Microbiology Research Journal International

22(6): 1-10, 2017; Article no.MRJI.39626
ISSN: 2456-7043
(Past name: British Microbiology Research Journal, Past ISSN: 2231-0886, NLM ID: 101608140)

Change in Antibiotic Susceptibility Pattern of Clinical Bacterial Isolates from Two Hospitals in Dhaka, Bangladesh over a Period of Three Years

Mahnaz Hossain Fariba[1], Abdullah Bashar Sami[1], Debanjan Bhattacharjee[2], Sajal Kumar Saha[3], Sitesh Chandra Bachar[4], M. Aftab Uddin[1] and Ruhul Haque Kuddus[1,5*]

[1]Department of Genetic Engineering and Biotechnology, University of Dhaka, Dhaka, Bangladesh.
[2]Department of Mathematics, Utah Valley University, Orem, UT 84058, USA.
[3]Department of Clinical Pharmacy and Pharmacology, University of Dhaka, Dhaka, Bangladesh.
[4]Department of Pharmaceutical Technology, University of Dhaka, Dhaka, Bangladesh.
[5]Department of Biology, Utah Valley University, Orem, UT 84058, USA.

Authors' contributions

This work was carried out in collaboration among all authors. The corresponding author conceived the theme of the study. Each of the authors contributed in designing and executing the study. Author DB conducted statistical analyses, and the last five authors critically evaluated the manuscript. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/MRJI/2017/39626
Editor(s):
(1) Eliton da Silva Vasconcelos, Department of Physiological Sciences, Federal University of São Carlos – UFSCar, Rod. Washington Luiz, São Carlos, SP, Brazil.
Reviewers:
(1) Bilkisu Ilah Garba, Usmanu Danfodiyo University & Usmanu Danfodiyo University Teaching Hospital, Nigeria.
(2) Hussaini Ibrahim Mohammed, Ahmadu Bello University, Nigeria.
Complete Peer review History: http://www.sciencedomain.org/review-history/23410

Original Research Article

Received 24th November 2017
Accepted 23rd February 2018
Published 3rd March 2018

ABSTRACT

Introduction: It is established that improper use of antibiotics leads to rapid development of bacterial antibiotic resistance. We investigated changes in the antibiotic susceptibility pattern of pathogenic bacteria in a megacity where improper antibiotic use is common.
Methodology: Data on the antibiotic susceptibility pattern of clinical isolates to 28 commonly used antibiotics was obtained from two hospitals at time point A (Jan-Dec 2009, or Nov-Dec 2010) and at about 12-36 months later (time point B), and the data were compared using the one-sided test for equality of proportions. For large samples, tests using Z-score and normal distribution were conducted; for small samples, Fisher's exact test was performed.

**Corresponding author: E-mail: Ruhul.kuddus@uvu.edu;*

Results: Of the 194 different pairs of isolate clusters compared; 66.5% of the cluster-pairs showed no change in the antibiotic susceptibility pattern. Of the remaining 33.5% of the isolate clusters, the time point B clusters showed a significant decrease in antibiotic susceptibility in 21.1% of the cases, and a significantly higher susceptibility in 12.4% of the cases, compared to the corresponding time point A clusters. The decreased antibiotic susceptibility was observed in 20.0% of the Gram-negative and 24.1% of the Gram-positive bacterial isolate clusters; and the increased antibiotic susceptibility was observed in 10.0% of Gram-negative and 18.5% of Gram-positive bacterial clusters.

Conclusions: Antibiotic resistance and susceptibility of Gram-positive and Gram-negative pathogenic bacteria may significantly change over a period as short as 1-3 years. Continuous vigilance of such changes in a region may allow development of regional strategies for rational antibiotic use.

Keywords: Antibiotic-susceptibility; antibiotic-resistance; equality of proportions test; Fisher's exact test.

1. INTRODUCTION

With an estimated population of 14.4 million and a population density of about 45,000/ square km, Dhaka City, the capital of Bangladesh, is one of the most densely populated megacities of the world [1]. The warm and moist climate, congested living and working environments, inadequate preventive and curative healthcare infrastructures, lack of adequate clean drinking water, lack of adequate sewage and industrial waste disposal processes, low literacy and inadequate public awareness on infection risks [2], create an ideal condition for infections and rapid dissemination thereof, in the city. Infectious diseases are the most common maladies in Bangladesh and antibiotics are the most commonly prescribed medications in the city [3,4,5]. Antibiotics are dispensed mostly based on 'best guess' clinical assessment and not on diagnosis of the etiological agents [4,5] and, in some cases, irrationally [6]. Because of the severe shortage of qualified medical providers [7], antibiotics and other controlled drugs can be prescribed by underqualified or unqualified healthcare providers [7,8,9]. Self-medication, antibiotic abuse and use of antibiotics in treating viral or protozoan infections or non-infectious diseases have also been reported [10]. Although illegal, many drugstores in the country sell antibiotics without a prescription [11]. Lack of strict quality control of antibiotics, adulteration of drugs, incomplete dosing, and financial inability of many citizens to complete the prescribed dose of antibiotics [11] further complicate the issue. Although intensive animal farming and use of antibiotics in animal feed are yet not significant problems in Bangladesh, such farms are emerging [12]. Finally, most of the hospitals of the city are resource-strained and overcrowded with many patients and their personal caregivers. As expected, the rate of healthcare-associated infections in Dhaka is quite high [13]. All these factors may confer antibiotic resistance to pathogenic bacteria.

The present study investigated if the antibiotic resistant pathogenic bacteria remain resistant to the antibiotics indefinitely under the ongoing environmental conditions or some of the resistant strains become antibiotic-sensitive over a reasonable time frame.

2. MATERIALS AND METHODS

2.1 Collection of Data on Antibiotic Susceptibility Pattern

Data on antibiotic susceptibility of clinically relevant bacterial isolates was collected from the pathology and/or microbiology laboratories of two major hospitals. Data from BIRDEM General Hospital (122 Kazi Nazrul Islam Avenue, Dhaka 1000) was from publicly available sources [14,15]. Data from Square Hospital Ltd. (18F, Bir Uttam Qazi Nuruzzaman Sarak, Dhaka 1205) was obtained as unpublished Monthly Reports (2009-2011). We obtained written permission from the Square Hospital to process, analyze, interpret and publish the processed data. The pathology/microbiology departments of the two hospitals isolated pure culture of the bacterial isolates from clinical samples, identified the strains using standard microbiological methods (colony morphology, staining, biochemical tests and immunochemical tests), and conducted drug susceptibility tests following standard techniques [16,17]. Drug susceptibility was conducted typically using disc diffusion method

following the NCCLS guidelines [18]. The discs were obtained from Oxoid (Oxoid Ltd., Basingstoke, Hampshire, UK). The discs were tested on reference bacterial strains before using them to test the newly isolated strains [17].

2.2 Statistical Analyses

We reorganized the obtained raw data to facilitate statistical analyses. For each hospital, tables were prepared showing the susceptibility (in percentage) of the bacterial isolates to the clinically relevant doses of the antibiotics. If resistivity was recorded, susceptibility was computed by subtracting resistance from 100. We wanted to investigate any significant changes of antibiotic-susceptibility between the two given time points for each of the isolates to each of the tested antibiotics. Here time point A precedes time point B. We performed statistical hypothesis testing procedure for equality of two proportions for two independent samples. Our hypothesis was: The proportion of susceptibility at the time point A is greater than that of the time point B. If the sample size is large enough, a 'large sample procedure' was performed. The test statistic that was computed for large samples is given by the Z-score,

$$Z_0 = \frac{\hat{p}_A - \hat{p}_B}{\sqrt{\hat{p}(1-\hat{p})}\sqrt{\frac{1}{n_A}+\frac{1}{n_B}}}$$

Where, $\hat{p} = \frac{n_A \hat{p}_A + n_B \hat{p}_B}{n_A + n_B}$, n_A and $\hat{p}_A$ are sample size and proportion of susceptibility during Time Point A; n_B and $\hat{p}_B$ are sample size and proportion of susceptibility during time point B. The probability of observing a standard normal deviate value greater than Z_0 was calculated. The right tail area exceeding 0.95 is indicative of a significant increase in susceptibility, while right tail area less than 0.05 is indicative of a significant decrease in susceptibility to the antibiotic. For large samples, 95% confidence intervals for difference in proportion were also calculated for all bacteria-antibiotic combinations and archived as supplementary data.

If the sample size was small, Fisher's exact test was performed. In Fisher's exact the probability function is

$$P[X = x] = \frac{\binom{n_A}{x}\binom{n_B}{n-x}}{\binom{n_A + n_B}{n}}$$

Here, *X* denotes the number of occurrences in Time A, and *n* denotes the total number of occurrences. For an observed value of *x,* we calculated either $P(X \geq x)$ or $P(X \leq x)$. For brevity, the tables only show $P(X \geq x)$. Notably, in small sample situations, $P(X \geq x) > 0.95$ is not necessarily a significant increase in susceptibility. For a small sample, the susceptibility has increased significantly if $P(X \leq x) < 0.05$, while it has decreased significantly if $P(X \geq x) < 0.05$. We considered a nominal significance level of 5% in drawing our conclusions. All calculations were done using MS Excel (Microsoft, Redmond, Washington, USA) and Minitab (Minitab Inc., State College, Pennsylvania, USA).

3. RESULTS

3.1 Reorganization of the Raw Data on Antibiotic Susceptibility

The raw data on bacterial isolates susceptible to various antibiotics were reorganized to facilitate statistical analyses. A representative segment of the reorganized data is shown in Table 1. The rest of the processed data has been archived. The number of isolates at time point A and time point B, respectively, for BIRDEM Hospital were the following: 335 and 1,439 *Escherichia coli* isolates; 94 and 457 *Klebsiella sp.* isolates; 53 and 324 *Acinetobacter sp.* isolates; 74 and 429 *Pseudomonas sp.* isolates; 71 and 312 *Staphylococcus aureus* isolates; and 275 and 159 *Enterococcus sp.* isolates. The number of isolates at time point A and time point B, respectively, for Square Hospital were the following: 1,247 and 1,522 *E. coli* isolates; 383 and 647 *Klebsiella sp.* isolates; 229 and 184 *Salmonella typhi* isolates; 109 and 96 *S. paratyphi* A isolates; 66 and 118 *Proteus sp.* isolates; 48 and 110 *Enterobacter sp.* isolates; 9 and 18 *Citrobacter sp.* isolates; 9 and 8 *Serratia sp.* isolates; 190 and 338 *S. aureus* isolates; 291 and 464 coagulase-negative staphylococci (ConS) isolates; 248 and 423 Enterococci isolates; and 7 and 58 group D non-enterococci (GDNE) isolates.

3.2 The Change in Antibiotic Susceptibility Observed at BIRDEM Hospital

The change in antibiotic susceptibility of some pathogenic bacteria from Nov-Dec 2010 to Jan-Jul 2012 as observed in BIRDEM Hospital is

shown in Table 2. The data indicates that the susceptibility of *E. coli* significantly decreased to augmentin but significantly increased to cefixime, co-trimoxazole, netilmicin, piperacillin and tazobactam-piperacillin; that of *Klebsiella sp.* isolates significantly decreased to colistin but significantly increased to piperacillin; that of *Acinetobacter sp*. isolates significantly decreased to amikacin, gentamicin, imipenem, and tazobactam-piperacillin but significantly increased to piperacillin; that of *Pseudomonas sp.* isolates significantly decreased to ceftazidime, ciprofloxacin, colistin, co-trimoxazole, gentamicin, imipenem, and netlimicin, but significantly increased to augmentin, piperacillin and nitrofurantoin; that of *Staphylococcus aureus* isolates significantly decreased to amikacin, augmentin, erythromycin, and rifamycin but significantly increased to cephalexin, netlimicin, nitrofurantoin, and oxacillin; and that of *Enterococcus sp.* isolates significantly decreased to amikacin and penicillin but significantly increased to nitrofurantoin. In total, 14 of the 54 clusters (or 25.9%) of the Gram-negative isolates showed decreased susceptibility, and 10 of the 54 (or 18.5%) of the Gram-negative isolates showed increased susceptibility. In the same hospital, 6 of the18 clusters (or 33.3%) of the Gram-positive isolates showed decreased susceptibility, and 5 of the 18 (or 27.8%) clusters of the Gram-positive isolates showed increased susceptibility. Antibiotic susceptibility of the rest of the isolate clusters (30 Gram-negative and 7 Gram-positive, or 51.4% of the 72 isolate clusters) in this hospital remained unchanged.

3.3 The change in Antibiotic Susceptibility Observed at Square Hospital

The change in antibiotic susceptibility of some pathogenic Gram-negative bacteria from Jan-Dec 2009 to Jan-Dec 2011 as observed in Square Hospital is shown in Table 3. The data indicates that the susceptibility of *E. coli* significantly decreased to amikacin, cefepime, cefixime, ceftriaxone, cefuroxime, ciprofloxacin, imipenem, and nitrofurantoin; that of *Klebsiella sp.* isolates significantly decreased to amikacin, imipenem, and nitrofurantoin, that of *Salmonella typhi* isolates significantly decreased to ciprofloxacin; that of *Salmonella paratyphi* A isolates significantly increased to ampicillin and co-trimoxazole; that of *Proteus sp.* isolates significantly decreased to cefuroxime, that of *Enterobacter sp.* isolates significantly increased to co-trimoxazole, and that of *Serratia sp.* isolates significantly increased to tetracycline. In total, 14 of the 86 clusters (or 16.3%) of the Gram-negative isolates showed decreased susceptibility, and 4 of the 86 clusters (or 4.7%) of the Gram-negative isolate clusters showed increased susceptibility to some of the tested antibiotics. The susceptibility of 68 of the 86 clusters (79.1%) of Gram-negative bacterial isolates remained unchanged to the other tested antibiotics.

Table 1. An example of processed data showing changes in antibiotic susceptibility of a bacterial pathogen (*Escherichia coli)* isolated at BIRDEM between the time periods Nov-Dec 2010 (point A) and Jul-Dec 2012 (point B)

Antibiotics	Point A (n=335)	Point B (n=1280)	Test (pA>pB) right tail prob.	Change*
Amikacin	85.16	87.90	0.9130	NC
Augmentin	16.52	7.9	0.0000	D
Cefixime	12.00	29.1	1.0000	I
Ceftazidime	35.25	31.3	0.0815	NC
Ceftriaxone	30.45	30.0	0.4358	NC
Ciprofloxacin	19.10	18.8	0.4497	NC
Co-trimoxazole	34.59	40.2	0.9709	I
Gentamicin	68.79	66.3	0.1919	NC
Imipenem	97.91	97.1	0.2071	NC
Netilmicin	65.44	76.6	0.9999	I
Nitrofurantoin	86.03	84.1	0.5704	NC
Piperacillin	0	70.06	1.0000	I
Tazo-piperacillin	50	76.60	1.0000	I

**- NC- not changed, I- significantly increased, D- significantly decreased*

Table 2. Change in the susceptibility pattern of gram-positive and gram-negative bacteria isolated between Nov-Dec 2010 and Jul-Dec 2012 from pathogenic samples collected at BIRDEM. Shown are the right tail probabilities

Antibiotics	*E. coli*	*Klebsiella sp.*	*Acinetobacter sp.*	*Pseudomonas sp.*	*S. aureus*	*Enterococcus sp.*
Amikacin	0.9130	0.0967	0*	0.0244*	0.035*	0.0064*
Ampicillin	N/A	N/A	N/A	N/A	N/A	0.7736
Augmentin	0*	0.4702	0.8824	0.9775‡	0.005*	NA
Cephalexin	NA	NA	NA	NA	0.9999‡	NA
Cefixime	1.0000‡	0.2871	0.9255	NA	NA	NA
Ceftazidime	0.0815	0.6031	0.5163	0*	NA	NA
Ceftriaxone	0.4358	0.5700	0.3547	0.4983	NA	NA
Ciprofloxacin	0.4497	0.7629	0.4865	0.0026*	NA	NA
Colistin	N/A	0*	0.1088	0*	NA	NA
Co-trimoxazole	0.9709‡	0.5073	0.9323	0*	NA	NA
Erythromycin	NA	NA	NA	NA	0.0256*	NA
Fusidic acid	NA	NA	NA	NA	0.5547	NA
Gentamicin	0.1919	0.4571	0.0161*	0.0257*	0.8871	0.5166
Imipenem	0.2071	0.1968	0*	0*	NA	NA
Netilmicin	1.0000‡	0.6571	0.8646	0.0154*	1.000‡	0.177
Nitrofurantoin	0.5704	0.4649	0.2839	1.0000‡	0.9895‡	0.9470‡
Oxacillin	NA	NA	NA	NA	0.9993‡	NA
Penicillin	NA	NA	NA	NA	NA	0.0016*
Piperacillin	1.0000‡	1.0000‡	0.9811‡	0.9998‡	NA	NA
Tazo/piperacillin	1.0000‡	0.0793	0*	0.353	NA	NA
Rifamycin	NA	NA	NA	NA	0.0090*	NA
Vancomycin	NA	NA	NA	NA	AS	0.9172

*‡-The susceptibility increased significantly; *-The susceptibility decreased significantly; AS- 100% of the isolates were sensitive during both time periods; NA- Data not available*

Table 3. Change in the antibiotic susceptibility pattern of gram-negative bacteria between Jan-Dec 2009 and Jan-Dec 2011 from pathogenic samples collected at Square Hospital. Shown are the right tail probabilities and conclusions

Antibiotics	*E. coli*	*Klebsiella sp.*	*S. typhi*	*S. parat.*	*Proteus sp.*	*Enterobacter sp.*	*Citrobacter sp.†*	*Serratia sp.†*
Amikacin	0.0000*	0.0000*	NA	NA	0.4251	0.6012	0.6954	1.0000
Amoxiclav	0.0782	0.9159	NA	NA	0.1187	0.2238	0.9642	0.5294
Ampicillin	NA	NA	0.3254	1.0000‡	NA	NA	NA	NA
Cefepime	0.0075*	0.5864	AS	0.8243	0.5117	0.732	0.6716	1.0000
Cefixime	0.0044*	0.4253	AS	0.8243	0.0601	0.4953	0.9358	1.0000
Ceftriaxone	0.0099*	0.5125	AS	0.8243	0.5976	0.682	0.8379	1.0000
Cefuroxime	0.0019*	0.4248	NA	NA	0.0127*	0.7577	0.9341	0.5633
Ciprofloxacin	0.0270*	0.4624	0.0133*	0.7916	0.3576	0.8963	0.9765	0.7353
Co-trimox.	0.8685	0.562	0.8810	0.9999‡	0.1722	0.9518‡	0.6716	0.8167
Gentamicin	0.8580	0.7995	NA	NA	0.2801	0.6756	0.8379	1.0000
Imipenem	0.0161*	0.0000*	AS	AS	0.9089	0.6901	0.279	AS
Nitrofurantoin	0.0007*	0.0000*	NA	NA	0.0242	0.5827	0.2438	NA
Tetracycline	0.2462	0.8955	NA	NA	0.1029	0.8899	0.8294	0.9910‡

*‡-The susceptibility has increased significantly; *-The susceptibility has decreased significantly; AS- 100% of the isolates were sensitive during both time periods; NA-Data not available. S. typhi-Salmonella typhi, S. parat.-Salmonella paratyphi A. †- Based on Fisher's exact test due to small sizes of samples. For small samples, the susceptibility is considered to have increased significantly if $P(X \leq x) < 0.05$, or decreased significantly if $P(X \geq x) < 0.05$ (i.e. the right tail probability value of 1.0000 does not necessarily mean a change in drug susceptibility).*

Table 4. Change in the antibiotic susceptibility pattern of gram-positive bacteria between Jan-Dec 2009 and Jan-Dec 2011 from pathogenic samples collected at Square Hospital. Shown are the right tail probabilities and conclusions

Antibiotics	*S. aureus*	CoNS	Enterococci	GDNE†
Cefuroxime	0.0017*	0.9994‡	NA	NA
Ciprofloxacin	0.0003*	0.1234	0.9791‡	0.0138*
Co-trimoxazole	0.0033*	0.0984	NA	NA
Gentamicin	0.0789	0.3842	0.3473	0.7064
Linezolid	AS	AS	0.9035	AS
Nitrofurantoin	0.8647	0.1682	0.9999‡	AS
Oxacillin	0.0010*	0.9999‡	NA	NA
Penicillin	0.2365	0.5	0.9999‡	0.8923
Rifampicin	0.0104*	0.197	0.7279	0.6267
Tetracycline	0.2142	0.0055*	NA	NA
Vancomycin	AS	AS	0.7527	AS

*‡-The susceptibility has increased significantly; *-The susceptibility has decreased significantly; AS-100% of isolates were sensitive during both time periods; NA-Data not available. CoNS- coagulase-negative staphylococci isolates; GDNE- group D non-enterococci. †-Based on Fisher's exact test due to small sizes of samples. For small samples, the susceptibility is considered to have increased significantly if $P(X \leq x) < 0.05$, and decreased significantly if $P(X \geq x) < 0.05$*

The change in antibiotic susceptibility of some pathogenic Gram-positive bacteria from Jan-Dec 2009 to Jan-Dec 2011 as observed in Square Hospital is shown in above Table 4. The data indicates that the susceptibility of *S. aureus* isolates significantly decreased to cefuroxime, ciprofloxacin, co-trimoxazole, oxacillin and rifamycin; that of coagulase-negative staphylococci (CoNS) isolates significantly decreased to tetracycline but significantly increased to cefuroxime and oxacillin; that of Enterococci isolates significantly increased to ciprofloxacin, nitrofurantoin and penicillin; and that of group D non-enterococci (GDNE) isolates significantly decreased to ciprofloxacin. In total, 7 of the 36 clusters (or 19.4%) of the Gram-positive bacterial isolates showed decreased susceptibility, and 5 of the 36 clusters (or 13.9%) of the Gram-positive isolates showed increased susceptibility to some of the tested antibiotics. Antibiotic susceptibility of 24 of the 36 (or 66.7%) of the Gram-positive bacterial isolate clusters remained unchanged to the other tested antibiotics.

4. DISCUSSION

Antibiotic resistance of pathogenic bacteria has reached a crisis point, and improper use of antibiotics has been identified as a major contributor to the crisis [19,20]. We attempted to examine the dynamics of bacterial drug susceptibility patterns in Dhaka City where improper use of antibiotics is common [21]. When improperly used, the antibiotics select the resistant strains and the resistant strains soon disseminate in the population. Lateral DNA transfer further assists the spread of antibiotic resistance [22]. The infections caused by the resistant strains result in increased hospitalizations, treatment failures and increased mortality [23]. This may explain the rapid change in antibiotic resistance among pathogenic bacteria. In the present study, many of the bacterial isolates obtained at time point B showed significantly decreased susceptibility to 21 of the 28 antibiotics, compared to the isolates of the same taxonomic groups collected at time point A, within about 1-3 years. In the two different hospitals, the drug-susceptibility changes were observed in some identical and some non-identical species. For example, a significant increase in susceptibility of *E. coli* to cefixime was observed in BIRDEM Hospital, but in Square Hospital, the same species was found to have a significant decrease of susceptibility to the same drug. This can be explained by the poorly defined species boundaries among bacteria, rapid horizontal gene transfer among related and unrelated bacterial species, and the preponderance of random genetic drift within the populations of bacterial species [24], amplification of the drug-resistance genes of the genome [25], and most certainly other unknown processes.

Bacteria tends to carry a precise genome with a very few genome-wide repeats and pseudogenes [24]. In the absence of a selective pressure, bacteria rapidly lose genes through a process

known as deletional bias [25,26]. In a country like Bangladesh, where agricultural use of antibiotics (and thus the concentration of contaminating environmental antibiotics) is insignificant, selective pressure through antibiotic use can, at best, be transitional. Thus, rapid changes in antibiotic susceptibility (i.e. increased antibiotic susceptibility of some previously resistant strains) can also be expected. In the present study, many of the bacterial isolate-clusters showed significantly increased susceptibility to 14 of the 28 tested antibiotics within about a two-year period. However, as were the cases for changes in drug-resistance, the changes in drug-susceptibility were observed, in some same or some different species in the two hospitals. For example, *S. aureus* showed a significant increase in susceptibility to oxacillin in BIRDEM Hospital, but the same species showed a significant decrease in the susceptibility to the same drug in Square Hospital. This again can be reconciled by the unusual structure of bacterial species boundary, and random genetic drift among small populations of bacteria [24,27]. Laehnemann and coworkers [25] recently observed that the copy number of the amplified antibiotic resistance genes rapidly drops from the genome of antibiotic-resistant bacteria after removal of the antibiotics.

This study showed that pathogenic bacteria can become more resistant or more susceptible to commonly used antibiotics in a relatively short time. In this study, a higher fraction of the isolates (i.e. 41 of the 194 or 21.1%) showed reduced susceptibility to the antibiotics, compared to isolate clusters (24 of 194, or 12.4%) that became more susceptible to the antibiotics. In this study 13 of the 54 isolate clusters (or 24.1%) of Gram-positive bacteria became less susceptible, and 10 of the 54 isolate clusters (or 28.5%) of Gram-positive bacteria became more susceptible to some of the antibiotics. In the same time span, 28 of 140 isolate clusters (or 20.0%) of Gram-negative bacteria became less susceptible (i.e. more resistant), and 14 of 140 isolate clusters (or 10.0%) of the Gram-negative bacteria became more susceptible to some of the antibiotics. Thus, the study indicated that drug susceptibility or resistance changed more rapidly among Gram-positive bacteria compared to the Gram-negative bacteria. Antibiotic susceptibility or resistance of a large majority (129 of 194, or 66.5%) of the isolate clusters remained unchanged in the study period.

Although rapid increase of antibiotic susceptibility of pathogenic bacteria is good news, we are uncertain about the clinical utility of these observations, given the rapidity of genetic changes among pathogenic bacteria [27,28]. We also acknowledge the limitations imposed by the modest sample size of this study. Despite these shortcomings, it is tempting to suggest that a new strategy of antibiotic use may prevent some currently ineffective but previously effective antibiotics from going obsolete. The global crisis of bacterial drug-resistance most certainly will require non-conventional or “practical not perfect” mitigation approaches [29]. The strategy proposed here is one that may ask a complete withdrawal of antibiotics that are having increased incidences of resistance only to release the drugs after a period when the drugs are found more uniformly effective. Such a rational approach is consistent with the executive summary items 3 and 4 of The Chennai Declaration [30] to tackle the global microbial drug-resistance problem. It has been recently observed that plasmid-mediated antibiotic resistance may linger in the bacterial populations in the absence of antibiotic-based selection pressure because of a relatively high rate of conjugation among bacteria [31]. Thus, periodic stoppage of antibiotics use alone may not eliminate bacterial antibiotic resistance. However, such an approach, together with application of regimens that may induce plasmid loss and inhibit bacterial conjugation [31] may substantially reduce the burden of the resistance genes and help keep the relevant antibiotics and their manufacturing processes from going obsolete.

5. CONCLUSION

We collected data on antibiotic susceptibility pattern of pathogenic Gram-positive and Gram-negative bacterial isolates from two hospitals for over a period of about three years. Our study indicates that both antibiotic susceptibility and resistance of Gram-positive and Gram-negative bacterial pathogens may significantly change over a period as short as 12-36 months. Such a quick flux may offer opportunity of devising strategies of rational antibiotic use in some developing countries. Since antibiotic-resistant pathogens may turn antibiotic-sensitive over time even in an environment where antibiotic abuse is common, continuous vigilance on bacterial antibiotic-susceptibility may keep effective and affordable antibiotics clinically relevant.

ACKNOWLEDGEMENTS

The authors are grateful to the administrators of BIRDEM Hospital and Square Hospital for sharing data, Ms. Candice Seguine of Utah Valley University for data processing, and Mrs. Monika Baker of Utah Valley University for editing the manuscript. RHK was supported by a US Fulbright Scholar Program Grant by the Bureau of Educational and Cultural Affairs (ECA) of the U.S. Department of State.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Rahman M. An urban reader. Dhaka, University Press; 2016.
2. Central Intelligence Agency. The CIA World Fact Book. New York, Skyhorse Publishing; 2017.
3. Alam MM, Parveen F, Ara F, Iqbal MJU, Saha RR. Prescribing trends in the outpatient department in a tertiary hospital in Bangladesh. Bangladesh Med J. 2011;40(2):8-12.
4. Faiz MA, Basher A. Antimicrobial resistance: Bangladesh experience. Regional Health Forum. 2011;15:1-8.
5. Biswas M, Roy DN, Tajmim A, Rajib SS, Hossain M, Farzana F, Yasmen N. Prescription antibiotics for outpatients in Bangladesh: A cross-sectional health survey conducted in three cities. Ann Clin Microbiol Antimicrob. 2014;13(15). DOI: 10.1186/1476-0711-13-15
6. Sutradhar KB, Saha A, Huda NH, Uddin R. Irrational use of antibiotics and antibiotic resistance in southern rural Bangladesh: Perspectives from both the physicians and patients. Ann Res Rev Biol. 2014;4(9): 1421-1430.
7. Ahmed SM, Hossain MA, RajaChowdhury AM, Bhuiya AU. The health workforce crisis in Bangladesh: Shortage, inappropriate skill-mix and inequitable distribution. Hum Resour Health. 2011;9(3). DOI: 10.1186/1478-4491-9-3
8. Alam GA, Shahjamal MM, Al-Amin AQ, Azam MN. State of pharmacy education in Bangladesh. Trop J Pharm Res. 2013; 12(6):1107-112.
9. Rakib A, Sarwar MS, Zannah S, Khanum S, Rashid M. A survey of the role of community pharmacists in Dhaka City, Bangladesh. Bangladesh Pharm J. 2015;2:137-141.
10. Nishat C, Rashedul MI, Mehedi H, Rouf MM. Prevalence of self-medication of antibiotics among people in Bangladesh. Indian J Pharm Practice. 2012;5(4):65-72.
11. Islam MS. Therapeutic drug use in Bangladesh: Policy versus practices. Indian J Med Ethics. 2013;10(3):24-25.
12. Akond MA, Alam S, Hassan SMR, Shirin M. Antibiotic resistance of *Escherichia coli* isolated from poultry and poultry environment of Bangladesh. Int J Food Safety. 2009;11:19-23.
13. Mohiuddin M, Huq JA, Hoq MM, Huq F. Microbiology of nosocomial infections in tertiary hospitals in Dhaka City and its impact. Bangladesh J Med Micr. 2010;4(2): 32-38.
14. BIRDEM Hospital. Bacteria and Antibiogram Report Nov-Dec 2010;1(1). Dhaka, BIRDEM Hospital Department of Microbiology; 2010.
15. BIRDEM Hospital. Bacteria and Antibiogram Report January-July 2012. BIRDEM Med J. 2013;3(1):63-66.
16. Colle JG, Miles RS, Watt B. Tests for the identification of bacteria. In: Collee JG, Fraser AG, Marmion BP, Simmons A, editors. Mackie and McCartney Practical Microbiology 14th Ed. New York, Churchill & Livingstone; 1996.
17. Shaida K, Borhan SB, Hasin H, Rahman S, Sultana S, Barai L, Jilani MSA, Huq A. Trends of antibiotic susceptibility of *Salmonella enterica* servoar typhi and paratyphi in an urban hospital of Dhaka City over six years period. Ibrahim Medical College J. 2011;5(2):42-45.
18. Clinical Laboratory Standard Institute. Performance standards for antimicrobial disc susceptibility testing: 14th informational supplement M100-S14. CLSI, Wayne PA, CSLI; 2004.
19. Rossolini GM, Arena F, Pecile P, Pollini S. Update on the antibiotic resistance crisis. Current Opinion in Pharmacol. 2014;18:56-60.
20. Martens E, Demain AL. The antibiotic resistance crisis, with a focus on the United States. J Antibiotics. 2017;70:520-526.

21. Rahman MS, Huda S. Antimicrobial resistance and related issues: An overview of Bangladesh situation. Bangladesh J Pharmacol. 2014;9:218-224.
22. Davies J, Davies D. Origins and evolution of antibiotic resistance. Micro Biol Mol Biol Rev. 2010;74(3):417-433.
23. Ventola CL. The antibiotic resistance crisis, Part 1: Causes and threats. Pharm Ther. 2015;40(4):277-283.
24. Kuo CH, Moran NA, Ochman H. The consequences of genetic drift for bacterial genome complexity. Genomes Res. 2009;19(8):1450-1454.
25. Laehnemann D, Peña-Miller R, Rosenstiel P, Beardmore R, Jansen G, Schulenburg H. Genomics of rapid adaptation to antibiotics: Convergent evolution and scalable sequence amplification. Genome Biol Evol. 2014;6(6):1287-1301.
26. Mira A, Ochman H, Moran NA. Deletional bias and the evolution of bacterial genomes. Trends Genet. 2001;10:589-596.
27. Gillings MR, Paulsen IT, Tetu SG. Genomics and the evolution of antibiotic resistance. Ann NY Acad Sci. 2017;1388(1):92-107.
28. Wilson DJ. Insights from genomics into bacterial pathogen populations. PLOS Pathog. 2012;8(9).
DOI: doi.org/10.1371/journal.ppat.1002874
29. Chennai Declaration Team. Chennai declaration: 5-year plan to tackle the challenge of anti-microbial resistance. Indian J Med Micro. 2014;32(3):221-228.
30. Ghafur A, Mathai D, Muruganathan A, Jayalal JA, Kant R, Chaudhary D, Prabhash K, Abraham OC, Gopalakrishnan R, Ramasubramanian V, Shah SN, Pardeshi R, Huilgol A, Kapil A, Gill J, Singh S, Rissam HS, Todi S, Hegde BM, Parikh P. The Chennai declaration: A roadmap to tackle the challenge of antimicrobial resistance. Indian J Cancer. 2013;50:71-3.
31. Lopatkin AJ, Meredith HR, Srimani JK, Pfeiffer C, Durrett R, You L. Persistence and reversal of plasmid-mediated antibiotic resistance. Nature Comm. 2017;8:1689.
DOI: 10.1038/s41467-017-01532-1

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history/23410