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Antibiotic Resistance Pattern of Clinical Isolates - *Escherichia coli*, *Enterobacter*, *Pseudomonas* and *Staphylococcus aureus* in the Western of Bangladesh

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Abstract: The present study was carried out to analyze the antibiotic susceptibility of four pathogenic bacteria *Escherichia coli, Enterobacter, Pseudomonas and Staphylococcus aureus* respectively. *Escherichia coli* strains from 35 samples, *Staphylococcusaureus* strains from 35 samples, *Enterobacter* strains from 39 samples and *Pseudomonas* strains from 39 samples were isolated from 200 suspected infected individuals. Pure cultures of isolate were done by isolating single colony from the stored bacteria. Identification of strains were confirmed by various microscopic, colonial and biochemical tests. Finally identified four varieties of pathogenic strains *Escherichia coli*, *Enterobacter, Pseudomonas* and *Staphylococcus aureus* were subjected to the antibiotic sensitivity test by antibiotic disc diffusion method. Approximately 15 commonly used antibiotics were used in the tests. For this study it was observed that 94% of *Escherichia coli* were resistant to Cefixime, 86% to Cefuroxime Sodium, 77% to Ceftriaxone, 71% to Ceftazidime, 66% to Amoxycillin-Clavulanic acid and Ciprofloxacin, 63% to Levofloxacin, 57% to Doxycycline, 49% to Co-trimoxazole andonly 37% to Gentamicin. No *Escherichia coli* samples were found resistant against Meropenem having highest sensitivity (100%). Only 7 *Escherichia coli* samples were resistant to Amikacin having sensitivity 80% and 10 *Escherichia coli* samples were resistant to Nitrofurantion with the third highest sensitivity 71%. *Staphylococcus aureus* were observed to show maximum resistant (100%) towards Azithromycin, next to Ceftriaxone 74%, Ciprofloxacin and Oxacillin 71%, Co-trimoxazole 63%, Levofloxacin 57% and Amoxycillin-Clavulanic acid 49%. Only 5 *Staphylococcus aureus* samples were resistant to Gentamicin with a maximum sensitivity 86% and 7 *Staphylococcus aureus* samples were resistant to Amikacin having second highest sensitivity 80% and third highest sensitivity Cephalexin 63%. *Enterobactor* showedmaximum resistant towards Amoxycillin-Clavunic acid 100%, Cefixime 100%, Ceftadizime 100%, Ceftriaxone 95%, Amikacin 85%, Co-trimoxazole 79%, Ciprofloxacine 77%, Doxycyclin 72%, Gentamicin51%, Levofloxacin 46%, and Nitrofurantion 41%. There were found to be no *Enterobacter* Samples that resistant to Meropenem having highest sensitivity (100%). Only 16 *Entarobacter* samples were resistant to Nitrofurantion having second highest sensitivity59% and third highest sensitivity Levofloxacin 54%. *Pseudomonus* were observed to maximum resistant towards Amoxicillin 100%, Ceftazidime 100%, Cefixime 100%, Doxycycline 100%, Co-trimoxazole 100%, Ciprofloxacin 83%, Ceftriaxone 83%, Levofloxacin 83%, Nitrofurantion 83%, and Amikacin 67%. There were found to be no *Pseudomonus* Samples that resistant to Meropenem having highest sensitivity (100%). Only 13 *Pseudomonus* samples were resistant to Gentamicin with a maximumsensitivity 67%.

Keywords: Resistant Microbes, *Escherichia Coli*, *Enterobacter*, *Pseudomonas* and *Staphylococcus Aureus*

1. Introduction

Microbes developed resistance primarily in the hospitals. Resistantmicrobesimpose more risk to the human health when it spread in the community. Infections with resistant microbes not only result in greater morbidity and mortality, but also increase the health care costs. [1]

Inadequate access to effective antimicrobials, incomplete therapy and questionable quality of medicine increase theemergence of resistance. [2]

Rate and frequency of infectious diseases are much more higher in Bangladesh because the country is situated in the sub-tropical zone. Bangladesh, with a high degree of antibiotic resistance, poses a regional and global threat. In Chittagong at Bangladesh in 2003, its was found that typhoid patients were unresponsive to second-line therapy (ciprofloxacin). First-line therapy was not even attempted because of existing resistance. [3]

Different studies have demonstrated irrational antibiotic prescribing by physicians, a habit of self-medication among patients, and the indiscriminate use of antibiotics in agriculture and farming in different parts of the country. [4-6]

Different studies in revealed that there is polypharmacy, high use of antimicrobials, vitamins and injectables in hospitals and very low generic prescribing. [7-11]

The sporadic, uncontrolled and unnecessary uses of antibiotics increasingthe number of multi-drugsresistant pathogenic strains. That's why the treatment of these diseases become harder than the earlier stage. Therefore the selection of appropriate antibiotic for the treatment of these diseases is the pre-requisite. Thus we aim to know the antibiotic resistant pattern of four most common pathogenic bacteria *Escherichia coli, Enterobacter, Pseudomonas* and *Staphylococcus aureus* and also to make awareness among the prescribing doctors as well as the patients. The present study was carried out to analyze the antibiotic susceptibility of four pathogenic bacteria, *Escherichia coli*, *Enterobacter, Pseudomonas* and *Staphylococcus aureus*. Thirty five samples of *Escherichia coli* strains, thirtynine samples of *Enterobacter* strains, thirty nine samples of*pseudomonas* strainsand thirty five samples of *Staphylococcus aureus* strains were isolated from more than 200 suspected infected persons.

2. Materials and Methods

Sampling sites: A total of 200 bacterial samples (urine, pus and exudates, feces, urogenital swab, and vaginal swab) were collected from many patients suspected for suffering from urinary tract infection and staphylococcal infection for the isolation and identification of *Escherichia coli*, *Enterobacter, Pseudomonas* and *Staphylococcus aureus*respectively in the microbiology laboratory of Amin diagnostic centers, Kushtia, Bangladesh.

Sample collection from urine: 'Midstream' clean specimens of urine in a sterile container were collected from both male and female patients suffering from UTI. Anogenital region was cleaned with antiseptics such as chlorhexidine or cetrimide. Specimens from adult patients were collected carefully in a sterile test tube. Specimens from infants were collected with a sterilized test-tube.

Sample of pus and exudates: Pus and exudates were collected from the abscesses, wound etc. with a sterile swab stick in a sterilized container. At least three swabs were taken from the exact site.

Sampling from urogenital swab: After opening the urethral, it was cleaned by using a swab moistened with sterile physiological saline with the help of expert technician of the laboratory. The urethral was massaged by gently above down-wards. To collect the discharge, a sterile swab was used.

Collection of sample from vaginal discharge: Clean, dry, leak-proof containers were given to the patient and request him to collect a specimen of vaginal discharge with help of sterilized swab stick.

Transportation of sample: After collection, all the samples were transported to the laboratory immediately in an insulating foam box with ice.

Bacteriological analysis: A small portion of the suspected specimen like urine, pus and exudates from abscesses, wound, stool, urethral, cervical, urogenital swab etc. mixed with 0.5 ml of normal saline and shake gently to make suspension. Then0.1 ml of that suspension and urine were inoculated on the solid surface ofMaconkey agar (Hi-Media, India). All samples were incubated for 24 hours at 37°C in three triplications of Maconkey agar for successful isolation of typical colonies. Identification was done according to Buchanan and Gibbons (1974) following a series of biochemical tests included gram staining, tests for oxidase, indole, citrate, catalase and coagulase. [12]

Drug Sensitivity Test. Single disc diffusion method (Bauer et al. 1966) was used to examine bacterial susceptibility to antimicrobial agents. A total of 16 antibiotic discs (Oxoid LTD. Basingstoke Hampshire, UK) with Amikacin (30µg), Amoxicillin (30µg), Azithromycin (15µg), Ceftazidime (10µg), Ceftriaxone (30µg), Ciprofloxacin (5µg), Cotrimoxazole (25µg), Cefuroxime Sodium (30µg), Cefixime (5µg), Cephalexin (30µg), Doxycycline (30µg), Gentamicin (10µg), Levofloxacin 5 (µg), Meropenem (10µg), Nitrofurantion (300µg) and Oxacillin (1µg) were used. By the standard method of inoculation, the top of a single and well-isolated colony was touched with a sterile loop and the growth was inoculated into 2 ml of Mueller–Hinton broth (Hi-Media, India). The broth culture was then allowed to incubate at 37°C for 4 hours to obtain the young culture. The turbidity of actively growing broth cultures was then adjusted to a 0.5 McFarland standard and then a sterile cotton swab was dipped into the adjusted suspension within 15minutes and

excess broth was purged by pressing and rotating the swab firmly against the inside of the tube above the fluid level. The swab was then spread evenly over the entire surface of the plate of Mueller–Hinton agar (Hi-Media, India) to obtain uniform inoculums. The plates were then allowed to dry for 3 to 5 minutes. Antibiotics impregnated discs were then applied to the surface of the inoculated plates with sterile forceps. Each disc was gently pressed down onto the agar to ensure complete contact with the agar surface. Even distribution of discs and minimum distance of 24 mm from center to center were ensured. Five discs (four antibiotics discs and one blank disc as control) were placed in each Petridis. Within 15 minutes of the application of the discs, the plates were inverted and incubated at 37°C. After 16 to 18 hours of incubation, the plates were examined, and the diameters of the zones of complete inhibition to the nearest whole millimeter were measured. The zone diameter for individual antimicrobial agents was then translated into susceptible, intermediate and resistant categories according to the interpretation table of the Becton Dickinson Microbiology Company, USA. [13].

3. Results

After primary collection, through cultural morphological and biochemical tests only those strains which were found to be *Escherichia coli*, *Enterobacter, Pseudomonas* and *Staphylococcusaureus* were taken for the antibiotic sensitivity and resistance study. It was found that 35 samples out of 48 suspected samples were *Escherichia coli*, 39 samples out of 50 suspected samples were *Enterobacter*, 39 samples out of 50 suspected samples were *pseudomonas* and 35 samples out of 52 suspected samples were *Staphylococcus aureus.*

The bacterial strains of these four pathogenic bacteria such as *Escherichia coli*, *Enterobacter, pseudomonas* and *Staphylococcus aureus*were identified and isolated, then they were cultured to see the antibiotic sensitivity and resistant pattern by using commonly used antibiotic that are prescribed for their treatment.

A total of 148 samples were selected and subjected to various morphological and biochemical tests followed by serological identification. The biochemical tests for identification of *Escherichia coli*, *Enterobacter, Pseudomonas* and *Staphylococcus aureus* isolates from infected individuals are summarized in Table 1.

Table 1. *Biochemical tests used for identification of Escherichia coli, Enterobacter, Pseudomonas and Staphylococcus aureus.*

Biochemical Test Properties	***Escherichia coli*Reaction**	***Enterobacter* Reaction**	***Pseudomonas* Reaction**	***Staphylococcus aureus*Reaction**
Gram Staining	G^-, Rod shape	G^-, Rod shape	G^-, Rod shape	G^+, cocci
MacConkey agar	pink	pink	colorless	Pale pink (grow in macConkey agar without crystal violet)
Oxidase Test	-	-	+	-
Indole Test	+	-	-	-
Citrate Test	-	+	+	+
Catalase Test	+	+	+	+
Coagulase Test	-	-	-	+

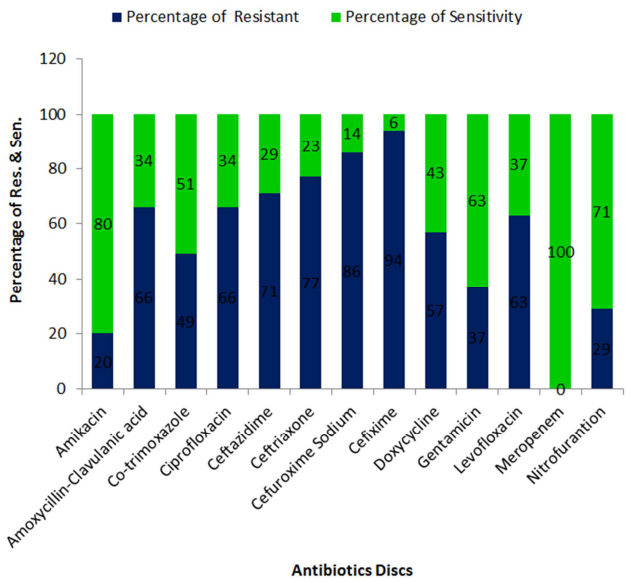

Figure 1. *Resistant and Sensitivity Pattern of Antibiotic for E.coli.*

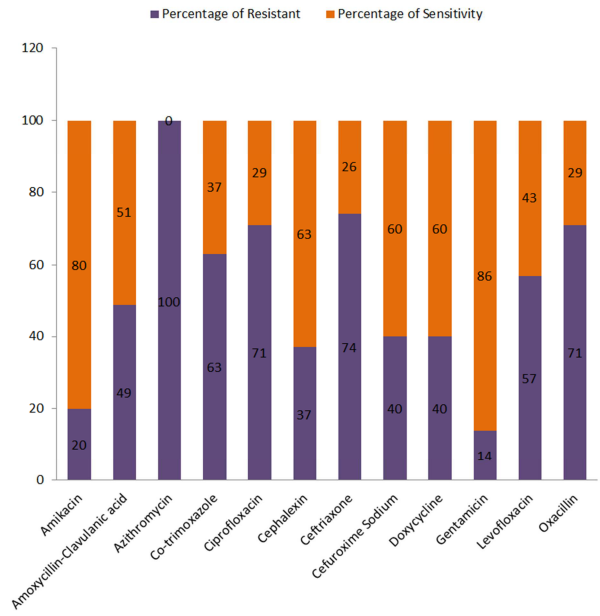

Figure 2. *Resistant and Sensitivity Pattern of Antibiotic for S.aureus.*

In figure 1 maximum resistant of *Escherichia coli* was

found against Cefixime (94%), Cefuroxime Sodium (86%), Ceftriaxone (77%) and Ceftazidime (71%). On the other hand, the bacteria showed minimum resistant against Meropenem (0%), Amikacin (20%), Nitrofurantion (29%) and Gentamicin (37%). The bacteria showed moderate resistance against the rest of the antibiotics which were used for the experiment.

In figure 2 Staphylococcus aureus showed maximum resistant against Azithromycin (100%), Ceftriaxone (74%), Ciprofloxacin (71%) and Oxacillin (71%). Whereas the bacteriashowed minimum resistant against Gentamicin (14%), Amikacin (20%) and Cephalexin (37%). The bacteria showed moderate resistant against rest of the antibiotics which were used in the experiment.

In figure 3 Enterobacter showed maximum resistant against Amoxycillin-clavunic acid (100%), Ceftadizime (100%), Cefixime (100%), Ceftriaxone (95%) and Amikacin (85%). Whereas minimum resistant were found against Meropenem (0%), Nitrofuration (41%) and Levofloxacin (46%). The bacteria showed moderate resistant against rest of the antibiotics which were used in the experiment.

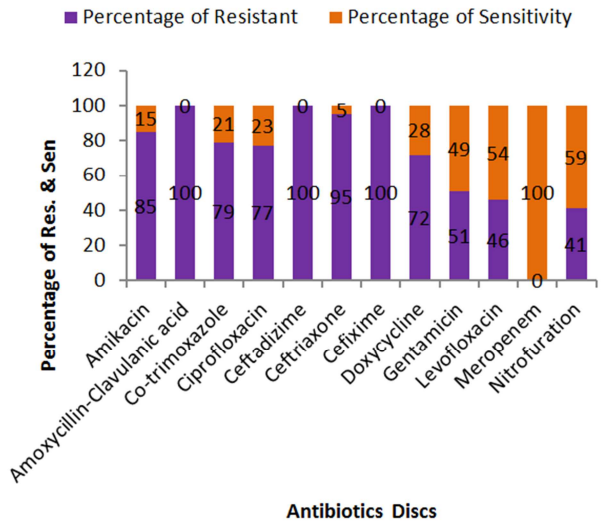

Figure 3. *Resistant & Sensitivity Pattern of Antibiotic for Enterobacter.*

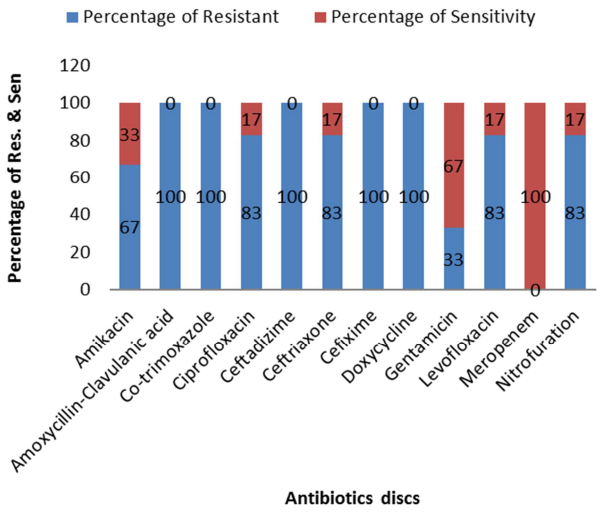

Figure 4. *Resistant and Sensitivity Pattern of Antibiotic for Pseudomonas.*

In figure 4 Pseudomonus showed maximum resistant against Amoxycillin-clavunic acid (100%), Ceftadizime (100%), Cefixime (100%), Co-trimoxazole (100%) and Doxycycline (100%). Where as it showed minimum resistant against Meropenem (0%) and Gentamicin (33%). It showed moderate resistant against rest of the antibiotics used for the study.

4. Discussion

Antibiotic resistant bacteria has become amajor threat to reduce the effectiveness of antibiotics worldwide. [14-16] The exposure of multiresistant organisms isincreasing, especially in the developing world. [17] WHO, placed great emphasis on increasing the awarenessabout antimicrobial resistance (AMR). [18-20]

In the present study we found that 66% *E.coli* samples were resistant to Amoxicillin, 66% to Ciprofloxacin, 71% to Ceftazidime and 37% to Gentamcin. Similarly they reported that 36% *S.aureus* were found to be resistant towards Amoxicillin, 6% to Azithromycin, 8% to Ciprofloxacin and 58%to Oxacillin. But we found that 100% *S.aureus*samples were resistant to Azithromycin, 49% to Amoxicillin, 71% to Ciprofloxacin, 71% to Oxacillin. Present data clearly indicate that the resistant capacity of the *E.coli* and *S.aureus*increasingday by day. On the other hand *Enterobacter* showed maximum resistant against Amoxycillin-clavunic acid (100%), Ceftadizime (100%), Cefixime (100%), Ceftriaxone (95%) and Amikacin (85%). *Pseudomonus* showed maximum resistant against Amoxycillin-clavunic acid (100%), Ceftadizime (100%), Cefixime (100%), Co-trimoxazole (100%) and Doxycycline (100%).

5. Conclusion

Our study results clearly showed that Cefixime, Cefuroxime and Ceftriaxone were failed or almost failed to treat the *E.coli* infection while Meropenem, Amikacin and Nitrofurantion were found to be most effective for the treatment of *E.coli* infection successfully. Similarly Amoxicillin, Cotrimoxazole, Ceftriaxone, Ceftazidime, Cefixime, Doxycycline, Nitrofurantionwere failed or almost failed to treat the urinary tract infection while Meropenem, and Gentamicin were found to be most effective for the treatment of urinary infection. On the other handAzithromycin, Ceftriaxone, Ciprofloxacin and Oxacillinwere failed or almost failed to treat the *Staphylococcal* infection while Gentamicin and Amikacin were found to be most effective for the treatment of *Staphylococcal* infection successfully. So the previous and present data clearly indicate that random, uncontrolled and antibiotic abuse become a bigthreatfor the treatmentof bacterial infection.

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