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Research article

# Study of resistant pattern and comparative analysis of different generation of antibiotics against various pathogens

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**Abstract**

The research was done on resistant pattern of pathogens in various pathological samples against 1st to 4th generation of antibiotics. Female were more prone to infectious than male. Gram negative bacteria were more infectious than gram positive bacteria. The 1st to 4th generation of antibiotics group were used like- 1st to 4th generation of cephalosporins, amionoglycosides, monobactams, macrolides, carbepenems, quinolones, oxazolidinones, penicillin, 2nd and 3rd generation of penicillin combination. On this research 1st generation cephalosporin, 2nd generation of penicillin combination, 3rd generation of penicillin combination, 4th generation of cephalosporins were mostly resistant and these all drugs are taken by orally.

## Introduction

Antibiotic resistance is serious threat in worldwide. A world without effective antibiotics is terrifying. The increasing prevalence of antibiotic resistance is a cause of serious concern and requires an international approach to its management. The world Health Organization (WHO) and the European Commission (EC) have recognized the importance of studying the emergence and determinants of resistance and the need for strategies for its control [1]. Antibiotic resistance occurs when an antibiotic has lost its ability to effectively control or kill bacterial growth; in other words, the bacteria are "resistant" and continue to multiply in the presence of therapeutic levels of an antibiotic. With the discovery of antimicrobials in the 1940s, scientists prophesied the defeat of infectious diseases that had plagued humankind throughout history. However, the remarkable healing power of antibiotics invites widespread and often inappropriate use. This misuse and overuse of antibiotics leads to antibiotic resistance among bacteria and consequent treatment complications and increased healthcare costs [2]. In developing countries, acquired bacterial resistance to antimicrobial agents is common in isolates from healthy persons and from persons with community-acquired infections. Complex socioeconomic and behavioral factors associated with antibiotic resistance, particularly regarding diarrheal and respiratory pathogens, in developing tropical countries, include misuse of antibiotics by health professionals, unskilled practitioners, and laypersons; poor drug quality; unhygienic conditions accounting for spread of resistant bacteria; and inadequate surveillance [3]. In Bangladesh antibiotic resistance is also threat and the same factors like other developing countries are responsible for antibiotic resistance. Rational use of antibiotics can improve this situation. Pathogenic bacteria are bacteria that cause bacterial infection. Bacteria have been the cause of some of the most deadly diseases and widespread epidemics of human civilization. Smallpox and malaria, diseases caused by other microbes, have killed more humans than bacterial diseases, but diseases such as tuberculosis, typhus, plague, diphtheria, typhoid, cholera, dysentery and pneumonia have taken a large toll of humanity. Water purification, immunization (vaccination) and antibiotic treatment have reduced the morbidity and the mortality of bacterial disease in the Twenty-first Century, at least in the developed world where these are acceptable cultural practices [4]. The present research was undertaken to follow the resistant pattern of the various pathogens in some 1st to 4th generation of antibiotics group and compare to the male or female those had more prone to bacterial infection

## Experimental

### Methodology

This was a cross sectional research and the research was conducted in July 2015 to September 2015 in a local diagnosis laboratory at microbiology department in Dhaka, Bangladesh. Sample size were 204 (were, p=0.15, q= 0.85, z= 1.96 and d= 0.5, $n = \frac{z^2pq}{d^2}$ = 195; where p = Prevalence value, q= Prevalence value – 1, d= Margin of

error and z =Z-value; and to adjust the anticipated non response rate of 5%. Finally our calculated sample size found 204). The research instrument age were 01 to 85, sample were Urine, Blood, Sputum, Pus and HVS, media were MacConkey agar, Blood agar, Cystine Lactose Electrolyte Deficient (CLED) agar, Xylose Lysine De-oxycholate (XLD) agar, Muller Hinton agar and different types of antibiotic. All samples were collected from infected patient at diagnosis and comparison their sensitivity pattern and comparison the antibiotics with their generation. All generation are compare with female and male positive sample. Standard microbiological and biochemical tests were performed in order to identify the isolated microbes. Different antibiotics group were used such as- aminoglycosides, 1st generation ceplalosporins, monobactams, penicillin combination, macrolides, carbepenems, 2nd generation cephalosporins, quinolons, oxazolidinones, penicillines, penicillin combination, 3rd generation cephalosporins and one 4th generation antibiotics were cephalosporins.

A sterile needle and sterile Petri dish with media were used for inoculation after 24 hours identified the bacteria the antimicrobial agent was added to another plate, it was done placing disk. After inoculation of the agar plate, Petri dishes were kept into incubator because it provides environmental condition ($35^0C \pm 2^0C$) for the growth of microorganism. Incubations of plates in $CO_2$ are not good for growth of microbes because it can decrease the pH of the agar. After overnight incubation (18-24hr) plates was kept outside of incubator and result was observed. The agar plates which were kept outside the incubator the diameter of zone of inhibition was measured by using slide calipers' in millimeter. Usually measurement is done by naked eye and it is done by holding the plate a few inches above a black, nonreflecting surface illuminated with reflected light. Result has to record in result sheet, if the diameter of the zone cannot be read, then measurement from the center of the disk to a point on the circumference of the zone where a distinct edge is present(the radius) has to done and multiplied the measurement by 2 to determine the diameter. Growth on the edges of the disk is considered as 0 mm.

Disk diffusion method was used to determine the susceptibility or sensitivity of various microorganisms against antimicrobial agents. Here pathogenic microorganisms were grown in laboratory settings and disk impregnated with antimicrobial agent has to apply on the culture plate and growth of micro organism around the disk has to observe. Disks should not be placed closer than 24 mm (center to center) on the agar plate. Ordinarily, no more than 12 disks should be placed on a 150 mm plate or more than 5 disks on a 100 mm plate. Disks should be avoided placing it close to the edge of the plate as the zones will not be fully round and can be difficult to measure. Each disk must be pressed down with forceps to ensure complete contact with the agar surface or irregular zone shapes may occur. If the surface of the agar is disrupted in any way (a disk penetrating the surface, visible lines present due to excessive pressure of the spreader against the plate during inoculation, etc.) the shape of the zone may be affected. After overnight incubation plate was taken out from incubator and measured the zone of inhibition with scale and vainer scale [5].

## Results and Discussion

This research carried out only on the clinical isolates to observe their sensitivity pattern on the clinically isolated used some 1st to 4th generation of antibiotics; those are mostly used in the regular life and compare the sample with male and female.

### Compare infected female and male

In the research maximum female had 24% bacterial infection out of 204 samples and maximum 21-40 years male had 12% bacterial infection out of 204 samples. 41-60 years of female were less infected 4% out of 204 samples same 41-60 years of male were less infected 45 out of 204 samples. The female mean values were 1.354 and male mean values were 2.447. So, 1 to 20 years female had prone to infection than other age of female and male (Table 1). Out of 204 samples, female were 40% infected in urinary tract infection and male had only 11% urinary tract infection, but out of 204 samples, only 13% male had infection in blood and 7% female had blood infection. And the samples pus, sputum, HVS or PRS was showed less infection both male female patients out of 204 samples (Table 2). Out of 204 samples, the 41% female patient were infected by Gram negative (Gv-) bacterial and 21% female patient were infected by Gram positive (Gv+) bacteria and 28% male were infected by Gv+ and only 16% male were infected by Gv- bacteria (Table 3).

**Table 1. Frequency of infection in different age group of female and male patients**

| AGE | Female frequency (%) | Mean | Male frequency (%) | Mean |
|---|---|---|---|---|
| 1-20 | 24 | | 8 | |
| 21-40 | 13 | 1.354 | 12 | 2.447 |
| 41-60 | 4 | | 6 | |
| 61-80 | 17 | | 9 | |

**Table 2. Frequency of infection in different samples of female and male patients**

| Sample | Female (%) | Male (%) |
|---|---|---|
| Urine | 40 | 11 |
| Sputum | 7 | 8 |
| Blood | 9 | 13 |
| Pus | 3 | 1.90 |
| HVS/PRS | 2 | 1 |

**Table 3. Percentage of microbes in female and male patients**

| Microbs | Female (%) | Male (%) |
|---|---|---|
| Gv- | 41` | 16 |
| Gv+ | 21 | 28 |

### First generation of antibiotics resistant pattern

In here, three antibiotics groups were used to follow their resistant pattern. The aminoglycosides, 1st generation cephosporins, monobactum were used. Cephalosporins showed mostly resistant 65% whereas aminoglycosides less and monobactum showed 53% and 63% less resistant in the Gv- bacteria. But aminoglycoside showed mostly resistant 47% whereas cephalosporins and monobactum showed less resistant in the Gv+ bacteria. So, cephalosporins have Cephalexin (CL), Co-thrimoxazole (SXT) were mostly resistant antibiotic group for Gv- bacteria. The average value of the resistant pattern of Gv- bacteria were 63% and Gv+ bacteria were 37% (Table 4).

**Table 4. The average value of the resistant pattern of Gv- and Gv+ bacteria against 1st Generation Antibiotics**

| 1ST Generation | Gv- (%) | Gv+ (%) |
|---|---|---|
| **aminoglycosides** | **53** | **47** |
| **cephalosporins** | **65** | **35** |
| **monobactams** | **63** | **28** |
| **Average** | **63** | **37** |

### Second generation of antibiotics resistant pattern

In here, four antibiotics groups were used to follow the resistant pattern on the Gv+ and Gv- bacteria. Penicillin combination were mostly resistant in Gv- bacteria (83%) whereas second generation cephalosporins were 61%, carbepenems were 58% and macrolides were 0% in the Gv- bacteria. Again, macrolides were mostly resistant in the Gv+ bacteria 100% whereas penicillin combination were 17%, carbepenems were 41%, cephalosporins were 39% in the Gv+ bacteria. The average value of the Gv- bacteria resistant pattern were 51% and the Gv+ bacteria resistant pattern were 49%. So, the penicillin combination has Amoxyclave (AMC) and macrolides have Clarythromycin (CLR), Erythromycin (E) and Tetracycline (TE) were mostly resistant antibiotics (Table 5).

**Table 5. The average value of the resistant pattern of Gv- and Gv+ bacteria against 2nd Generation Antibiotics**

| 2ND Generation | Gv- (%) | Gv+ (%) |
|---|---|---|
| p. combination | 83 | 17 |
| Macrolides | 0 | 100 |
| Carbepenems | 58 | 41 |
| cephalosporins | 61 | 39 |
| **Average** | **51** | **49** |

### Third generation of antibiotics resistant pattern

In here five antibiotics group were used to follow the resistant pattern, the cephalosporins, quinolons, oxazolidinones, penicillin and penicillin combination were used. The penicillin combination was mostly resistant in Gv- bacteria 100% whereas oxazolidinones were 98%, cephalosporins were 67%, quinolons were 50% and penicillin were 0% in Gv- bacteria. Again, penicillin were mostly resistant in the Gv+ bacteria 100% whereas cephalosporin were 32%, quinolons were 50% oxazolidiones were 2% in Gv+ bacteria. The average resistant value of Gv- bacteria were 63% and Gv+ bacteria were 37%. So the antibiotic group penicillin combination have Piperacillin (PRL) and the penicillin have Cloxacillin, Cloxacillin (OB) were mostly resistant antibiotic (Table 6).

**Table 6. The average value of the resistant pattern of Gv- and Gv+ bacteria against 3rd Generation Antibiotics**

| 3RD Generation | Gv- (%) | Gv+ (%) |
|---|---|---|
| **cephalosporins** | **67** | **32** |
| **Quinolons** | **50** | **50** |
| **oxazolidinones** | **98** | **2** |
| **Penicillines** | **0** | **100** |
| **p. combination** | **100** | **0** |
| **Average** | **63** | **37** |

### Fourth generation of antibiotics resistant pattern

Only group cephalosporins 4th generation was used and this group antibiotic was highly resistant in Gv- bacteria 93% and less resistant in Gv+ bacteria 7%. Cephalosporins 4th generation have Ciprofloxacillin (CIP), Levofloxacillin (LEV) and Moxifloxacillin (MXF) were most resistant (Table 7).

**Table 7. The average value of the resistant pattern of Gv- and Gv+ bacteria against 4th Generation Antibiotics**

| 4TH Generation | Gv- (%) | Gv+ (%) |
|---|---|---|
| **Cephalosporins 4TH Generation** | 93 | 7 |

So, this research were conducted that 1-20 age of female mostly infected in Gv- bacteria and most of the infection were urinary tract infection. Male patient were infection in the 21-41 age group but male were less infected in Urinary Tract Infection (UTI) they were mostly infected in the septicemia. Female and male both patients were in 41-60 age groups less infectious. Out of 204 samples, Gv- bacteria were more prone to infection than Gv+ bacteria. The first generation antibiotic CL is work on bacterial cell wall in peptidoglycan layer and SXT is work on double inhibition of bacterial folic acid synthesis and both are work as a bactericidal method, both can take orally. Second generation of antibiotic AMC is work on bacterial enzyme and work as a bactericidal method and in the

macrolide group antibiotic CLR, E work binding with bacterial 50S subunit of rRNA complex and TE binding to the 30S rRNA subunit, all macrolide group antibiotics are work as a bactericidal method. AMC, CLR, E, TE all antibiotics can take orally but these antibiotics were more resistant than other antibiotics. Third generation of antibiotic PRL is work in bacterial cell wall and it can take as an injection. Another antibiotic OB is work in bacterial cell wall and it can take orally both are work as bactericidal method but both were resistant than other antibiotics.

Antimicrobial agents are substances produced by microorganisms, which suppress the growth of or kill other microorganisms at very low concentrations. This definition excludes other natural substances which also inhibit microorganisms but are produced by higher forms (e.g. antibodies) or even those produced by microbes but are needed in high concentration. Now many antibiotics and their analogues have been synthesized, so both synthetic and microbiologically produced drugs need to be included together, however it would be more meaningful to use the term Antimicrobial Agent to designate synthetic as well as naturally obtained drugs that attenuate microorganisms [6]. Antimicrobial agents acting only on a single or limited group of microorganisms are said to have a narrow spectrum. For example, isoniazid is active only against mycobacterium. It is the term applied to antibiotics that are effective against gram-positive organisms and also against a significant number of gram negative bacteria. For example, ampicillin is considered to have an extended spectrum, because it acts against gram-positive and some gram negative bacteria. It is the term applied to antibiotics that are effective against a wide variety of microbial species [7]. Antibiotic resistance can develop at any one or more of steps in the processes by which a drug reaches and combines with its target. Thus, resistance development may develop due to: (i) Reduced entry of drug into pathogen: The outer membrane of gram negative bacteria is a permeable barrier that excludes large polar molecules from entering the cell. Small polar molecules including many antibiotics enter the cell through protein channels called porins. Absence of, mutation in, or loss of favored protein channel can slow the rate of drug entry into a cell or prevent entry altogether, effectively reducing drug concentration at the target site. If the target is intracellular and the drug requires active transport across the cell membrane, a mutation or phenotypic change that slows or abolishes this transport mechanism can confer resistance; (ii) Enhanced export of antibiotic by efflux pump: Microorganisms can over express efflux pumps and then expel antibiotics to which the microbes would otherwise be susceptible. A wide variety of efflux pumps provide antimicrobial resistance to bacteria; (iii) Release of microbial enzymes that destroy the antibiotic: Drug inactivation is a common mechanism of drug resistance. Bacterial resistance to aminoglycosides and to β-lactam antibiotics usually is due to production of an aminoglycoside-modifying enzyme or β-lactamase respectively; (iv) Alteration of microbial proteins that transform pro-drugs to the effective moieties and alteration of target protein: A common consequences of either single point or multiple point mutations is change in amino acid composition and confirmation of target protein. This change leads to a reduced affinity of drug for its target, or of a pro-drug for the enzyme that converts the pro-drug to active drug. Such alteration may be due to mutation of the natural target, target modification, or acquisition of a resistant form of the native susceptible target. Microbes can also develop alternative pathways to those inhibited by the antibiotic, which may be due to- (i) Genetic alterations leading to drug resistance: Acquired antibiotic resistance requires the temporary or permanent gain or alteration of bacterial genetic information. Resistance develops due to the ability of DNA to undergo spontaneous mutation or to move from one organism to another; (ii) Spontaneous mutations of DNA: Chromosomal alteration may occur by insertion, deletion or substitution of one or more nucleotides within the genome. The resulting mutation may persist, be corrected by the organism, or be lethal to the cell. If the cell survives, it can replicate and transmit its mutated properties to progeny cells. However, mutations that produce antibiotic resistant strains can result in organisms that may proliferate under certain selective pressures [8].

In Europe, antimicrobial resistance of invasive pathogens has been monitored by the European Antimicrobial Resistance Surveillance System (EARSS) where they reported data from 1962 invasive isolates of *E. coli*: resistance to ampicillin, co-trimoxazole, ciprofloxacin and gentamicin was found in 58.46%, 32.91%, 17.19% and 6.39%of isolates, respectively [1]. In one study of United States, 705 isolates of *Enterococci* sp. was collected. They identified two *E. faecalis* isolates resistant to vancomycin. *E. faecium* isolates were significantly resistant to penicillin, ampicillin, piperacillin, imipenem, and ciprofloxacin. Antimicrobial susceptibility patterns vary among species of enterococci, and these organisms, while commonly resistant to high-level aminoglycosides, can also acquire resistance to vancomycin or the ability to produce 13-lactamase. Because of these diverse antimicrobial resistance mechanisms, successful treatment and control of enterococcal infections with current antimicrobial agents are becoming increasingly difficult [9]. A study of deparment of medical microbiology, The London hospital microbiology did on *Klebsiella* sp. They found that extended-spectrum β-lactamases (ESBLs) resistant to ceftazidime, ceftriaxone, cefuroxime, some isolates highly sensitive and others

very resistant to ciprofloxacin, piperacillin/ tazobactam and aminoglycosides [10].

## Conclusion

The research confirmed the view that patients who had G-ve infection were mostly resistant. Most of the resistant bacteria are taken by orally. In Bangladesh, there are many patient are taken antibiotics so easily sometime without any prescription and that is the major cause of bacteria genetically recombinant against the antibiotics. Also, bacteria create antigen against antibiotics. Education, conscious and awareness can solve or slow down the problem. So (i) educate patients and the general community on the appropriate use of antimicrobials; (ii) educate patients on the importance of measures to prevent infection, such as immunization, vector control, use of antibiotics, etc.; (iii) educate patients on simple measures that may reduce transmission of infection in the household and community, such as hand-washing, food hygiene, etc.; (iv) encourage appropriate and informed health care seeking behavior; (v) encourage prescribers and dispensers to educate patients on antimicrobial use and the importance of adherence to prescribed treatments; (vi) improve antimicrobial use by supervision and support of clinical practices, especially diagnostic and treatment strategies; can improve to slow down the resistant status.

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