Hospital in Dhaka, Bangladesh between January 2005 and October 2006. Antimicrobials susceptibility testing was performed by Kirby Bauer's disc diffusion method and reported according to CLSI guidelines. Of the 1592 different tested samples, 779 showed growth of pathogens, among which the most prevalent gram negative bacilli were *Escherichia coli* (71%) followed by *Pseudomonas* (8%) and *Klebsiella* (5%). *Staphylococcus aureus* (11%) was found as the most important gram positive resistant pathogens. The resistance pattern among gram negative bacilli was observed high against ampicillin, cephalexin, cotrimoxazole, nalidixic acid and ciprofloxacin. *Staphylococcus* showed almost same trend except ciprofloxacin and nalidixic acid. Most of the isolates were resistant to 4 or more number of antibiotics. Available antimicrobials with good activity against resistant pathogens include ceftriaxone and imipenem. The role of fluoroquinolones in the empiric treatment of bacterial infections is also being limited by new resistance patterns and increasing resistance levels, resulting a considerable economic and health burdens on our country.

Key words: Antimicrobials susceptibility, Pathogens.

Introduction:

The antimicrobial resistance in common pathogens of hospital and community-acquired infections are now a worldwide concern[1,2]. This global problem is particularly pressing in developing countries, where the infectious disease burden is high and cost constraints prevent the widespread application of newer, more expensive agents. Since the discovery, antimicrobial drugs have shown dramatic efficacy for the control of bacterial infections[3]. However, it was soon evident that bacterial pathogens have a remarkable ability to mutate and acquire resistance genes from other organisms and thereby develop resistance to antimicrobial drugs.Resistant infections adversely affecting on mortality, increase treatment costs, facilitate disease spread into the community and lengthen duration of illness[4,5]. Initially, organisms resistant to multiple drugs were found mostly in hospitals, because of most frequent use of antibiotics, but resistance is currently found almost as frequently in the community. The indiscriminate use as well under/over dosing of antimicrobial drugs has resulted in drug resistances that seriously threatens the control of infectious diseases and reverse the medical advances of last half century.

Changes in the types of pathogens isolated in serious infections might also affect resistance patterns because different bacterial species inherently have differing antimicrobial susceptibilities[6]. A study between 1973 and 1994 performed by The European Organization for Research and Treatment of Cancer trials documented that Gram-negative pathogens were the dominant bloodstream isolates from 1973 to 1985, but Gram-positive isolates were more

Correspondence:
Dr. Nurun Nahar Mawla
Assistant Professor, Department of Microbiology
Delta Medical College & Hospital
Mirpur-1, Dhaka-1216, Bangladesh.
Phone: +880 1711 877 044
Email: drnnmawla@gmail.com.

common in later years[7]. This change in pathogen dominance coincided with the 1985 introduction of the third generation cephalosporins, ceftazidime and ceftriaxone and was probably driven by the widespread use of these drugs[8].

In this study, we build a picture of the situation of antimicrobial resistance for common bacterial pathogens in a tertiary level medical college hospital of a developing country like Bangladesh to guide the clinician in selecting the best antimicrobial agent for an individual patient in a low-cost, less-available set-up. The current magnitude of the problem and the speed with which new resistance phenotypes have emerged increases the public health significance of this issue. This study helps to accumulate epidemiological information for public health importance also.

Materials And Methods:

Over a 22 month period (January 2005 to October 2006), we collected and tested 1592 of which, 871 in 2005 and 721 in 2006 different samples of urine, pus, wound swab, blood, stool, high vaginal swab and pleural fluid in the laboratory of Microbiology department of Sir Salimullah Medical College and Hospital (SSMCH), Dhaka, Bangladesh. All valid samples coming from different wards and outdoors of SSMCH were included in the study but we excluded those samples with inadequate information, contaminated or improperly taken and which gave doubtful results indicating contamination.

Standard methods for isolation and identification of the organisms were carried out with every sample. Blood agar and MacConkey agar were used for isolation. Triple Sugar Iron (TSI), Motility Indole Urea (MIU), citrate, catalase, coagulase, oxidase, optochin and bacitracin sensitivity were done to identify pathogenic organisms.

Antimicrobial susceptibility of Gram positive and Gram negative bacteria were determined by Disk-diffusion method of Bauer and co-workers with use of commercially prepared antibiotic disks[9].

A suspension of the test organism is prepared in sterile saline equivalent to a 0.5 McFarland standard using isolated colonies and incubated at 37°C for 2 hours. Using a sterile cotton swab, the organism is inoculated into Mueller-Hinton agar plate, spread evenly over entire surface. By a needle, appropriate antibiotic disks were applied firmly to the surface of the agar, with an equal distance apart from each other. A 90 mm plate usually accommodates 6 disks. After overnight incubation of the plates, the diameter of 'zone of inhibition (areas of no growth of bacteria by inhibitory effect of drugs in disks)' is measured and reported as Susceptible (S), Intermediate (I) or Resistant (R) according to 'The Clinical Laboratory Standard Institute' (CLSI) guidelines.

Trends in isolation as well as antimicrobial susceptibilities of isolated pathogens were determined using t-test for trend in the SPSS (Statistical Package for the Social Science) program 11.5 version. A P value ≤0.05 was considered significant for all comparisons.

Results:

During the study period from January 2005 to October 2006, a total 1592 samples were received from patients who ranged in age from 1day to 85 years with a mean age of 22 years of which 779 (49%) samples were found culture positive.

Among the positive samples, urine (59%); mean percentage value) had highest isolated pathogenic organisms followed by pus (17%), wound swab (7%), blood (5%), stool (4%) and high vaginal swab (4%). Sputum, throat swab and pleural fluid showed insignificant isolations.

Escherichia coli was the highest growing gram negative bacteria in both 2005 (67.04%) and 2006 (76.05%).Gradually isolation of *Pseudomonas* (8.13%, 8.08%), *Klebsiella* (5.42%, 4.79%) and *Salmonella* (2.48%, 1.14%) decreased. Growth of *Shigella* and *Proteus* were not significant. *Staphylococcus* was highest isolated Gram positive bacteria in both the year (14% & 8%), while other Gram positive bacteria were found insignificant (Table-1).

Table-1: Bacterial pathogens isolated in SSMCH, Dhaka, Bangladesh from January 2005 to October 2006

Organisms	Year					
	2005 (445)	%	2006 (334)	%	Total	Percentage (no. of total isolates, N=779)
Gram negative						
Escherichia Coli	297	67	254	76	551	71
Pseudomonas	36	8	27	8	63	8
Klebsielle	24	5	16	5	40	5
Salmonella	11	2	4	1	15	2
Shigella	2	0	2	1	4	1
Proteus	6	1	2	1	8	1
Gram positive						
Staphylococcus	60	14	26	8	86	11
Streptococcus	7	2	3	1	10	1
Enterococcus	2	0	0	0	2	0

The resistance rate of gram negative bacteria to ampicillin (P<0.004), cephalexin (P<0.004), ciprofloxacin (P<0.015), chloramphenicol (P<0.012) and nalidixic acid (P<0.003) increased in 2006 compare to 2005. Though ceftriaxone also showed higher resistance in 2006 for Escherichia Coli, *Pseudomonas*, and *Klebsiella* (Table-2), but it is still effective against these organisms. In 2006, susceptibility to tetracycline for gram positive bacteria was not tested as it found sensitive in all isolates of 2005, while nitrofurantoin sensitivity test was added and, *Pseudomonas*, and *Klebsiella* showed significant resistance (Table-2) to this drug.

Table-2: Percentage of antimicrobial resistance in gram negative bacteria isolates.

	E.coli		Pseudomonas		Klebsiella		Salmonella	
	2005	2006	2005	2006	2005	2006	2005	2006
Antibiotic	(297)*	(254)	(36)	(27)	(24)	(16)	(11)	(4)
Ampicillin	70	91	68	89	79	100	70	75
Ceftriaxone	46	51	36	47	42	45	0	0
Cephalexin	74	72	50	84	74	73	60	75
Ciprofloxacin	54	61	45	26	53	55	20	25
Chloramphenicol	38	44	73	68	32	45	90	25
Gentamicin	27	0	41	16	42	0	40	0
Cotrimoxazole	24	0	0	74	0	82	0	50
Nalidixic Acid	57	71	45	63	63	45	20	50
Imipenem	0	1	0	5	11	0	0	0
Tetracycline	0	-	32	-	0	-	0	-
Nitrofurantoin	-	34	-	63	-	55	-	25

* Values in parentheses are the number of isolates

Gram positive bacteria, represented by *Staphylococcus* showed increase resistance rate to ampicillin from 40% to 81% (P<0.004), followed by cotrimoxazole (23% to 52%; P<0.001), ciprofloxacin (30% to 48%; P<0.001) and erythromycin (21% to 43%; P<0.005). Tetracycline and Penicillin sensitivity were not performed on 2006. Staphylococcus was also found sensitive to Imipenem, chloramphenicol and gentamicin (Table-3).

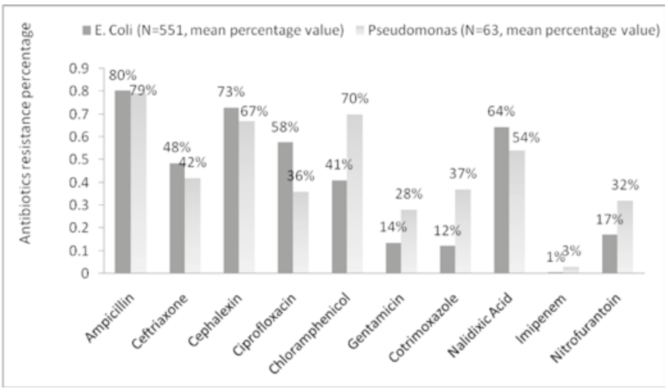
Table-3: Percentage of antimicrobial resistance in gram positive bacteria isolates.

	Staphylococcus	
	2005	2006
Antibiotics	(60)*	(26)
Ampicillin	40	81
Ceftriaxone	0	24
Cephalexin	44	30
Ciprofloxacin	30	48
Chloramphenicol	28	33
Gentamicin	14	14
Erythromicin	21	43
Cotrimoxazole	23	52
Imipenem	0	10
Tetracycline	14	-
Penicillin	37	-

* Values in parentheses are the number of isolates

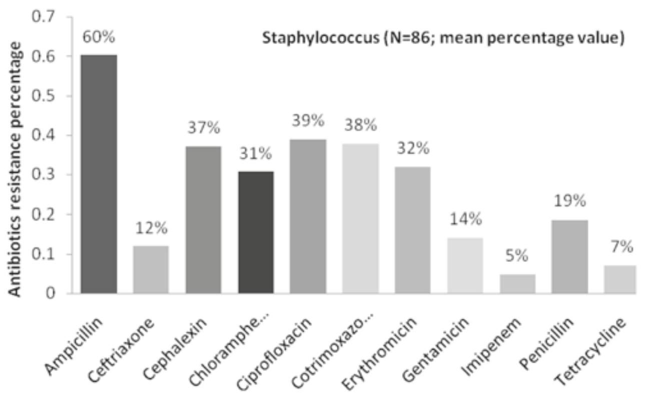
Among total 551 *Escherichia coli*, highest resistance was showed to ampicillin (80%; mean percentage value), followed by cephalexin, nalidixic acid and ciprofloxacin. Imipenem had lowest resistance rate about 1% only. *Pseudomonas* was found resistant to more drugs like ampicillin (79%), chloramphenicol (70%), cephalexin (67%), nalidixic acid (54%) and, ceftriaxone (42%) in compare to *Escherichia coli*. Rates of resistance to Imipenem (3%) were also higher than *Escherichia coli* but this drug remain effective against *Pseudomonas*. Cotrimoxazole and nitrofurantoin have significant resistance rate of 74% and 63% respectively in 2006 (Figure I).

Figure-I: Antimicrobial resistance pattern in *Escherichia coli* and *Pseudomonas* isolates from January 2005 to October 2006.



It was evident from figure-II, that nearly all strains of *Staphylococcus* in this study (N=86) were highly resistant to ampicillin (60%) followed by ciprofloxacin, cotrimoxazole, cephalexin and erythromycin. Study showed Ceftriaxone, Gentamicin and Imipenem as drug of choice for proper treatment of *Staphylococcus* infections.

Figure II: Antimicrobial resistance pattern in *Staphylococcus isolates.*



Discussion:

Antibiotic resistance is a major clinical problem in treating infections caused by different microorganisms. The resistance to the antimicrobials has increased over the years[10]. This study shows the antibiotic resistance patterns of microbial species isolated from patients with hospita acquired infections suggests that the rate of resistance to a variety of antimicrobials commonly used is significantly higher in a developing country like Bangladesh. Resistance rates are typically higher in developing countries (up to 99) as compared to developed countries (<20%)[11]. In the developing world ampicillin, cephalexin, nalidixic acid, ciprofloxacin, tetracycline, cotrimoxazole, and chloramphenicol are widely used to treat common infections like diarrhoea, urinary tract infections, sore-throat, enteric fever etc because of their low cost and ready availability. Our analysis confirms that the first three of these antibiotics no longer have a role in the treatment of these common infections.

Ampicillin is no longer an effective drug, almost all organisms (>80%) in this study shows resistance to it. Reports of penicillin-resistant *Staphylococcus aureus* have first appeared by 1944, and today virtually all *Staphylococcus* are resistant to natural penicillin's as well as aminopeniciliins[12,13]. Also high rates of resistance of enteric pathogens to ampicillin have remained relatively constant over many years[14].

Our analysis found that ciprofloxacin is widely used in Bangladesh to treat many infections without prescription and is likely to result in high prevalence of resistance. Though since discovery, *Escherichia coli* was susceptible to Ciprofloxacin, but in our study of 2005-2006, the resistance rate was found 58%, which make this fluoroquinolone not suitable for treatment of *Escherichia coli* infection further. Increasing resistance also limits it's use for treatment of *Pseudomonas* and *klebsiella*. Though in this study, salmonella showed 20% resistance to Ciprofloxacin, but still it could be one of the treatment option for Salmonella.

Gentamicin is still effective against many bacteria, but nalidixic acid has lost its efficacy against major organisms. The 1990s have been notable for the emergence of resistance among enteric pathogens to nalidixic acid and fluoroquinolones. In our 2 years study, the most frequently isolated gram-negative bacilli were *Escherichia coli* and *pseudomonas*, among which *Escherichia coli* (P<0.003) accounted more resistance to nalidixic acid (64%) than *pseudomonas* (P<0.002) with 14% increase in 2006 from 2005 (Table-2). This is consistent with the findings of previous studies[15]. However, our other study isolates also showed resistance to ampicillin, cephalexin, cotrimoxazole, chloramphenicol, nalidixic acid and ciprofloxacin which support the previous data of other community based studies[16].

By 1993, many organisms had developed resistance to all commonly prescribed antibiotics leaving the expensive ciprofloxacin, ceftriaxone and imipenem as the only available alternatives[14]. Higher resistance rate to all antibiotics used in this study with the exception of imipenem may be explained as uncontrolled consumption of these antibiotics during the past decade in our region[17-19]. In the present study overall imipenem resistance were 11% for *Klebsiella* in 2005, whereas, other isolates were found to be sensitive to imipenem. For treating *Pseudomonas* infection, imipenem is the only choice though 5.26% resistance was found among our study isolates of 2006.

This study had limitation where isolates were obtained from various populations in different locations coming to only one hospital in Bangladesh. However, it remains likely that the general trends documented in this study are representative of the overall resistance trends for our country, but more comprehensive confirmatory studies will need to be conducted.

Commonly prescribed antimicrobial agents are lifesaving when used appropriately, but it's indiscriminate use faster emergence of bacterial resistance that results in an equivalent burden of one new case of the disease, since the infection must be retreated. Actually, second line therapies are more costly, require more complicated dosing, have more side-effects, and may need a greater degree of medical attention. Therefore, the true cost of curing one resistant infection exceeds that for two non-resistant ones, with less assurance of success[20,21]. So, this study also highlights the need to develop newer classes of antibiotics to treat diseases.

It is important to keep in mind that the principle purpose of monitoring antibiotic resistance trends among different pathogens is to provide clinicians with data that can be used to select appropriate treatment regimens. Monitoring antimicrobial susceptibility pattern help to recommend antibiotic restriction appropriately, provide a guideline for prophylactic use of antibiotics and preventing its indiscriminate uses. So, our key prevention strategies should be diagnosing and treating infection effectively through rational use of antimicrobials.

Conclusion:

It is quite alarming to note that almost all of the isolates included in this study were found resistant to four or more antibiotics. A continued emphasis is required on the prevention of emergence and spread of resistance through rational antibiotic use. Also strategies should be taken to facilitate the earlier identification of resistant organisms and minimize the delay in administration of appropriate antibiotic therapy. Therefore, it is an important issue to be addressed by the policy makers to formulate a strict antibiotics prescription policy for our country.

Reference:

1. Stuart BL., Bonnie M, Susan S, Deborah R, and Joanne D. High Frequency of Antimicrobial Resistance in Human Fecal Flora. Antimicrob. Agents Chemother. 1988; 32(12):1801-1806.
2. Farrar, W. E. Antibiotic resistance in developing countries. J. Infect. Dis. 1985; 152:1103-1106.
3. Howard SG, and Robert CM Jr. Antimicrobial-Drug Resistance. The New England Journal of Medicine. 1997; 335(19):1445-1453.
4. Iruka NO., Ramanan L., Zulfiqar AB., Adriano GD., Philip J., Thomas FO., Ariel PM., and Keith PK. Antimicrobial resistance in developing countries: recent trends and current status (Part-1). Lancet Infect Dis. 2005; 5: 481-93.
5. Laxminarayan R. Battling resistance to antibiotics and pesticides: an economic approach. Washington, DC: Resources for the Future, 2003.
6. Jones RN, Pfaller MA. Bacterial resistance: a worldwide problem. DiagnMicrobiol Infect Dis. 1998; 31:379-388
7. Klastersky J. Science and pragmatism in the treatment and prevention of neutropenic infection. J Antimicrob Chemother. 1998; 41(suppl D):13-24
8. Jones RN. Contemporary antimicrobial susceptibility patterns of bacterial pathogens commonly associated with febrile patients with neutropenia. Clin Infect Dis. 1999; 29:495-502.
9. Bauer AW, Kirby WM, Sherris JC, andTurck M. Antibiotic susceptibility testing by a standardized single disc method. Am J ClinPathol. 1966;45:493-6.
10. Gales AC, Jones RN, Turnidge J, Rennie T, Ramphal R. Characterization of Pseudomonas aeruginosa isolates: occurrence rates, antimicrobial susceptibility patterns and molecular typing in the global SENTRY antimicrobial surveillance program 1997-1999. Clin Infect Dis. 2001; 32(S1):46-55.
11. Lipsitch M, Samore MH. Antimicrobial use and antimicrobial resistance: a population perspective. Emerg Infect Dis. 2002; 8:347-54.
12. Chambers HF. The changing epidemiology of Staphylococcus aureus? Emerg Infect Dis. 2001; 7:178 - 182.
13. Neu HC. The crisis in antibiotic resistance. Science. 1992; 257:1064-1073.
14. Charles W.H, Jeffrey M.G, Apichai S, Chittima P, and Peter E. Trends in Antibiotic Resistance Among Diarrheal Pathogens Isolated in Thailand Over 15 Years. Clinical Infectious Diseases 1998; 26:341-5.
15. Manges AR, Natarajan P, Solberg OD, Dietrich PS, Riley LW. The changing prevalence of drug-resistant Escherichia coli clonal groups in a community: evidence for community outbreaks of urinary tract infections. Epidemiol Infect. 2006; 134(2):425-31.
16. Dimitrov TS, Udo EE, Emara M, Awini F, Passadilla R. Etiology and antibiotic susceptibility patterns of community-acquired urinary tract infections in Kuwait hospital. Med PrincPract. 2003; 13:334-339.
17. Mudur G. Drug resistant cholera in India attributed to antibiotic misuse. BMJ. 2000; 321:1368-9.
18. Svetlansky I, Liskova A, Foltan V, Langsadl L, Krcmery V. Increased consumption of fluoroquinolones is not associated with resistance in Escherichia coli and Staphylococcus aureous in the community. J AntimicrobChemother. 200; 48:457-458.
19. Hillier SL, Magee JT, Howard AJ, Palmer SR. How strong is the evidence that antibiotic use is risk factor for antibiotic-resistant, community-acquired urinary tract infection. J AntimicrobChemother. 2002; 50:241-247.
20. Carmeli Y, Eliopoulos G, Mozaffari E, Samore M. Health and economic outcomes of vancomycin-resistant enterococci. Arch Intern Med. 2002; 162: 2223-28.
21. Carmeli Y, Troillet N, Karchmer AW, Samore MH. Health and economic outcomes of antibiotic resistance in *Pseudomonas aeruginosa*. Arch Intern Med.1999; 159: 1127-32.