ORIGINAL ARTICLE

Analysis of Cumulative Antibiotic Sensitivity Testing Data (Antibiogram) of Anwer Khan Modern Medical College Hospital

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ABSTRACT

Background: Monitoring of antimicrobial resistance at local level is a very crucial aspect for clinical decision making,infection control interventions and antimicrobial-resistance containment strategies in this era of rising superbugs. The hospital antibiogram constructed by standardized method is a summary of antimicrobial susceptibilities of local bacterial isolates, with periodic review.

Methods: This is a prospective observational study conducted in Microbiology Laboratory of Anwer Khan Modern Medical College Hospital (AKMMCH) over a period of three months from January to March 2021. All the samples which were received in the microbiology laboratory for aerobic bacterial culture and antibiotic sensitivity testing were considered in this study. The bacterial isolates from diagnostic clinical samples were subjected to culture in appropriate media for isolation of pathogens by standard methods and antibiotic sensitivity test (AST) was performed according to CLSI guidelines by modified Kirby Bauer disk diffusion techniques.

Results: A total of 2917 samples were analysed in the microbiology department of AKMMCH for a period of three months of which 513(17.59%) were culture positive.Among the cultured samples of urine, respiratory sample, blood and pus, wound swabs, the rate of bacterial isolation was 266 (51.85%) ,161 (31.38%), 31 (6.04%) and 33 (6.4%) respectively. Most of the clinical isolates were Gram-negative bacilli404 (78.75%). The predominant isolate was *Escherichia coli* 186(36.26%) followed by *Pseudomonas spp* 102 (19.88%), *Klebsiellaspp* 79 (15.4%) and *S. aureus* 66 (12.87%). Antibiotic sensitivity in case of Gram-negative bacteria ranges from 30%-61% to amoxicillin-clavulanic acid, 18%-94% to third generation cephalosporin, 47%-75% to aminoglycosides, 27% -70% to ciprofloxacin, 26-70% to cotrimoxazole, 33-73% to nitrofurantoin, 27-69% to piperacillin- tazobactam and 100% to colistin. However, 53% of *Escherichia coli* and 58% of *Klebsiella spp*. were resistant to 3rd generation cephalosporins due to extended-spectrum beta-lactamase (ESBL) production. The carbapenem resistance in *Escherichia coli* was found 12%, *Klebsiella spp* 26%, *Pseudomonas spp* 29% and highest in *Acinetobacter* 46%. No superbug was detected in this study period.

Conclusions: Antimicrobial susceptibility of various pathogen for different antibiotic is variable. Prompt antimicrobial therapy in an infection makes a lot of difference between recovery and death and most of the time prevents long term disability. Hence, antibiotic policy is one of the mandatory requirements and making an antibiogram is the first step before framing antibiotic policy.

Key words: Antibiogram, Antibiotic Resistance, Antimicrobial Susceptibility Test

Introduction

Antimicrobial resistance (AMR) is emerging as a major threat to public health and has been estimated that 10 million deaths annually will be due to AMRby 2050.[1] With increasing antimicrobial resistance worldwide, it is crucial to monitor drug resistance at the local level to support clinical decision making, infection-control interventions, and antimicrobial-resistance to containment strategies Development of

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mechanisms by the microorganisms to evade antimicrobial action leads to development of AMR.[2,3] Though the development and spread of antibiotic resistance is multifactorial,misuse and overuse of antibiotics are the major factors contributing to AMR. So continuous surveillance is necessary to explore the current situation of antibiotic resistance globally. Resistance pattern of organism varies from one country to another and within the country. Systematic data are lacking in many developing countries of the world.[4]

The antibiogram of a hospital is a periodic summary of antimicrobial sensitivity of local microbial isolates in the hospital's microbiology laboratory.[5,6] The most frequent use of it is in guiding initial empirical antimicrobial therapy for the management of infections in patients for whom microbiological test data do not yet exist.[7,8] For the ongoing management of prolonged infections, clinicians should rely on culture and antimicrobial susceptibility test results previously available for the patient and an understanding of the likelihood of the emergence of an antimicrobial-resistant strain during therapy. There are other applications for the analysis of susceptibility test data like monitoring the emergence of antimicrobial resistance, guiding therapy choices for subsequent infections and identifying isolates with specific antimicrobial resistance phenotypes.[9]

As per the recommended protocols by CDC (center for disease control), the adequate information regarding the sensitivity pattern of various isolates in specific localities is necessary for initial prescription. Therefore, this study had been planned to acquire the knowledge about sensitivity pattern of various isolates in various specimen of AKMMCH.

Methods

This is a prospective observational study conducted in Anwer Khan Modern Medical College Hospital. The antibiogram was prepared by the microbiology laboratory of the hospital, based on the CLSI M39 – A4 guidelines.[9] The data was collected for a period of only three months from January to March, 2021. All the samples (included blood, urine, respiratory secretions, stool, various types fluids, genital specimens, pus, wound swab and various others) which were received in the microbiology laboratory for aerobic bacterial culture and sensitivity testing were considered. The bacterial isolates from diagnostic clinical samples were subjected to culture in respective media for isolation of potential pathogens. Isolates were identified by standard methods and antibiotic sensitivity was performed according to CLSI guidelines by modified Kirby Bauer disk diffusion techniques.[11,12] The isolates per patient in the period was analysed irrespective of the body site from which the specimen was obtained and the antimicrobial susceptibility pattern was considered. Repeat isolates were alsoincluded as segregation was not possible. In accordance with CLSI M39-A46 recommendations all the antimicrobials which were routinely tested for an isolate were analysed for cumulative antibiogram preparation. The percentage of isolates that were susceptible only was included. The percentage of isolates with intermediate susceptibility was excluded.

The data was analysed using EXCEL and expressed using descriptive statistics such as counts and percentage.

Results

A total of 2917 culture specimens from various department of AKMMCH were received in the microbiology laboratory for bacteriological results, of which 513 (17.59%) showed culture positivity (Fig 1).Among them 67.83% (n= 348) were from outpatient department, 26.5% (n= 136) were indoor department and 5.65% (n=29) from ICU. Table 1

The largest number of bacterial isolations was seen in urine specimens (266/1423) followed by respiratory sample (161/435) and blood (31/816) Table 2. In this study, most of the identified isolates were Gram-negative bacilli 404 (78.75%) while the remaining 100 (19.49%) were Gram-positivecocci and 1.7% were yeast (Fig 2).The distribution of growth in culture positive samples in the study by specimen type is summarized in Table 3.The most frequently identified isolate was *Escherichia coli* 186 (36.26%) followed by *Pseudomonas spp* 102 (19.88%), Klebsiellaspp 79 (15.4%) and *S. aureus* 66 (12.87%).

The overall AST profile of the isolates is presented in Table 4 and Table 5.For urinary pathogen we used Fosfomycin but this data is not included here as it described elsewhere. In this study, antibiotic sensitivity observed were 30%-61% to amoxicillin clavulanic acid, 18%-94% to third generation cephalosporin, 47%-75% to aminoglycosides, 27% -70% to ciprofloxacin, 26-70% to cotrimoxazole, 33-73% to nitrofurantoin, 27-69% to piperacillin- tazobactam and 100% to colistin in Gram negative bacteria. However, 53% of *Escherichia coli* and 58% of *Klebsiella spp.* were resistant to 3rd generation cephalosporins due to extended-spectrum beta-lactamase (ESBL). The carbapenem resistance in E*scherichia coli* was found 12%, *Klebsiella spp* 26%, P*seudomonas spp* 29% and highest in *Acinetobacter* 46%. Of special interest, 70.59% *Salmonella* was ciprofloxacin sensitive and only 41% was azithromycin sensitive. No vancomycin resistant *Staphylococcus aureus* (VRSA) and *Enterococci* (VRE) was detected. But unfortunately, MRSA analysis could not be included in this study due to lack of uniform data for AST of oxacillin or cefoxitin.

In case of specimens received from ICU,Gram-negative bacilli (79.31%) were the most common pathogens, with an incidence of *Pseudomonas* 34.5% followed by *Klebsiella spp.* 24.14%, *Escherichia coli* 10.3% and Acinetobacter10.3%. Antibiotic sensitivity showed that colistin is the most effective antibiotic with around100% sensitivity for *Klebsiella, E. coli, Pseudomonasand Acinetobacter* while *carbapenems* sensitivity was extremely low, showing 43%,80% and 100% meropenemresistance for *Klebsiella, Pseudomonas* and *Acinetobacter respectively* Fig 3. Fungal infections in ICU represented 10%.

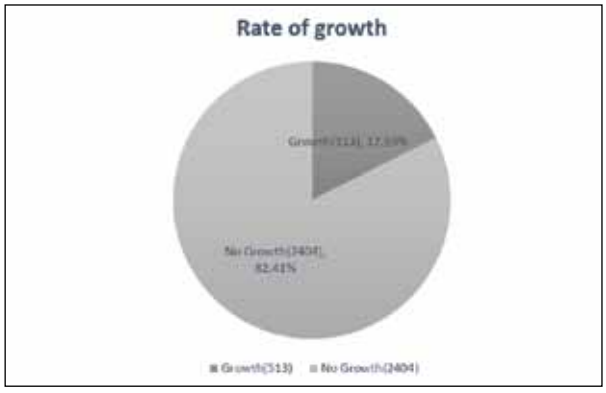


Figure 1- Total samples cultured in the study

Table 1: Distribution of sample according to patient location

Patient location	Urine (%)	Blood (%)	Respiratory Sample (%)	Pus & wound swab (%)	Genital Sample (%)	Others (%)	Total (%)
OPD	206 (77.44)	19 (61.29)	72 (44.72)	31 (93.94)	16 (100)	4 (66.67)	348 (67.84)
IPD	54(20.30)	7 (22.58)	71 (44.10)	2 (6.06)	0	2 (33.33)	136 (26.51)
ICU	6(2.26)	5 (16.13)	18 (11.18)	0	0	0	29 (5.65)
Total	266(51.85)	31(6.04)	161(31.38)	33(6.40)	16(3.11)	6(1.17)	513

Table 2: Percentages of culture positive specimens received in microbiology laboratory

Specimen	Total specimens n (%)	Positive specimen n (%)
Urine	1423(48.78)	266(51.85)
Blood	816(27.97)	31(6.04)
Respiratory samples	435(14.91)	161(31.38)
Genital samples	75(2.57)	16(3.11)
Pus and wound swab	79(2.70)	33(6.4)
Others	89(3.04)	6(1.16)
Total	2917	513

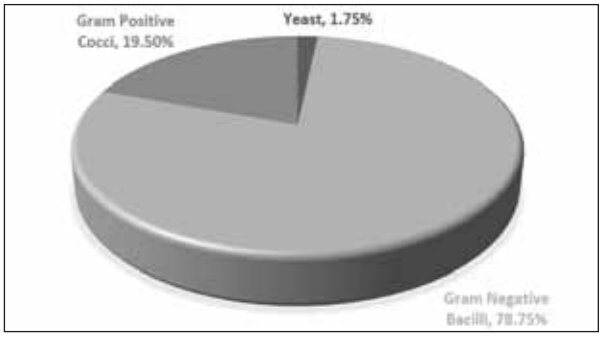


Figure 2- Percentage of various isolates among culture positive samples

Table 3: Percentagesof bacterial isolates (%) from various specimens

Organism	Urine n=266	Respiratory Sample n=161	Blood n=31	Pus & wound swab n=33	Genital Sample n=16	others n=6	Total n=513
Acinetobacter	5(1.88)	5(3.11)	1(3.23)	0	0	0	11(2.14)
Pseudomonas	45(16.92)	46(28.57)	6(19.35)	4(12.12)	0	1(16.67)	102(19.88)
Escherichia coli	150(56.39)	7(4.35)	5(16.13)	11(33.33)	10(62.5)	3(50)	186(36.26)
Klebsiella	12(4.51)	61(37.89)	2(6.45)	4(12.12)	0	0	79(15.4)
Enterococcus	07(2.63)	1(0.62)	0	0	1(6.25)	0	9(1.75)
S. aureus	30(11.28)	17(10.56)	0	14(42.42)	14(18.75)	2(33.33)	66(12.87)
S.Typhi	0	0	17(54.84)	0	0	0	17(3.31)
S. pneumoniae	0	20(12.42)	0	0	0	0	20(3.90)
S. saprophyticus	2(0.75)	0	0	0	0	0	2(0.39)
Proteus	9(3.38)	0	0	0	0	0	9(1.75)
S. agalactiae	2(0.75)	0	0	0	1(6.25)	0	3(0.58)
Candida	4(1.5)	4(2.48)	0	0	1(6.25)	0	9(1.75)

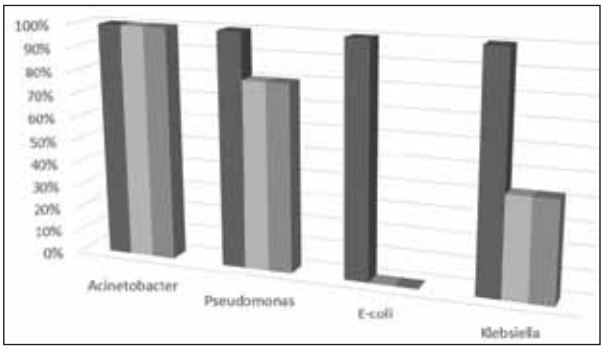
Table 4: Antibiogram (% sensitivity) of Gram-negative bacteria

Antimicrobial agent	% sensitivity					
	E.coli n=186	*Klebsiella* n=79	*Pseudomonas* n=102	*Acinetobacter* n=11	*S. typhi* n=17	*Proteus* n=9
Amoxiclav	61.29	46.84	30.39	45.45	100	100
Piperacillin-tazobactam	69.35	58.23	66.67	27.27	0	0
Ceftriaxone	46.24	40.51	25.49	18.18	100	88.89
Ceftazidime	51.61	49.37	29.41	18.18	94.12	0
Cefixime	32.26	27.85	23.53	27.27	64.71	66.67
Meropenem	88.17	69.62	65.69	54.55	100	100
Amikacin	75.81	54.43	47.06	0	0	0
Azithromycin	0	0	0	0	41.18	0
Ciprofloxacin	43.01	35.44	26.47	27.27	70.59	55.56
Cotrimoxazole	52.15	40.51	21.57	36.36	0	0
Nitrofurantoin	73.12	0	0	45.45	0	33.33
Colistin	100	100	100	100	0	33.33

Table 5: Antibiogram (% sensitivity) of Gram-positive bacteria

Antimicrobial agent	% sensitivity				
	S. aureus n=66	*S. saprophyticus* n=2	*S. pneumoniae* n=20	*Enterococcus* n=9	*S. agalactiae* n=3
Amoxiclav	93.94	100	100	100	100
Cotrimoxazole	36.36	100	10	66.67	0
Ceftriaxone	72.73	50	100	66.67	33.33
Cefixime	69.09	50	40	44.44	0
Levofloxacin	15.15	0	45	33.33	0
Ciprofloxacin	28.79	50	0	33.33	0
Meropenem	92.42	100	100	100	100
Vancomycin	100	0	100	100	0

Figure 3- Resistance Pattern of the most Prevalent Bacteria in ICU



Discussion

A cumulative antibiotic sensitivity test(antibiogram) report is a periodic summary of susceptibility rates of the commonly isolated organisms in a health care setup. It plays a key role at the bedside, in deciding the antimicrobial therapy for a patient. The knowledge of the local antimicrobial resistance patterns is vital to introduce appropriate infection control measures to check the spread of these resistant organisms as well as to prevent the emergence of new drug resistant bugs.[12] Unfortunately, the easy availability and familiarity, affordable prices and ignorance of the impending consequences of antimicrobials, has resulted in use and misuse of antimicrobials and aided the persistent expansion of multidrug resistant microbes, leading to the loss of efficacy of these "magic drugs". Inappropriate antibiotic usage triggers the selection and rapid emergence of drug resistant bacteria like ESBLs, which in turn spread in the community by horizontal gene transfer.[13]

In this three-month period study, among various types of samples bacterial isolation rate was very low (6.04%) in blood sample. It might be due to prior antibiotic use before doing AST or large number of blood sample was collected from OPD suspect or technical fault.The isolation rate of Gram-negative bacteria (78.73%) was higher than Gram positive bacteria (19.49%). This finding is similar to the study done at BIRDEM.[4] The cause of predominant isolation rate of the Gram-negative organism among hospitalized patient might be due to selective pressure of broad-spectrum antibiotics causing persistent of drug resistance genes or plasmids, virulence factors like flagella, capsule, outer membrane in this class compared to Gram positive bacteria.[14] The most frequently identified isolate was Escherichia coli followed by *Pseudomonas spp, Klebsiella spp* and *S. aureusin* the study. We depend on colony morphology and limited available biochemical tests for bacterial identification. So, it was not possible to identify various other causative pathogens and all isolated bacteria up to species level.

Among different antibiotics tested for Gram positive cocci, amoxicillin – clavulanic acid and vancomycin is very much effective antibiotic against *S. aureus* and *Enterococcus spp,* which was in agreement with Dharmapalan et al study.[15]

In this study, Antibiotic sensitivity observed were 30%-61% to amoxicillin clavulanic acid, , 18%-94% to third generation cephalosporin, 47%-75% to aminoglycosides, 27% -70% to ciprofloxacin, 26-70% to cotrimoxazole, 33-73% to nitrofurantoin, 27-69% to piperacillin-tazobactam in case of Gram-negative bacteria. Several recent reports suggest high rate of resistant organisms among hospitalized patients of Bangladesh.[16-18] Misuse and overuse of the antibiotics, high consumption rate, easy accessibility of antibiotics (OTC), lack of hospital antimicrobial policy and concrete regulatory body for antibiotic stewardship program at national level are important factors for this increasing rate of antibiotic resistance in Bangladesh.[4,19] It is alarming that 53% *Escherichia coli* and 58% *Klebsiella* developed resistant to 3rd generation cephalosporins due to extended-spectrum beta-lactamase (ESBL) production. A study conducted in a referral hospital of Dhaka city had also noted 43.2% *Escherichia coli* and 39.5% *Klebsiella* were ESBL positive.[20] The emergence and rapid dissemination of carbapenem resistant organism (CRO) is now global health threat.[21,22] In our study, the carbapenem resistance in *Escherichia coli* was found 12%, *Klebsiella spp* 26%, *Pseudomonas spp* 29% and highest in *Acinetobacter* 46%. Multi drug resistant bacteria are difficult to treat as the treatment options are limited .High prevalence of ESBLs limited the therapeutic options for drug resistant organisms causes increase consumption of carbapenems. However, long term hospitalization, frequent use of invasive medical devices have also increased carbapenem resistance.[23]

Treatment failures with ciprofloxacin have emerged in Bangladesh and other countries due to infection with nalidixic acid resistant *Salmonella Typhi* or NARST. NARST has decreased susceptibility to ciprofloxacin but in this study, it was 70% sensitive to ciprofloxacin and only 41% sensitive to azithromycin. It does not correlate with other study where NARST was 80 to 90%.[4,24] However, antibiotic resistance is an increasingly serious threat to global public health that requires coordinated action of people, health workers, pharmacists, policy makers, scientists and industry to minimize emergence and spread of antibiotic resistance globally.[25]

Since the study was relied on laboratory data it was not possible to correlate clinical profile and underlying disease condition of patients, risk factors or their source of infections.

Conclusion

Though no superbug is detected, the high rate of different resistant strain herald continuous monitoring for containment of antimicrobial resistance as there is very few new antibiotics in trial worldwide.The use of antibiograms to help in selecting empirical antibiotic therapy for suspected infection is a well-established practice. This is the first study to describe the analysis of antibiogram results of AKMMCH and provide the information of microorganisms and antibiotics susceptibility for the AKMMCH. Strict adherence to the infection control practices and judicious use of antimicrobial therapy remains the only way to counter the threat of antimicrobial resistance.

Conflict of interest: We have no conflict of interest.

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