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Original Article

Antimicrobial Resistance pattern of Common Bacterial Pathogens in Tertiary Care Hospitals in Dhaka City.

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Abstract

Bacterial pathogens were isolated from pus, wound swab, urine, blood and throat swab. A total of 300 samples were collected from Sir Salimullah Medical College & Mitford Hospital (SSMC & MH), BIRDEM and National Medical College (NMCH) and processed following standard microbiological methods. Antibiotic susceptibility testing were performed on pure culture isolates by employing Kirby-Bauer disc-diffusion method for the commonly used antibiotics. 326 (93.33%) bacterial pathogens were isolated from 300 patients. Single bacterial pathogen was present in 78% cases and mixed bacterial pathogens were in 15.40% cases. *Staphylococcus aureus* was the predominant species (38.66%) followed by *Escherichia coli* (38%), *Pseudomonas spp.* (13.33%), *Proteus spp.* (8.33%), CoNS (7.66%), *Serratia spp* (2.85%), *Klebsiella spp.* (2.00%) and *Acinetobacter spp.* (0.97%). Resistance rate towards amoxicillin, ciprofloxacin, co-trimoxazole and ceftriaxone were high among both Gram-positive and Gram-negative isolates. However, both groups showed good susceptibility to gentamicin and levofloxacin. S. aureus and CoNS showed 100% sensitivity to vancomycin and all isolated Gram negative organisms showed 98-100% sensitivity to imipenem.These results indicate that gentamicin and levofloxacin may be convenient alternative antimicrobial agent for both Gram-positive and Gram-negative bacteria and vancomycin for Gram positive and imipenem for Gram negative bacteria as well

Key words: Antimicrobial resistance

Introduction:

Bacterial infections continue to be important causes of morbidity and mortality. The growth of drug resistance among bacteria has made several antibiotics useless in treating certain infections for which they were formerly curative. There is a phenomenal increase in antibiotic resistant bacterial pathogens which is one of the major problems facing medicine and science today. There are many reasons for this alarming phenomenon and one of them is widespread and indiscriminate use of antibiotics which have been implicated in the development of serious problems of resistance to the older and less expensive antimicrobial agents. This resistance is associated with longer duration of hospital stay and greater hospital mortality [1]. A large number of bacterial pathogens have remarkable ability to develop resistance to a variety of drugs by genetic mutation, plasmid mediated transfer, enzymes production etc.Infections caused by resistant pathogens will make the therapeutic options for treatment rather difficult or virtually impossible [2]. The knowledge of prevailing susceptibility patterns is therefore vital to the selection and use of antibiotics and also for the development of prescribing policies [3]. This study was conducted to identify the common and clinically significant bacteria and to determine their antimicrobial susceptibility pattern.

Materials And Methods:

This study was carried out in the department of Microbiology, Sir Salimullah Medical College, from January to December 2011. Total 300 samples were collected from inpatient and outpatient department of SSMC & MH, BIRDEM and NMC&H from the patients with various clinical diagnoses such as abscess, urinary tract infections, wound sepsis, sore throat, septicaemia etc. Total 326 bacterial pathogens were isolated and identified by conventional methods.The antimicrobial susceptibility test was carried out by using Kirby-Bauer's disc diffusion method according to the Clinical and Laboratory Standards Institute (CLSI) guidelines 2007 [4].

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Susceptibility Testing:

Antimicrobial disc susceptibility tests of the isolates were performed according to the recommendations of National Committee for Clinical Laboratory Standards (NCCLS) [5] using the following antibiotic discs: amoxycillin (10ug), oxacillin (1ug), cloxacillin (30µg), cefoxitin (30µg), cephalexin (CL), ceftriaxone (30µg), ceftazidime, cefotaxime, levofloxacin (5µg) ciprofloxacin (5µg), gentamicin (10µg), tetracycline (30µg), erythromycin (15µg), cotrimoxazole (1.25µg), vancomycin (30µg), and imipenem (10µg). Control organisms used as standards were Staphylococcus aureus MR-108, Escherichia coli NCTC 10418 and local sensitive strains for other isolated bacteria were used.

Inoculum standardization

With a sterile wire loop 3-5 isolated colonies of similar appearance were transferred to screw-capped tube containing 4 ml of sterile normal saline, turbidity of which was then adjusted to 0.5McFarland turbidity standard which correspond to 1.5x 10^8 organisms per ml.

Inoculation of test plate and disc placement [4]

Mueller Hinton agar plates were dried in an incubator at 37°C for 30 minutes before use. Within 15 minutes after standardization of inoculum, a sterile cotton swab was immersed into the bacterial suspension. The excess broth was removed by retaining the swab with firm pressure against the inner side of the tube above fluid level. The swab was then streaked evenly on the surface of the plate in three different planes by rotating the plate approximately 60° each time to get a uniform distribution of inoculum. The inoculum was allowed to dry for 15 minutes at room temperature with lid closed. The antimicrobial discs were then placed on the inoculum surface by a sterile fine pointed forceps 10-15 mm away from the edge of the petridishes and having 24 mm gap between the discs. The plates were incubated at 37°C (35°C for oxacillin) for 24 hours.

Measurement of inhibition zone:

After incubation , each plate was examined, and the diameter of compete inhibition zone were measured with the help of a scale placed on the undersurface of the petri dish. The end point of the inhibition zone was that point where there was complete inhibition of growth observed by naked eye. Measurement of diameter in millimeter was made in two directions at right angle to each other through the center of each disc and the average of the two readings were taken [5]

Interpretation of zone

The zone of inhibition in growth produced by each antimicrobial agent on the test organisms were compared with that produced on the control organism. Depending on the diameter of the clear zone of inhibition around disc on the test organisms, they were categorized into sensitive (S) and resistant (R) to the representative antimicrobial agents [6].

Results:

Three hundred and twenty six bacterial pathogens were recovered and identified from 300 various clinicalspecimens. Infections with one or multiple organisms were present in about 280 (93%) cases. Single organism was isolated from 234 (78%) cases and remaining 46 (15.33%) cases yielded multiple organisms. These included 116 pus (38.66%), 103 wound swabs (34.33%), 36 urine (12.00%), 35 blood (11.66%) and 10 throat swab (3.35%).

Gram-negative bacilli accounted for 187 (57.36%) of the isolates while 139(42.64%) were Gram-positive cocci. *Staphylococcus aureus* was the predominant species (38.66%) followed by *Escherichia coli* (38%), *Pseudomonas spp.* (13.33%), *Proteus spp.* (8.33%), CoNS (7.66%), *Serratia spp* (2.85%), *Klebsiella spp.* (2.00%) and *Acinetobacter spp.* (0.97%). In multiple infection the most frequent combination of organism are *Esch. Coli* and *Pseudomonas spp.*

In vitro activities of different antibiotics against the bacterial isolates are illuminated in Tables 2 and 3.Resistance rates were higher among both Gram-positive cocci and Gram-negative bacilli. *E. coli* was highly resistant to amoxicillin (71.92%) followed by co-trimoxazole (68.42%), gentamicin, tetracycline, ciprofloxacin (57% in each) and ceftriaxone (49%). It showed slightly lower resistance to levofloxacin (38%). However, it was highly sensitive to imipenem (98%). *Pseudomonas. Proteus* and *Kebsiella spp* were highly resistant to amoxicillin (83-92%) followed by co-trimoxazole and tetracycline. *Pseudomonas spp.* were moderately resistant to ceftazidime (40%) and cefotaxime (52%). *Proteus* showed higher rate of resistance to gentamicin (60%) and ciprofloxacin (52%). *Pseudomonas spp.* were 97.50% sensitive to imipenem whereas *Proteus* and *Klebsiella* showed 100% sensitivity to it.

In this study, higher level of resistance to commonly used antibiotics was showed by *S. aureus*. It was highly resistant to amoxicillin (96%), ciprofloxacin (87%), co-trimoxazole (87%) and erythromycin (81%). Moderate resistance was showed in case of levofloxacin (56%), ceftriaxone, and gentamycin (51% in each). Lower resistance was observed in oxacillin (28.45%) and cefoxitin (25. 86%). CoNS were highly resistant to amoxycillin (86.96%) and co-trimoxazole and erythromycin (78.27% in each). Both *S. aureus* and CoNS showed 100% sensitivity to vancomycin.

Table-I: Frequency of isolated organisms from different samples

	Frequency of isolated organisms (%)							
Samples	S. aureus	E. coli	CoNS	Pseudomonas	Proteus	Klebsiella	Acinetobacter	Serratia
Pus (116)	55 (47.41)	40 (34.48)	07 (6.03)	02 (1.72)	02 (1.72)	00	00	00
Wound swab (103)	41 (39.80)	61 (59.22)	16 (15.53)	26 (25.24)	13 (12.62)	02 (1.94)	01 (0.97)	00
Blood (35)	11 (31.42)	00	00	03 (8.57)	00	00	00	01 (2.85)
Urine (36)	08 (22.22)	13 (36.11)	00	09 (25.00)	10 (27.77)	04 (11.11)	00	00
Throat swab (10)	01 (10.00)	00	00	00	00	00	00	00
Total (300)	116 (38.66)	114 (38.00)	23 (7.66)	40 (13.33)	25 (8.33)	06 (2.00)	01 (0.97)	01 (2.85)

Figure within parentheses indicate percentage

Table-II: Antimicrobial resistance pattern of isolated Gram negative organisms

	Number of Resistant Organism (%)			
Antimicrobial agent	E. coli N=114	Pseudomonas N=40	Proteus N=25	Klebsiella N=6
AML	82 (71.92)	37 (92.50)	23 (92.00)	05 (83.34)
CN	49 (43.00)	16 (40.00)	15 (60.00)	02 (33.34)
SXT	78 (68.42)	21 (52.50)	22 (88.00)	04 (66.66)
CRO	56 (49.13)	17 (42.50)	12 (48.00)	01 (16.66)
CAZ	44 (38.60)	16 (40.00)	07(28.00)	02 (33.34)
CTX	36 (31.00)	21 (52.50)	07 (28.00)	00(00)
CL	61 (53.50)	19 (47.50)	14 (56.00)	02 (33.34)
T	65 (57.01)	24 (60.00)	19 (76.00)	04 (66.66)
CIP	65 (57.00)	14 (35.00)	13 (52.00)	03 (50.00)
LE	44 (38.60)	16 (40.00)	12 (48.00)	02 (33.34)
IPM	02(1.76)	01 (2.50)	00 (00)	00 (00)

Figure within parentheses indicate percentage

Table-III: Antimicrobial resistance pattern of isolated Gram positive organisms.

	Number of Resistant Organism (%)	
Antimicrobial agent	S. aureus N=116	CoNS N=23
AML	112 (96.53)	20 (86.96)
OX	33 (28.45)	05 (21.73)
OB	82 (27.58)	16 (69.56)
FOX	30 (25.86)	04 (17.39)
CRO	56 (48.27)	07 (30.43)
CL	86 (74.14)	10 (43.48)
SXT	102 (87.94)	18 (78.27)
CN	56 (48.27)	05 (21.73)
T	90 (77.58)	07 (30.43)
E	95 (81.90)	18 (78.27)
CIP	101 (87.07)	10 (43.48)
LE	66 (56.90)	10 (43.48)
VAN	00 (00)	00 (00)

Figure within parentheses indicate percentage

FOX= cefoxitin OX= oxacillin
AML = Amoxycillin T = Tetracycline CN= Gentamicin
CIP = Ciprofloxacin LE = Levofloxacin SXT= Co-trimoxazole
CRO= Ceftriaxone CL= Cefalexin IPM= Imipenem
CAZ = Ceftazidime CTX = Cefotaxime

Discussion:

S. aureus was the most frequently isolated organism (38.66%) in the present study. Among them most of the *S. aureus* were isolated from pus. This result is almost similar to the findings of Sittu *et al* (2003) [7] and Thanni *et al* from Nigeria (2003) [8] reporting staphylococcal infection rate of 35% and 37% respectively. *E. coli* was the second most common organism isolated in this study (38%) followed by *Pseudomonas* (13.33%) and *Proteus spp.* (8.33%).

In this study, the antibiograms of organisms showed varying susceptibility pattern. All the isolated Gram negative organisms such as *E. coli, Pseudomonas, Proteus* and *Klebsiella* are highly sensitive to imipenem ranged from 97.50% to 100%. *E. coli, Pseudomonas* and *Proteus* are moderately sensitive to levofloxacin, ceftriaxone and gentamycin. Sensitivity was lower for co-trimoxazole, ciprofloxacin and tetracyclin while it was least sensitive to ampicillin. Almost similar susceptibility pattern of these organisms were reported by Saini *et al* in 2004 [9]. *Acinetobacter* and *Serratia* were 100% sensitive to imipenem, levofloxacin, tetracyclin, ceftriaxone and co-trimoxazole.

Among the Gram positive organisms, *S. aureus* showed the highest sensitivity to vancomycin (100%) followed by oxacilin, cefoxitin and ceftriaxone. They are moderately sensitive to gentamycin (51.73%) followed by levofloxacin (43.10%). Sensitivity was lower for ciprofloxacin, co-trimoxazole, tetracycline and erythromycin which was below 20-40%. It was least sensitive to amoxicillin (3.47%). This result is in agreement with Dashgupta (2003) [10] and Rahman *et al* (1997) [11] from Bangladesh and Saini *et al*(2003) [9] from India. CoNS were susceptible to oxacillin, gentamycin, ceftriaxone, tetracycline and ciprofloxacin. However it was lower for amoxicillin, co-trimoxazole and erythromycin. All CoNS showed 100% sensitivity to vancomycin.

Conclusion:

In this study, all the organisms showed higher level of resistance to different drugs. It has demonstrated that, the call for judicious use of antibiotics cannot be over emphasized. This study, the resistance of *S. aureus, E. coli, Pseudomonas, Proteus,* and *Klebsiella* to different drugs, was further estimated that,a situation that favours their continuous existence in hospital environment. This high rate of resistance is probably connected with widespread, indiscriminate and inappropriate use of antibiotics, which have been implicated in the development of serious problems to the older and less expensive antimicrobial agents. Therefore it is necessary to ensure availability of guideline regarding the selection of antibiotics, recycling of antibiotics and strengthening the detection of resistant strains. Furthermore, continuous surveillance of changes in resistance pattern of these bacteria to antibiotics is of important.

Reference:

1. Smyder, JW, IC McDonald and R Van Brik, Common bacteria whose susceptibility toantimicrobials is no longer predictable. J. Med. Liban, 2000. 48(4): 208-214.
2. Collignon, P,. Antibiotic resistance. MedicalJ. Australia, 2002.177(6): 325-329.
3. El-Astal, Z, Bacterial pathogens and theirantimicrobial susceptibility in Gaza strip, Palestine. Pakistan J. Med., 2004.20(4): 365-370.
4. CLSI. Performance standards for antimicrobial testing; 17th informational supplement.2007.CLSIM100-S17.CLSI Wayne, PA.
5. Washington JA. Susceptibility test, agar dilution method. N: Lannet EH, Dalus A, Sauser WJ, editors. Mannual of clinical microbiology, 4th edition, Washington: American society for microbiology, 1985; 967-997.
6. Villanova PA. Performance standards for antimicrobial disc susceptibility tests. In: National Committee for Clinical Laboratory Standards. Approved standard.7thedn. National Committee for Clinical Laboratory Standards: 2000. P. M2-A7.
7. Sittu AO, Kolawale DO and Oyedepo EA. A study of wound infection in two health institution in Ile-Ife, Nigeria. Afr Biomed Res; 2003. 5: 97-102.
8. Thanni LO, Osinupebi OA and Deji-Agboola M. Prevalence of bacterial pathogens in infectected wounds in a tertiary hospital, 1995-2001: any change in trend? J Natl Med Assoc; 2003.95(12): 1189-1195.
9. Saini S, Gupta N, Lokvear A and Griwarn MS. Surgical infection: A microbiological study. Braz J Infect Dis; 2003.8(2): 118-125.
10. Dashgupta S. Pattern of hospital wound infections and comparative study of antimicrobial sensitivity and current trend of antibiotic used. Dissertation, BCPS, 2003.
11. Rahman MM, Gomes DJ, Chowdhury N, Hasan Z, Rahman SR, and Haq F.. Drug sensitivity pattern of aerobic bacteria isolated from infected wounds of 40 diabetic and 60 non-diabetic patients. J Bang Med Col. 1997; 2(1):20-25.