Bacteriological Spectrum of Different Infections and their Antibiogram at NICVD, Dhaka

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Key Words: *Antibiogram, antibiotic sensitivity pattern, aerobic bacteria.*

Abstract

Background: *One of the major causes of death in the current era is the infectious diseases. Aerobic bacteria are one of the most commonly isolated organisms from hospitalised patients.* ***Objectives:*** *The aim of the present study was to observe the infections caused by aerobic bacteria and their antibiotics susceptibility pattern.*

Methods: *This retrospective study was carried out in the National Institute of Cardiovascular Diseases (NICVD) from January 2012 to December 2012 for a period of one year. Patients who were admitted in medical wards and medical ICU suffering from different infections were undertaken for this study. Proper thoroughly clinical examination, routine and specific investigations were done in each case. Microbiological samplings were tried on day 1, after completion of antibiotic therapy or in between as required. Aerobic bacterial culture and sensitivity tests were done according to clinical laboratory standard institute (CLSI) standard.*

Result: *A total of 660 samples were studied of which male (70.0%) were predominant than female (30.0%). The highest number of patients was in the age group of 30-60 years (54.0%) followed by 15-30 years (21.5%) and less than 15 years (13.0%). The mean age with standard deviation was 38.61±19.236 years. The most common isolated bacteria was* Escherichia coli *(40.1%) followed by* Pseudomonas *species (30.4%), coagulase negative* Staphylococcus *(19.0%) and coagulase positive* Staphylococcus *(5.9%); however, beta-haemolytic* Streptococcus *(4.2%) was detected. Urine culture has yielded* Pseudomonas *species (13.3%),* E. coli *(71.1%) and CNS (15.0%). From pus* Pseudomonas *species (37.3%) was isolated mostly which was 62 cases followed by* E. coli *(31.3%), CNS (19.3%) and CPS (7.2%).* Pseudomonas *species was resistant to penicillin, amoxycillin and vancomycin and ~50% resistant to cotrimoxazole, cefuroxim, ceftriaxone, piperacillin, azythromycin, cephalexin, netelmycin and pfloxacillin.*

Conclusion: *In the conclusion, majority bacteria are resistant to commonly used antibiotics.*

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Introduction:

Aerobic bacteria isolated from different clinical specimens have developed resistance against the major classes of antibiotics.[1] Many of these resistance mechanisms are widespread among common pathogens and cause considerable concern in several clinical situations in which treatment options have become very limited. Cardiovascular intervention is very critical to handle. A great cardiovascular surgery by an expert surgeon can go in vein due to the infection and will go more serious if that infection is caused by resistant one. It is a great concern that extended-spectrum â-lactamase-producing *Escherichia coli* and *Klebsiella pneumoniae* are increasingly reported[2-4] and have been associated with high mortality in adults.[5] These ESBL are frequently resistant to broad-spectrum cephalosporins.[1] Similarly, the incidence of methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant *Enterococcus faecium* has increased noticeably.[6] Even among new antibiotic classes like oxazolidinones and the ketolides, there are reports of resistant bacteria.[1]

The emerging resistance to the antibiotic creates problem during the treatment of infections among the cardiovascular disease patients.[7] Thus, there is always a demand of surveillance to monitor current resistance pattern of the commonly used antibiotics. The purpose of the present study was to see the current levels of antibiotic resistance to predominant pathogens encountered in different infections at a tertiary care hospital.

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Methodology:

This was a retrospective study conducted in the Department of Microbiology at National Institute of Cardiovascular Disease (NICVD), Dhaka to find out the aerobic bacterial agents causing different infections as well as their antibiotic sensitivity pattern. NICVD is the referral tertiary care hospital for cardiovascular diseases in Bangladesh. All patients at any age with both sexes presented with infections admitted at NICVD from July 2012 to June 2013 for a period of 01(One) year were enrolled for this study. Specimens were collected according to the type of infections. The most common specimen were blood, urine, pus, pericardial fluid, swab from wound, conjunctiva and throat. All these samples were received for microbiological examination. The specimens were inoculated into blood agar media, chocholate agar media, MacConkey's agar media and nutrient agar media. Bacterial species were identified by standard procedures.[8,9] Antibiotic sensitivity test was performed by disc diffusion method (Kirby-Bauer's technique) using commercially available antibiotic discs (HiMedia, India) according to appropriate antibiotics panel to a specific bacterial strain. The antibiotic discs were applied manually into the surface of the inoculated agar plate and were incubated into an aerobic incubator (Memmert Company Ltd., Germany) for 18 h at 37°C. The zones of inhibition for each antibiotic were measured in millimeters by measuring scale and were compared with values provided by the Clinical and Laboratory Standards Institute.[10] The commonly used antibiotic disks were amoxicillin, amikacin (30 mcg), ciprofloxacin (5 mcg), cotrimoxazole (1.25/23.75 mcg), ceftriaxone (30 mcg), cephalexin (30 mcg), ceftazidime (10 mcg), gentamycin (10 mcg), tobramycin (10 mcg), imipenem, netilmycin, vamcomycin (5 mcg). Statistical analysis was performed by SPSS 19.0 (Statistical Package for Social Science, Chicago, USA).

Results:

A total of 660 cases were studied of which male were predominant than female which were 462(70.0%) cases and 198(30.0%) cases respectively. The highest number of patients was in the age group of 30-60 years which was 357(54.0%) cases followed by 15-30 years and less than 15 years which were 142(21.5%) cases and 86(13.0%) cases respectively. The mean age with standard deviation was 38.61±19.236 years (range 1-90 years) (Table I).

Table-I

Distribution of Study Population According to Age and Sex (n=660)

Age Group	Sex		Total
	Male	Female	
<15	65(14.1%)	21(10.6%)	86(13.0%)
15-30	88(19.0%)	54(27.3%)	142(21.5%)
30-45	129(27.9%)	66(33.3%)	195(29.5%)
45-60	120(26.0%)	42(21.2%)	162(24.5%)
>60	60(13.0%)	15(7.6%)	75(11.4%)
Total	462(100.0%)	198(100.0%)	660(100.0%)

* Pearson Chi-Square has been done to see the association; p value= 0.018;
Mean age ± SD= 38.61±19.236 (Range: 1 to 90 years)

Among 660 clinical specimens the growth of bacteria was in 289(43.8%) cases and the rest 371(56.2%) specimens were yielded no growth (Table II).

Table-II

Rate of Bacterial Growth from Different Specimens (n=660)

Culture	Frequency	Percentage
Positive	289	43.8
Negative	371	56.2
Total	660	100.0

The most common isolated bacteria was *Escherichia coli* which was 116(40.1%) followed by *Pseudomonas* species, coagulase negative *Staphylococcus* and coagulase positive *Staphylococcus* which were 88(30.4%), 55(19.0%) and 17(5.9%) respectively; however, beta-haemolytic *Streptococcus* was detected in 12(4.2%) cases (Table III).

Table-III

Distribution of Different Isolated Bacteria from Positive Culture (n=289)

Isolated Bacteria	Frequency	Percentage
Escherichia coli	116	40.1
Pseudomonas species	88	30.4
CNS	55	19.0
CPS	17	5.9
Streptococcus pyogenes	12	4.2
Proteus species	01	0.4
Total	289	100.0

*Coagulase positive *Staphylococcus*= CPS
* Coagulase Negative *Staphylococcus*= CNS

Most common specimen was urine 276(41.8%) which had been given only 60(21.7%) positive growth. Pus, wound swab, blood and throat swab which were 229(34.7%) cases, 73(11.1%) cases, 36(5.5%) and 21(3.2%) cases respectively were yielded 166(72.5%), 43(58.9%), 3(8.3%) and 10(47.6%) cases positive growth. Culture of urine has yielded *Pseudomonas* species, *E. coli* and CNS which were 8(13.3%), 43(71.1%) and 9(15.0%) respectively. From pus *Pseudomonas* species was isolated mostly which was 62(37.3%) cases followed by *E. coli,* CNS and CPS which were 52(31.3%), 32(19.3%) and 12(7.2%) respectively. One *E. coli* and two *Pseudomonas* species were isolated from blood. In wound swab the most common isolated bacteria was *Pseudomonas* species, *E. coli* and CNS which were 12(27.9%), 11(25.6%) and 11(25.6%) (Table III).

Pseudomonas species was mostly resistant to penicillin, amoxycillin and vancomycin and ~50% resistant to cotrimoxazole, cefuroxim, ceftriaxone, piperacillin, azythromycin, cephalexin, netelmycin and pfloxacillin. It was found that *Pseudomonas* species was still more than 90.0% sensitive only to imipenem. *Escherichia coli* was more than 80.0% sensitive to only imipenem and amikacin. Coagulase negative *Staphylococcus* (CNS) was sensitive in imipenem, novobiocin and netelmycin. Coagulase positive *Staphylococcus* (CPS) was sensitive to only imipenem and cephalexin (Table V).

Table-IV

Distribution of most frequently received specimens according to Positive Bacterial Growth (n=289)

Isolated Bacteria	Pus	Urine	Blood	Wound Swab	Throat Swab	Others
Pseudomonas species	62(37.3%)	8(13.3%)	1(33.3%)	12(27.9%)	3(30.0%)	2(28.6%)
E. coli	52(31.3%)	43(71.1%)	2(66.7%)	11(25.6%)	3(30.0%)	5(71.4%)
CNS	32(19.3%)	9(15.0%)	0(.0%)	11(25.6%)	3(30.0%)	0(0.0%)
CPS	12(7.2%)	0(0.0%)	0(.0%)	5(11.6%)	0(0.0%)	0(0.0%)
Streptococcus pyogenes	7(4.2%)	0(0.0%)	0(.0%)	4(9.3%)	1(10.0%)	0(0.0%)
Proteus species	1(0.6%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)
Total	166(100.0%)	60(100.0%)	3(100.0%)	43(100.0%)	10(100.0%)	7(100.0%)

* Pearson Chi-Square has been done to see the association which is corrected by Fisher's Exact Test; p value (95% CI) = 0.026 (0.024-0.027); others= Pericardial fluid, endotracheal tube, sputum, stool, serous fluid; Coagulase positive *Staphylococcus*= CPS; Coagulase Negative *Staphylococcus*= CNS; *E. coli=Escherichia coli*

Table-V(a)

Distribution of isolated bacteria with Resistant Pattern

Bacteria name	Tobra	Genta	Ceftazi	Cotrimo	Cipro	Imipe	Cefuro	Ceftri	Amika
Pseudomonas	2.4	28.2	35.3	46.2	36.5	7.1	55.3	50.6	15.3
E coli	-	48.5	68.8	50.0	49.1	6.1	34.2	54.4	15.3
CNS	-	21.8	40.0	1.8	56.4	3.6	30.9	30.9	12.7
CPS	-	20.0	50.0	00	52.9	5.9	41.2	58.8	17.6
Strep pyogenes	-	41.7	66.7	-	41.7	-	16.7	58.3	25.0
Proteus species	-	100.0	100.0	-	-	-	-	-	-

*Tobra= tobramycin, genta= gentamycin, ceftazi= ceftazidime, cipro= ciprofloxacin, imipe= imipenem, cefuro= cefuroxim, ceftri= ceftriaxone, amika= Amikacin**,** *Staph aureus= Staphylococcus aureus, Staph saprop= Staph saprophyticus, E. coli= Escherichia coli*

Table-V(b)
Distribution of isolated bacteria with sensitivity pattern

Bacteria name	Piper	Penicil	Azyth	Amoxy	Cephale	Clarith	Vancom	Netel	Pfloxaci
Pseudomonas	55.3	8.2	30.6	29.4	41.2	-	7.1	9.4	47.1
E coli	47.9	13.2	32.5	26.3	45.6	-	1.8	7.0	50.0
CNS	58.2	10.9	52.7	14.5	20.0	3.6	12.7	5.5	58.2
CPS	64.7	-	29.2	17.6	52.9	-	11.8	11.8	58.8
Strep pyogenes	58.3	8.3	58.3	-	16.7	-	8.3	16.7	33.3
Proteus species	100.0	-	-	100.0	-	-	-	-	100.0

*Piper= Piperacillin, penicil= penicillin, azyth= azithromycin, cephale= cephalexin, clarith= clarithromycin, vancom= Vancomycin, novobio= novobiocin, netel= netelmycin, pfloxaci= pfloxacin, *Staph aureus= Staphylococcus aureus, Staph saprop= Staph saprophyticus, E. coli= Escherichia coli*

Discussion:

The antibiotic sensitivity pattern of organisms is changing very rapidly over a short period.[11] It is particularly true for developing countries like Bangladesh where antibiotics are prescribed irrationally not only by the medical practitioners but the antibiotics are also purchased directly from the chemists like medicine shop keepers without prescription.[7] Palikhe reported similar reason.[12] It has been advised that clinicians should be aware of the rising resistance of bacteria to commonly prescribed antibiotics as well as the profile of antibiotic resistance.[13] Therefore, for rational and appropriate use of antibiotics periodic evaluation of sensitivity pattern is essential.[14,15]

Male (70.0%) was predominant than female (30.0%) in this study. The specimen was collected from the cardiac referral tertiary care hospital in Bangladesh and regarding this cardiovascular diseases majority of the patients was male. That's why the findings of this study are correlated with this scenario. It is seen that the highest number of patients were in the age group of 30-60 years (54.1%) followed by 10-30 years (21.5%) and more than 60 years (11.4%). The mean age with standard deviation was 38.61±19.236 years (range 1-90 years). The overall growth positive rate (37.2%) in this study was in agreement with previous studies in Nepal.[11,12] *Escherichia coli* (40.1%) was the most common isolated bacteria followed by *Pseudomonas* species (30.4%), CNS (19.0%) and CPS (5.9%). It is also found that *Pseudomonas* species (37.3%), CNS (13.0%) and CPS (4.3%) were both found most commonly in pus. These findings agree with those reported by Anguzu and Olila on different infections where the most common wound contaminant was *Pseudomonas* species and *Staphylococcus aureus*.[16] The findings also agree with those of Buwembo who identified *Staphylococcus aureus* as the commonest causative agent of potentially contaminated wounds.[17] Nasal carriage of *S. aureus* is an important risk factor for infection of surgical site as the organism is a normal flora in the nostrils.[7] Again, it is found that with the disruption of natural skin barrier *Staphylococcus aureus*, which is a common bacterium on surfaces of the body, can easily find the way into breach of the skin surfaces. The high prevalence of *Pseudomonas* infection may be because it is an exogenous source of infection and it is assumed that infection with this organism may also be due to contamination from the hospital environment.[12]

It is found that 56.2% specimen had no bacterial growth. This could be due to prior use of antibiotic, or may be the normal healing process where the bacteria have been overpowered by body's defence mechanism, antimicrobial activity in patients circulation since all of them had been on antibiotic therapy post operatively at time of collecting the samples or adequate nursing care like use of antiseptics for cleaning the wounds.[12] It is also possible that some organisms could have been anaerobic bacteria that were missed as cultures were incubated aerobically. This condition could not therefore support growth of such organisms[12]. In this study the most

common specimen was urine (41.8%) followed by pus (34.7%), blood (5.5%) and wound swab (11.7%). From all pus specimens 72.5% have shown positive growth. Urine samples have shown 21.7% positive growth. Interestingly majority (91.7%) blood samples were shown negative growth. Reason of this may be due to prior intake of antibiotic leading to suppression of bacteria.

From pus specimen *Pseudomonas* species (37.3%) was most commonly found and next to this is the *E. coli* (31.3%). However, CNS and CPS were detected from pus in 19.3% and 7.2% respectively. *Streptococcus pyogenes* was isolated in 4.2% cases. Rahman et al has reported similar finding.[18] Almost similar findings was reported by Shrestha et al in Nepal and observed that bacterial growth in 35.7% of the pus samples collected from all age groups.[19] On the contrary, this was much higher than this present findings among adults as have been reported by Rajbhandari et al,[20] Rai et al,[11] Shrestha et al[19] and Chhetri et al.[21] This discrepancy could be because of the age of the subjects included in those studies. Another reason may be due to the sample size of the present study.

In this present study urine was yielded *E. coli* (71.1%) as most common bacteria followed by CNS (15.0%) and *Pseudomonas* species (13.3%). Similar result was reported by Rahman et al[18] and Rai et al.[11] *Pseudomonas* species was maximum resistant to penicillin, amoxycillin and vancomycin; however, resistant to cotrimoxazole, cefuroxim, ceftriaxone, piperacillin, azythromycin, cephalexin, netelmycin and pfloxacillin was also significant. However *Pseudomonas* species are still sensitive only to imipenem. The resistance observed in *Pseudomonas* species could also be attributed to irrational use of antibiotics for conditions that may not clinically indicate their use, over-the-counter sell of antibiotics in pharmacies without prescription by authorised practitioners, some new drug formulations which may be of poor quality and dumping of banned products into the market where the public may get access to them.[12] In view of the resistance observed, infections caused by MRSA can be expensive in terms of costs of treatment, morbidity and prolonged hospitalisation.[18]

Escherichia coli was sensitive to only imipenem and Amikacin. Similar result was reported by Rahman et al[18] that most of the antibiotics were resistant to *E coli* and mentioned that only ceftriaxone and ceftazidime were maximum sensitive. *Staphylococcus saprophyticus* was sensitive in imipenem, novobiocin and netelmycin. *Staphylococcus aureus* was sensitive to only imipenem and cephalexin. Similar sensitivity pattern was reported by Sharma, Kumari et al and Bhandari.[22-24] The resistance shown to amoxycillin and ampicillin may be due to the antibiotics having been in use for much longer time and their oral route of administration that affects their rate of absorption into blood stream.[12] Some of them were used as prophylaxis therefore increasing their use in patients. Over-use of antibiotics contributes to organisms developing resistance.[25] In another study in Bangladesh Shamsuzzaman et al has reported that there is a trend of antibiotic resistant among the *Pseudomonas* species, *E coli* and *Staphylococcus aureus* isolated from different samples and has shown that the resistant pattern gradually increases among the isolated bacteria from different clinical specimens.[26] This clearly indicates that antibiotic resistant is alarming to the community of this country. It is now an urgent need to develop antibiotic policy as soon as possible to save the future generation from these bugs.

Conclusion:

In the conclusion, majority bacteria are resistant to commonly used antibiotics. Since a high proportion of samples have positive cultures, infection control is recommended as a strategy to minimise spread of resistant organisms. Future studies should be extended to include cultures under anaerobic conditions to establish presence of other organisms that require such environment for growth. It is recommended that judicial antibiotic use should be carried out.

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