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# Emerging Antimicrobial Resistance amongst Common Bacterial Pathogens in Mymensingh Medical College Hospital

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## Abstract

**Antimicrobial resistance has been coming up as one of the challenging business for treating domiciliary and nosocomial infections. The present study compared rate of resistance towards different commonly used antibiotics for common bacterial pathogens isolated during 2001 to 2003. Regarding *Escherichia coli,* mentionable increase ($p<0.01$) of resistance was noted against ceftazidime (47% to 77%) and ceftriaxone (43% to 71%). Imipenem (2% to 1%) and pefloxacin (40% to 17%) showed decreased trend. For *Staphylococcus aureus*, marked increase in resistance was shown against almost all antibiotics except co-trimoxazole (55% to 57%). Mentionable increase in resistance ($p<0.05$ and $p<0.001$, respectively) was noted against ciprofloxacin (17% to 43%) and ceftriaxone (28% to 83%). Although, oxacillin resistance increased from 22% to 42% but no resistance against vancomycin was noted during this period. Strains of Pseudomonas species showed increase ($p<0.05$) in resistance against ciprofloxacin (47% to 71%), ceftriaxone (50% to 74%) and ceftazidime (39% to 58%). Carbenicillin showed decreased resistance (92% to 50%) and none of the strains was found resistant to imipenem. Emphasis was given towards judicial use of antibiotics by followig local antibiogram.**

**Key words: Emerging resistance, Common bacteria**

## Introduction

Antibiotic treatment alone is the sole or even major intervention in an integrated public health approach to infectious disease management and control. Conversely, increasing or high levels of resistance to the antibiotics, used for these purposes, pose the real problem of increased morbidity and mortality. Thus, the most obvious application of antibiotic susceptibility testing and surveillance is to facilitate use of the most appropriate treatment in infected individuals.[1]

Use of an antibiotic in a disease outbreak or in an individual infection is often commenced before the diagnosis is confirmed and almost always before the susceptibility pattern to the pathogen can be fully ascertained. To choose an antibiotic, the important consideration is the level of resistance to that antibiotic.[2] On the contrary, irrational prescribing, dispensing and consumption of medicines remain widespread, especially in the private sectors, despite having so many efforts. Such irrational use can also be a major source of impoverishment for poor populations as well as a hazard to health. It is particularly a serious public health problem in developing countries (like Bangladesh) where between 50-90% of drug purchases are made in the private sector without any prescription. Therefore, selling of antibiotics has become an unauthorized right of the druggists that has been silently creating devastating nature of bacterial drug resistance.[3]

Previously, we reported an alarming picture of drug resistance among *E. coli*, *S. aureus* and Pseudomonas species in our hospital settings as a part of the ARM program, launched by WHO through Director General of Health Services, Bangladesh. Pattern of resistance in *E. coli* was as follows: resistance towards ampicillin, cephradine, nalidixic acid, co-trimoxazole, ciprofloxacin and ceftriaxone were 84.6%, 64.1%, 62.6%, 51.5%, 37.8% and 19.6% respectively.

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Whereas, 69.9% and 66.6% of strains were sensitive to gentamicin and nitrofurantoin, respectively. Though resistance to ceftriaxone was the lowest (19.6%) but 36.4% strains were intermediate. In addition, over 50% strains appeared to be resistant towards combination of at least 2 antibiotics.[4]

In another report from the same settings, it was found that above 85% and 23% isolates of *S. aureus* showed resistance to penicillin and oxacillin, respectively indicating existence of methicillin-resistant *S. aureus* (MRSA).[5] More than 32% isolates appeared as intermediate towards erythromycin. Ciprofloxacin resistance was noticed in 15% isolates, whereas >75% isolates were sensitive to gentamicin. Another study reported of having quite high percentage of Pseudomonas strains sensitive towards ceftazidime (>71%), but over 30% strains showed intermediate sensitive pattern against ceftriaxone and resistance to ciprofloxacin. Sensitivity value towards gentamicin was good (>60%).[6]

It has been suggested to become careful regarding use of ciprofloxacin and 3rd generation cephalosporins. Still, in the local hospital, treating infection have been appearing as a great problem due to lack or non-response to multiple antibiotics. Local clinicians expressed that judicial use of antibiotics should be an optimistic approach to minimize the on-going scenario of antimicrobial resistance. Our experiences from the laboratory also correlated with their views. The present study was undertaken focusing on the prevailing situation of antimicrobial resistance in this locality so that clinicians can select appropriate antibiotics. Our aim was to insist on comprehensive antimicrobial therapy in the local hospital that might be one of the important ways of infection control.

## Methods

Antimicrobial susceptibility of 571 bacterial isolates from different clinical specimens (urine, wound swab, pus, vaginal swab, stool, throat swab, abdominal fluid and sputum) was analyzed in the present study. Aerobic culture and sensitivity tests were done in the department of Microbiology, Mymensingh Medical College (MMC) including specimens sent from outpatient (OPD) and inpatient department (IPD) of the same Medical College Hospital, during the period from April' 2001 to December' 2003. All specimens were inoculated into Blood agar and MacConkey's agar media and incubated at 37$^{0}$C overnight. Bacterial isolates were identified by colony morphology, staining, motility test and appropriate biochemical tests (catalase, coagulase, oxidase and others).[7,8] Antimicrobial susceptibility test was done by disk diffusion method according to the NCCLS.[9]

Briefly the method was as follows - isolated colonies of same morphology were suspended in sterile normal saline which was then adjusted to match the 0.5 McFarland turbidity standard. Within 15 minutes a sterile cotton swab was dipped into the adjusted suspension. The swab was rotated several times and pressed firmly on the inside wall of the tube above the fluid level and inoculated over the dried surface of a Muller Hinton agar plate. The inoculum was placed by streaking the swab over entire surface, rotating the plate approximately 60 degrees three times. Antimicrobial disks from a prefixed panel were dispensed onto the surface of the inoculated agar plate. The plates were inverted and placed in an incubator set at 35$^{0}$C. After overnight incubation, zones of inhibition were measured using a ruler and recorded accordingly. Results were reported either as sensitive (S), resistant (R) and intermediate (I). Data were entered and subsequently analyzed using the WHONET 4 program.

## Results

Comparison of resistance pattern of *E. coli* in different years shows gradual increase in resistance against almost all the antibiotics except imipenem (2% in 2001 and 1% in 2003) and pefloxacin (40% in 2001 and 17% in 2003). Mentionable increase was noted against ceftazidime (47% in 2001 and 77% in 2003) and ceftriaxone (43% in 2001 and 71% in 2003). (Table I)

Table I: Resistance pattern of *E. coli*

| Antibiotics | Percent of resistant isolates | | |
|---|---|---|---|
| | 2001 | 2002 | 2003 |
| Ampicillin | 97 | 99 | 97 |
| Co-trimoxazole | 74 | 66 | 82 |
| Nalidixic acid | 68 | 81 | 84 |
| Tetracycline | 63 | 79 | 87 |
| Ciprofloxacin | 52 | 59 | 59 |
| Ceftazidime | 47 | 64 | 77 |
| Ceftriaxone | 43 | 49 | 71 |
| Chloramphenicol | 40 | 50 | 60 |
| Pefloxacin | 40 | 71 | 17 |
| Gentamicin | 40 | 47 | 62 |
| Nitrofurantoin | 27 | 36 | 62 |
| Imipenem | 2 | 0 | 1 |

Comparison of resistance pattern of *S. aureus* in different years shows gradual increase in resistance against almost all the antibiotics except co-trimoxazole, where it was found to be 55% in 2001 and 57% in 2003. Mentionable increase was noted against ciprofloxacin (17% in 2001 and 43% in 2003) and ceftriaxone (28% in 2001 and 83% in 2003). Although, oxacillin resistance increased from 22% in 2001 to 42% in 2003, but no resistance against vancomycin was noted in any year. (Table II)

Table II: Resistance pattern of *S. aureus*

| Antibiotic | Percent of resistant isolates | | |
|---|---|---|---|
| | 2001 | 2002 | 2003 |
| Ampicillin | 97 | 100 | 100 |
| Penicillin G | 89 | 96 | 100 |
| Oxacillin | 22 | 17 | 42 |
| Co-trimoxazole | 55 | 60 | 57 |
| Ceftriaxone | 28 | 16 | 83 |
| Erythromycin | 20 | 25 | 62 |
| Gentamicin | 19 | 09 | 39 |
| Ciprofloxacin | 17 | 18 | 43 |
| Doxycycline | 17 | 31 | 50 |
| Cephalexin | 13 | 00 | 62 |
| Vancomycin | 00 | 00 | 00 |

Resistance pattern of Pseudomonas species in different years shows gradual increase against almost all the antibiotics except carbenicillin (92% in 2001 to 50% in 2003). Resistance of mentionable increased from 2001 to 2003 as noted against ciprofloxacin (47% to 71%), ceftriaxone (50% to 74%) and ceftazidime (39% to 58%). None of the strains showed resistance against imipenem. (Table III)

Table III: Resistance pattern of Pseudomonas species

| Antibiotic | Percent of resistant isolates | | |
|---|---|---|---|
| | 2001 | 2002 | 2003 |
| Carbenicillin | 92 | 61 | 50 |
| Cephachlor | 92 | 88 | 100 |
| Ceftriaxone | 50 | 36 | 74 |
| Ciprofloxacin | 47 | 52 | 71 |
| Gentamicin | 41 | 56 | 67 |
| Ceftazidime | 39 | 25 | 58 |
| Imipenem | 00 | 00 | 00 |

## Discussion

The WHO has identified antibiotic resistance as one of the major emerging public health problems and established monitoring system in different countries. In Bangladesh, antibiotic resistance monitoring (ARM) has been launched by a program (BAN-BCT) as a multicentre based record keeping and collection system. As a part of the program, the present study was conducted. Due emphasis was given by WHO on antibiotic resistance pattern among common bacteria, like *E. coli*, *S. aureus* and Pseudomonas species.[10] Accordingly, we reported resistance pattern of these three bacteria.

In this study, we reported resistance pattern of candidate bacterial strains for 3 consecutive years so as to have a comparative picture regarding available antibiotics. In this respect, almost all the strains of *E. coli* were resistant to ampicillin (97%) in all 3 years. We documented gradual rise of resistance to 3rd generation cephalosporins (ceftriaxone and ceftazidime) with respective values in 2001, 2002 and 2003 as 43%, 49% and 71%, respectively for ceftriaxone, 47%, 64% and 77%, respectively for ceftazidime. Corresponding resistance to ciprofloxacin were 52%, 59% and 59% respectively. Increased rates of resistance were also noticed against gentamicin, nitrofurantoin, nalidixic acid, co-trimoxazole and tetracycline. Imipenem was the only antibiotic appeared effective for almost all strains of common bacterial pathogens. In a study from the Netherlands (Sahm *et al*)[11], almost similar level of resistance was reported among isolates of *E. coli,* where resistance towards ampicillin, sulphamethoxazole- trimethoprim (SXT), cephalothin, ciprofloxacin and nitrofurantoin was 97.8%, 92.8%, 86.6%, 38.8% and 7.7%, respectively.

Our findings of 2003 compared well with that of ampicillin and SXT. But ciprofloxacin resistance was comparatively higher in all 3 years in our study. We observed gradual elevation of resistance to third generation cephalosporins that could not be compared exactly with the above mentioned study. Because, resistance towards cephalothin was not reported in their study. This finding should not be considered inconsistent as cephalothin resistance indicated the same phenomenon against the whole family of cephalosporin drug. Another multi-centre study, carried out in the BIRDEM, Dhaka, included bacterial strains sent from different regions of the country, during the year 2000, almost in the same period of our study, considered both 'intermediate' and 'resistant' results as resistance. In the same manner, we compared our data and values of ciprofloxacin resistance in *E. coli* isolated from BIRDEM hospital was 59.6%, from community samples of Dhaka city 53.6%, from Chittagong region 44.7% and from Rajshahi region 25.0%. Whereas, our value of 42.5% (09/99 - 03/01) was higher than Rajshahi, almost same with Chittagong but lower than those of BIRDEM and Dhaka city. Corresponding values for Pseudomonas species also showed variation (50.9% with 63.7%, 39.2%, 60.0% and 21.8% respectively). In the same period mentioned above, our study recorded 27.5% *S. aureus* strains resistant to ciprofloxacin which was much lower than value (75.9%) of BIRDEM, Dhaka, quite higher (7.7%) than Rajshahi and almost similar with Chittagong.

This variation should have been due to multifactorial cause, such as number of isolates, patient's condition, samples, test condition, transportation and preservation of strains. We could not compare our data of the period (09/01-08/02) with regional basis due to un-availability of any such reports. But, the increasing trend of ciprofloxacin resistance in every common bacterial isolates found in our study well compared with other studies from abroad. In this connection, a collaborative study among laboratories of Germany, Austria and Switzerland recorded increased rate of resistance in all common bacterial pathogens against ciprofloxacin.[12] One study from Korea also found high level of similar quinolone resistance and noted increasing trend.[13] Another study documented decreased sensitivity to fluoroquinolones among isolates after treating with suboptimal doses.[14] This phenomenon well supported our findings of increased resistance. Because, empirical and improper use of ciprofloxacin is a common happening especially in our community medical practice. On finding high level of quinolones resistance in Staphylocci isolates, one study elucidated mutational change at genomic level.[15] This observation was in good agreement with our explanation that prolonged and irrational exposure of the drug might have induced genomic change among bacterial pathogens of our locality.

Analyzing the findings obtained in the present study, it was concluded that we are not in a safe position regarding blind use of ciprofloxacin for curative purpose. Above all, increasing population of resistant bacteria will soon be wide-spread in our clinical fields and through a challenge to the community. Therefore, everybody needs to be methodical and rationale to select and prescribe antibiotics whenever necessary. A need for antibiotic policy is also felt.

The antibiotic susceptibility pattern of the present study recorded as >84% strains resistant to ampicillin. Rate of resistance for other antibiotics of common use were as follows: cephradine 64.1%, nalidixic acid 62.6%, co-trimoxazole 51.5%, ciprofloxacin 37.8% and ceftriaxone 19.6%. Gentamicin and nitrofurantoin appeared as the two most sensitive drugs having the values as 69.9% and 66.6% respectively. We compared our values with those of a multi-centre study in the BIRDEM, Dhaka [unpublished data] and found almost similar resistance pattern from different parts of the country. Another surveillance report from Virginia, USA in the year 2000, recorded as >90% of *E. coli* isolates were resistant to ampicillin and co-trimoxazole, >30% to ciprofloxacin and >78% to cephalothin. Nitrofurantoin was found to be sensitive in 92% isolates.[11]

Findings of the present study correlated well with that surveillance report excepting the value of nitrofurantoin. In our study, we did not use cephalothin, so we could not comment anything on resistance towards cephalothin. But our study found high degree of cephradine resistance having a beat mimicry with cephalothin resistance. One very much notable finding of the present study was that 37.0% strains showed intermediate sensitive results for ceftriaxone. Susceptibility as intermediate goes to the side of resistance. Because, higher doses of antibiotics are needed to treat infections caused by intermediate bacterial strains which in turn facilitates aggravation of resistance gene expression.[16] So, it has seriously appeared as emerging resistance towards costly and very effective third generation cephalosporins.

Urinary isolates of *E. coli* of the present study showed highest sensitivity (66.6%) against nitrofurantoin. Although the value was lower compared to Sahm *et al*[11] (92%), but rank as most sensitive drug was similar. The reason behind this might be due to low use of this drug for long period considering its toxicity and side effects. To the contrary, abuse, overuse or injudicious use of ampicillin, co-trimoxazole, ciprofloxacin and cephalosporins resulted higher magnitude of resistance. One phenomenon was carefully observed in our study that increases in the rate of resistance towards ciprofloxacin. In the year 1998, another study by Haque *et al*[17] recorded only 3% of urinary isolates resistant to ciprofloxacin, whereas present study yielded that value about 38%. Carelessness, misuse, erratic use and improper use of this drug for a long period in this country would have been thought as the cause for this rapid emergence of ciprofloxacin resistance. *E. coli* isolates in the present study showed second highest sensitive result (63.0%) against gentamicin. Ahmed *et al*[18] recorded

85% sensitive results, Haque *et al* [17] recorded 56 %. Although our study did not found same values as compared to the above mentioned studies but trend of increasing effectiveness of gentamicin towards *E. coli* is similar. The explanation for this increase sensitivity should be due to the fact that gentamicin is only available in injectable form. So, its use especially in community by rural practitioners is somehow restricted due to low acceptability of injection to the patients and also dose inconvenience is a cause. This minimal use might have caused lowering of resistance.

Selection of antibiotic is not itself a problem. The problem arises when bacteria causing disease withstand the chosen drug.[19] Today, antibiotic resistance has a significant impact on treatment of some of the leading causes of human death. In an environment exposed to an antibiotic, susceptible bacteria will decrease in numbers while resistant bacteria will continue to multiply.

In evolutionary terms, exposure to antibiotics exerts a selective pressure on bacterial populations. Plasmids and transposons may carry several resistance genes, each to a different class of antibiotics. All the genes will be transferred in the same event and this is called co-transfer. Exposure to anyone of these antibiotics will select for the presence of the entire genetic element.[20] Upon each treatment of an animal, not only the pathogen but also the entire normal flora is exposed to antibiotics. Commensals, or even environmental bacteria, will act as a reservoir of resistance genes. Unless specifically looked for, this reservoir will go unnoticed until the resistance genes pass into clinically relevant bacteria. Antibiotic resistance epidemiology will be influenced by the same factors that favour bacterial spread. The selective pressure in a population is the major risk factor. The fact that resistance epidemiology also has a molecular level (transposons or plasmids spread between bacteria) complicates the matter. Further, the phenomenon of co-selection must be considered.[21] Therefore, it is not surprising that the relation between antibiotic use and resistance is not always clear-cut.[2]

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