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Studies of the Impact of Occupational Exposure of Pharmaceutical Workers on the Development of Antimicrobial Drug Resistance

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Abstract: Studies of the Impact of Occupational Exposure of Pharmaceutical Workers on the Development of Antimicrobial Drug Resistance: Md. Moklesur Rahman SARKER, *et al*. Department of Pharmacy, School of Science, Primeasia University, Bangladesh—Objectives: Pharmaceutical workers involved with the production of antimicrobial drugs are exposed to various antimicrobial chemicals in different steps of manufacturing such as grinding, sieving, compression, granulation, mixing and filling. These exposures may lead to the development of multidrug resistance (MDR) in bacteria. Scientific reports on the occupational health hazard of pharmaceutical workers involved in manufacturing antibiotics are scarce. The present study aimed to compare the degree of bacterial resistance in pharmaceutical workers in Bangladesh to that of individuals not involved in the pharmaceutical field. **Methods:** Twenty male workers from five local pharmaceutical companies and twenty male subjects not involved in the pharmaceutical field (non-pharmaceutical subjects) were randomly selected. Nasal fluid, mucus/cough and stool specimens were collected from each subject and were cultured separately at 37°C for 24 hours to obtain bacterial growth. The cultured species were then identified, isolated and subjected to microbial sensitivity testing against 18 different antibiotics from eight different groups by the disk diffusion method. *Staphylococcus spp., Pseudomonas spp.* and *Escherichia coli* were identified and isolated from the culture of nasal fluids, mucuses and stools, respectively. **Results:** All the isolated species of bacteria exhibited significant enhancement of the degree of MDR in pharmaceutical workers compared with non-pharmaceutical subjects. Workers with a longer working history had greater degree of antibiotic resistance and vice versa. It can be certainly considered that the exposure of pharmaceutical workers to antibiotic agents resulted in a high incidence of multidrug resistance. **Conclusions:** Effective steps should be taken to minimize inherent exposure of pharmaceutical workers to antibiotics during work to prevent antimicrobial drug resistance.
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Key words: Antibiotic resistance, Antimicrobial drug resistance, *E. coli*, Misuse of antibiotics, Occupational health hazard, Pharmaceutical workers of Bangladesh, *Staphylococcus spp*

Antimicrobial drug resistance is a great problem for the treatment of infectious diseases all over the world. Resistance is increasing not only in low- and middle-income countries but also in high-income countries due to many reasons including the misuse of antibiotics and movement of infectious people all over the world[1)]. The use of poor quality, degraded, expired, counterfeit and adulterated drugs are also among the several prime reasons[2)]. In the above cases, the amounts of drugs administered into the human body fail to achieve the minimum effective concentration (MEC) levels; therefore, bacteria can easily develop resistance against the drugs when the drug is readministered. In general, workers in the pharmaceutical industries are constantly exposed to various chemical substances, such as organic solvents, vapors, dusts

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of various chemicals including active ingredients and excipients, used in the processing of final products. On exposure, the chemical substances enter the body through different routes, such as inhalation, ingestion and dermal penetration, which results in damage of many organic systems and carcinogenic, mutagenic and teratogenic effects, particularly in personnel working with potent chemicals including steroids, cytotoxic anticancer drugs and antibiotics, if careful and appropriate measures are not taken[3].

Pharmaceutical workers involved with the production of antibiotics are frequently exposed to the drug materials in the course of their manufacturing if exposure is not adequately controlled[4]. Prolonged exposure of workers to antimicrobial agents, especially dusts of those chemicals, results in the development of bacterial resistance against the exposed antibiotics. There is no routine program to test for antimicrobial resistance of pharmaceutical workers in Bangladesh. Therefore, the actual status of antimicrobial drug resistance (ADR) in pharmaceutical workers involved in the production of antimicrobials is totally unknown in Bangladesh. To date, no scientific report has been published on either the effect of occupational exposure of pharmaceutical workers to antibiotics or any other occupational adverse effects of chemical exposure on health in Bangladesh. The main problem of multiple drug resistance (MDR) is that it limits the choice of antibiotics available for the treatment of associated infections[5] and reduces the effectiveness of antimicrobial treatment. This leads to an increase in the morbidity, mortality and treatment cost of diseases[6, 7]. In this sense, pharmaceutical workers may have a greater risk of treatment of infectious diseases caused by the microbes, which may have already become resistant in them. Therefore, the aim of the present study was to assess the status of bacterial resistance in pharmaceutical workers engaged in the production of antimicrobial drugs in five pharmaceutical companies located in Dhaka, Bangladesh.

Materials and Methods

Chemicals and reagents

Nutrient agar, MacConkey agar, Müller-Hinton agar media and blood agar media base were purchased in powder form from Sigma-Aldrich GmbH, Germany. The final media for culture were prepared as per the instructions of the manufacturer and autoclaved for 15 minutes at 15 lbs pressure and 121ºC just before use except in case of blood agar media. For the preparation of blood agar media, 40 g of blood agar base powder was dissolved in 1,000 m*l* distilled water and autoclaved for 15 minutes. It was then cooled to 45–50°C, and 50 m*l* of aseptically collected defibrinated blood was added to it[8]. After mixing properly, the prepared blood agar media was poured into sterile petri plates for bacterial culture.

Selection of pharmaceutical workers and healthy volunteers

The study was conducted on twenty male workers (mean age: 36.5 ± 6.63 years) from five different pharmaceutical companies (average working experiences: 9.7 ± 3.8 years). All these were local pharmaceutical companies operated under license by a regulatory authority of Bangladesh, the Directorate General of Drug Administration (DGDA) of Bangladesh. These companies are supposed to follow the world health organization recommended guidelines for good manufacturing practices (WHO-GMP) as instructed and verified by the DGDA. None of the companies had any certification for manufacturing of quality medicines from an internationally recognized regulatory authority such as the United States Food and Drug Administration (US-FDA), Medicines and Healthcare Products Regulatory Agency (MHRA) of the United Kingdom and Therapeutic Goods Administration (TGA) of Australia. But two companies among the five had ISO-9001 certification. The ranks of the five companies were between 50 to 120 among the 197 pharmaceutical companies functionally operating in Bangladesh. The companies and the workers were selected randomly on a convenience basis. A total of 18 antibiotics were manufactured by the five selected pharmaceutical companies, and they were categorized into nine different antibiotic groups (based on chemical structure): penicillins (amoxicillin (30 μg/disk) and cloxacillin (5 μg/disk)), cephalosporins (1st generation, cephalothin (30 μg/disk) and cephradine (25 μg/disk); 2nd generation, cefuroxime (30 μg/disk); 3rd generation, cefixime (5 μg/disk)), aminoglycosides (kanamycin, streptomycin and neomycin having concentrations of 30, 10 and 30 μg/disk, respectively), glycopeptides (vancomycin (30 μg/disk)), macrolides (erythromycin (15 μg/disk) and azithromycin (15 μg/disk)), quinolones (ciprofloxacin (15 μg/disk) and levofloxacin (5 μg/disk)), tetracyclines (tetracycline (30 μg/disk) and doxycycline (30 μg/disk)), sulfonamides (cotrimoxazole (25 μg/disk)) and chloramphenicol (30 μg/disk). Pharmaceutical workers were exposed to these antibiotics during their manufacturing processes. Workers involved in at least one of several stages in the production of antibiotics and antimicrobial drugs, for example, dispensing, blending, filling of capsules and dry powder for suspensions, were considered in this study. The workers voluntarily participated and provided written informed consent. The inclusion criteria for the workers were as follows:

i) Workers who had been associated with the production of antibiotics for at least 3 years

ii) Workers who had not been treated with antibiotics for the last 3 months before the collection of samples
iii) Workers who were free from any kind of ailments including skin lesions and allergies

Twenty healthy male subjects not involved in the pharmaceutical field (non-pharmaceutical subjects; mean age: 34.25 ± 5.33 years) who met inclusion criteria ii) and iii) were randomly selected and recruited as controls for comparison of the data obtained from study subjects (pharma. workers) with their written informed consent. The study was performed as per the Declaration of Helsinki and according to the protocol approved by the ethical committee of the School of Science, Primeasia University, Dhaka, Bangladesh.

Collection of sample specimens

Nasal swabs, mucus and stool were aseptically collected at the homes of the subjects as samples using sterile swab sticks and plastic containers for both the study subjects and control volunteers in the morning; the subjects were asked to refrain from brushing their teeth, spitting and having breakfast before sample collection.

Inoculation of samples and isolation and identification of bacteria

Microorganisms were identified by morphological and biochemical tests. Nasal swabs and mucus were inoculated into blood agar, and stool was inoculated into MacConkey agar media for the detection of *Staphylococcus, Pseudomonas* and *E. coli*, respectively, which are usually prevalent in those kinds of samples. After sample inoculation, plates were incubated at 37°C for 24 hours. Aseptic conditions were maintained in every step of experiments. Each bacterium was isolated as a single colony and identified by colony-morphology, and isolates were confirmed by standard chemical tests (gram staining, catalase and oxidase tests)[9].

Determination of antimicrobial susceptibility

In vitro susceptibility of bacteria to different antibiotics was tested by the disk diffusion method[10, 11]. A single colony of identified bacteria was spread on a Petri dish containing Müller-Hinton agar media. Antibiotic disks were purchased from Oxoid, United Kingdom, and HiMedia, India. Seven to nine commercially available fixed-concentration, paper antibiotic discs were placed on the surface of inoculated agar medium in each Petri dish for incubation at 37°C for 24 hours[12–14]. At the end of culture, the diameters of the zones of inhibition were measure to the nearest whole millimeter as recommended by the National Committee for Clinical Laboratory Standards[15]. The sensitivity status of bacteria, defined as susceptible (S) (a status in which the growth of the bacteria is inhibited *in vitro* by the applied concentration of the antibiotic and the antibiotic dose is therapeutically effective), moderately susceptible (M) (bacterial response to the drug is lower, and the drug may fail to achieve a therapeutic response) and resistant (R) (bacteria are not responsive to the given antibiotic, which clearly indicates therapeutic failure) to antibiotics, was determined according to the specifications of the Clinical and Laboratory Standards Institute (CLSI)[14–16] which are listed in Table 1.

Determination of the impact of working history on bacterial resistance

The pharmaceutical workers (n=20) were categorized according to their number of working years in the pharmaceutical companies as follows: i) workers with a work tenure of 1–5 years (n=8), ii) workers with a work tenure of 6–10 years (n=7) and c) workers with a work tenure of >11 years (n=5). The percentage of resistant workers in each group was calculated by using the following formula:

$$\% \text{ of resistant} = \frac{\text{No. of resistant workers in a category}}{\text{Total no. of workers in a category}} \times 100$$

Statistical analysis

The data for sensitivity and the development of resistance among the pharmaceutical and healthy volunteers were compared and statistically analyzed by using the Chi-square test. A p-value of less than 0.05 was considered as significant ($*p<0.05$, $**p<0.01$, $***p<0.001$).

Results

Twenty pharmaceutical workers from five different pharmaceutical companies and twenty healthy volunteers were randomly selected for this study. Nasal fluid, mucus/cough, stool and blood samples were collected from the volunteers. On incubation and subsequent identification, *Staphylococcus spp., Pseudomonas spp.* and *E. coli* were isolated from the culture of nasal fluid, mucus and stool, respectively. The isolates were further confirmed by standard biochemical tests. No growth of microorganisms was found in the culture of blood.

The isolates analyzed to determine the antibiotic susceptibility pattern against widely used drugs in Bangladesh are presented in Tables 2, 3 and 4.

We compared the data obtained from the sensitivity and resistance profile of *Staphylococcus spp.* between the pharmaceutical workers and non-pharmaceutical healthy subjects. Moderate resistance data was not

Table 1. Zone diameter interpretive standards chart for the determination of antibiotic sensitivity and resistance status by the Disk Diffusion method[15)]

Name of antibiotics (dose)	Inhibitory zone diameter to nearest millimeter (mm)		
	Sensitive (S)	Moderately sensitive (MS)	Resistant (R)
Amoxicillin (30 μg/disk)	≥18	14–17	≤13
Cloxacillin (5 μg/disk)	≥25	22–24	≤21
Cephalothin (30 μg/disk)	≥18	15–17	≤14
Cephradine (25 μg/disk)	≥18	13–17	≤12
Cefuroxime (30 μg/disk)	≥23	15–22	≤14
Cefixime (5 μg/disk)	≥19	16–18	≤15
Kanamycin (30 μg/disk)	≥18	14–17	≤13
Streptomycin (10 μg/disk)	≥15	12–14	≤11
Neomycin (30 μg/disk)	≥17	13–16	≤12
Vancomycin (30 μg/disk)	≥12	10–11	≤9
Erythromycin (15 μg/disk)	≥23	14–22	≤13
Azithromycin (15 μg/disk)	≥18	14–17	≤13
Ciprofloxacin (15 μg/disk)	≥21	16–20	≤15
Levofloxacin (5 μg/disk)	≥17	14–16	≤13
Tetracycline (30 μg/disk)	≥15	12–14	≤11
Doxycycline (30 μg/disk)	≥14	11–13	≤10
Cotrimoxazole (25 μg/disk)	≥16	11–15	≤10
Chloramphenicol (30 μg/disk)	≥18	13–17	≤12

displayed in any cases because the moderately susceptible situation serves as a buffer zone between the susceptible and resistance stages. In this stage, the microbes have partially developed (also continuously developing) resistance against the antibiotic or the drug may fail to achieve a therapeutic response. The data of "moderately susceptible" cases were excluded from the analysis, as no clear conclusion could be made from them.

The degree of bacterial resistance was found to be significantly higher in the case of pharmaceutical workers compared with non-pharmaceutical volunteers against amoxicillin (p=0.007), cephalothin (p=0.020), kanamycin (p=0.011), streptomycin (p<0.001), neomycin (p=0.026), ciprofloxacin (p=0.001), levofloxacin (p=0.003) and tetracycline (p=0.004) (Table 2). Overall, the degree of resistance of *Staphylococcus spp.* against amoxicillin, cloxacillin, kanamycin and cotrimoxazole were high in case of both the pharmaceutical and non-pharmaceutical subjects. Exceptional results were also found; in contrast to pharmaceutical workers, the degree of resistance of *Staphylococcus spp.* in the non-pharmaceutical volunteers was found to be significantly higher against azithromycin (p=0.021) when compared between the two groups (Table 2).

As shown in Table 3, the degree of resistance of *Pseudomonas spp.* was found to be significantly higher in pharmaceutical workers compared with the non-pharmaceutical subjects against cephradine (p=0.027), kanamycin (p=0.012), streptomycin (p<0.001), neomycin (p=0.031), vancomycin (p<0.001), ciprofloxacin (p=0.022), tetracycline (p=0.031), doxycycline (p<0.001), cotrimoxazole (p=0.006), and chloramphenicol (p<0.001). In general, a high degree of resistance was observed to be developed by *Pseudomonas spp.* against amoxicillin, cefixime, tetracycline and cotrimoxazole in the case of both the pharmaceutical and non-pharmaceutical subjects.

The degree of resistance of *E. coli* against amoxicillin (p<0.001), cloxacillin (p=0.029), cephalothin (p=0.003), cephradine (0.009), cefuroxime (0.029), kanamycin (p<0.001), streptomycin (p<0.001), neomycin (p=0.021), vancomycin (p<0.001), erythromycin (p=0.005), levofloxacin (p<0.001), tetracycline (p<0.001), doxycycline (p=0.005) and chloramphenicol (p<0.001) was found to be significantly higher in the pharmaceutical workers compared with the non-pharmaceutical subjects (Table 4). On the other hand, the degree of resistance of *E. coli* against doxycycline and cotrimoxazole was found to be much higher in the case of both the pharmaceutical and non-pharmaceutical subjects.

The incidences of bacterial resistance in the work-

Table 2. Comparison of the antibiotic resistance profiles of *Staphylococcus spp.* derived from nasal fluid of 20 pharmaceutical workers and 20 non-pharmaceutical healthy subjects. The sensitivity was tested in culture at 37°C for 24 hours by measuring the zone of inhibition against each antibiotic

Name of antibiotics (dose)	Pharma worker frequency (percentage)	Non-pharma volunteer frequency (percentage)	Chi-square test value	*p*-value
Amoxicillin (30 μg/disk) (n=40)				
Sensitive	0 (0)	6 (33.3)	7.917	0.007**
Resistant	20 (100)	12 (66.7)		
Cloxacillin (5 μg/disk) (n=40)				
Sensitive	2 (11.1)	8 (40)	4.077	0.067
Resistant	16 (88.9)	12 (60)		
Cephalothin (30 μg/disk) (n=40)				
Sensitive	4 (25)	12 (66.7)	5.903	0.020*
Resistant	12 (75)	6 (33.3)		
Cephradine (25 μg/disk) (n=40)				
Sensitive	8 (40)	10 (55.6)	0.920	0.516
Resistant	12 (60)	8 (44.4)		
Cefuroxime (30 μg/disk) (n=40)				
Sensitive	4 (33.3)	6 (30)	0.039	1.00
Resistant	8 (66.7)	14 (70)		
Cefixime (5 μg/disk) (n=40)				
Sensitive	10 (55.6)	8 (44.4)	0.444	0.740
Resistant	8 (44.4)	10 (55.6)		
Kanamycin (30 μg/disk) (n=40)				
Sensitive	0 (0)	8 (40)	7.323	0.011*
Resistant	14 (100)	12 (60)		
Streptomycin (10 μg/disk) (n=40)				
Sensitive	0 (0)	14 (70)	19.950	<0.001***
Resistant	18 (100)	6 (30)		
Neomycin (30 μg/disk) (n=40)				
Sensitive	6 (30)	14 (70)	6.400	0.026*
Resistant	14 (70)	6 (30)		
Vancomycin (30 μg/disk) (n=40)				
Sensitive	6 (75)	20 (100)	5.385	0.074
Resistant	2 (25)	0 (0)		
Erythromycin (15 μg/disk) (n=40)				
Sensitive	6 (42.9)	8 (66.7)	1.474	0.267
Resistant	8 (57.1)	4 (33.3)		
Azithromycin (15 μg/disk) (n=40)				
Sensitive	18 (100)	14 (70)	6.413	0.021*
Resistant	0 (0)	6 (30)		
Ciprofloxacin (15 μg/disk) (n=40)				
Sensitive	0 (0)	12 (60)	11.520	0.001**
Resistant	12 (100)	8 (40)		
Levofloxacin (5 μg/disk) (n=40)				
Sensitive	0 (0)	6 (42.9)	9.495	0.003**
Resistant	18 (100)	8 (57.1)		
Tetracycline (30 μg/disk) (n=40)				
Sensitive	0 (0)	20 (100)	22.000	0.004**
Resistant	2 (100)	0 (0)		
Doxycycline (30 μg/disk) (n=40)				
Sensitive	4 (28.6)	12 (60)	3.265	0.092
Resistant	10 (71.4)	8 (40)		
Cotrimoxazole (25 μg/disk) (n=40)				
Sensitive	0 (0)	3 (15)	3.243	0.231
Resistant	20 (100)	17 (85)		
Chloramphenicol (30 μg/disk) (n=40)				
Sensitive	6 (30)	8 (57.1)	2.505	0.163
Resistant	14 (70)	6 (42.9)		

*p<0.05; **p<0.01; ***p<0.001.

Table 3. Comparison of the antibiotic resistance profiles of *Pseudomonas spp*. derived from mucus/cough of 20 pharmaceutical workers and 20 non-pharmaceutical healthy subjects. The sensitivity was tested in culture at 37°C for 24 hours by measuring the zone of inhibition against each antibiotic

Name of antibiotics (dose)	Pharma workers frequency (percentage)	Non-pharma volunteer frequency (percentage)	Chi-square test value	*p*-value
Amoxicillin (30 μg/disk) (n=40)				
Sensitive	0 (0)	2 (12.5)	1.875	0.485
Resistant	14 (100)	14 (87.5)		
Cloxacillin (5 μg/disk) (n=40)				
Sensitive	6 (37.5)	12 (66.7)	2.892	0.168
Resistant	10 (62.5)	6 (33.3)		
Cephalothin (30 μg/disk) (n=40)				
Sensitive	14 (77.8)	14 (70)	0.296	0.719
Resistant	4 (22.2)	6 (30)		
Cephradine (25 μg/disk) (n=40)				
Sensitive	10 (55.6)	18 (90)	5.797	0.027*
Resistant	8 (44.4)	2 (10)		
Cefuroxime (30 μg/disk) (n=40)				
Sensitive	6 (37.5)	8 (50)	0.508	0.722
Resistant	10 (62.5)	8 (50)		
Cefixime (5 μg/disk) (n=40)				
Sensitive	6 (33.3)	8 (44.4)	0.468	0.733
Resistant	12 (66.7)	10 (55.6)		
Kanamycin (30 μg/disk) (n=40)				
Sensitive	8 (57.1)	19 (95)	7.219	0.012*
Resistant	6 (42.9)	1 (5)		
Streptomycin (10 μg/disk) (n=40)				
Sensitive	0 (0)	14 (82.4)	21.024	<0.001***
Resistant	14 (100)	3 (17.6)		
Neomycin (30 μg/disk) (n=40)				
Sensitive	6 (42.9)	14 (82.4)	5.231	0.031*
Resistant	8 (57.1)	3 (17.6)		
Vancomycin (30 μg/disk) (n=40)				
Sensitive	0 (0)	18 (100)	26.000	<0.001***
Resistant	8 (100)	0 (0)		
Erythromycin (15 μg/disk) (n=40)				
Sensitive	4 (33.3)	8 (57.1)	1.474	0.267
Resistant	8 (66.7)	6 (42.9)		
Azithromycin (15 μg/disk) (n=40)				
Sensitive	8 (42.1)	10 (55.6)	0.669	0.517
Resistant	11 (57.9)	8 (44.4)		
Ciprofloxacin (15 μg/disk) (n=40)				
Sensitive	0 (0)	8 (50)	6.000	0.022*
Resistant	8 (100)	8 (50)		
Levofloxacin (5 μg/disk) (n=40)				
Sensitive	10 (62.5)	14 (77.8)	0.952	0.457
Resistant	6 (37.5)	4 (22.2)		
Tetracycline (30 μg/disk) (n=40)				
Sensitive	0 (0)	6 (30)	5.100	0.031*
Resistant	14 (100)	14 (70)		
Doxycycline (30 μg/disk) (n=40)				
Sensitive	0 (0)	12 (75)	17.500	<0.001***
Resistant	14 (100)	4 (25)		
Cotrimoxazole (25 μg/disk) (n=40)				
Sensitive	0 (0)	6 (37.5)	8.196	0.006**
Resistant	18 (100)	10 (62.5)		
Chloramphenicol (30 μg/disk) (n=40)				
Sensitive	0 (0)	18 (94.7)	29.179	<0.001***
Resistant	14 (100)	1 (5.3)		

*p<0.05; **p<0.01; ***p<0.001.

ers with longer working histories was found to be greater compared with the workers with shorter working histories and vice versa (Table 5). Overall, in the case of *Staphylococcus spp., Pseudomonas spp.* and *E. coli*, the percentage of workers who developed antibiotic resistance was found to be higher in workers having working histories more than 11 years compared with those having working histories of 6–10 years or 1–5 years. Similarly, the frequency of a higher percentage of resistant workers was found to be greater in the case of pharmaceutical workers having working histories of 6–10 years in comparison with those having working histories of 1–5 years.

Discussion

In this study, we evaluated the susceptibility of *Staphylococcus spp., Pseudomonas spp.* and *E. coli* bacteria isolated from culture specimens of nasal fluid, cough/mucus and stool from pharmaceutical and non-pharmaceutical subjects against all the antibiotics manufactured by the five investigated pharmaceutical companies. Our study demonstrated that all the isolated bacteria developed MDR not only in the pharmaceutical workers but also in the non-pharmaceutical healthy subjects. But the quantity and intensity of MDR in pharmaceutical subjects were higher than in the non-pharmaceutical volunteers (Tables 2, 3 and 4). Thus, our observation clearly demonstrated an enhanced antibiotic resistance status in the pharmaceutical workers involved in manufacturing antibiotics and antimicrobial drugs. Table 2 shows that the bacterial resistance statuses of streptomycin, ciprofloxacin, levofloxacin and tetracycline were highly significant in *Staphylococcus spp.* in pharmaceutical workers compared with in the non-pharmaceutical subjects. Pharmaceutical workers might be highly exposed to these antibiotics in any of their manufacturing processes. Therefore, these four antibiotics may be considered risk factors for occupational exposure during the manufacture of antibiotics. Similarly, streptomycin, vancomycin, doxycycline, cotrimoxazole and chloramphenicol may be considered risk factors for occupational health hazard in the development of antibiotic resistance by *Pseudomonas spp.* (Table 3). *E. coli* was found to have resistance to the highest number of antibiotics in the case of pharmaceutical workers compared with the non-pharmaceutical volunteers (Table 4). Hence, amoxicillin, cephalothin, cephradine, kanamycin, streptomycin, vancomycin, erythromycin, levofloxacin, tetracycline, doxycycline and chloramphenicol may be considered occupational risk factors for development of antibiotic resistance in pharmaceutical workers.

An exceptional observation in our findings is that the resistance status of *Staphylococcus spp.* against azithromycin was higher in the non-pharmaceutical subjects compared with the pharmaceutical workers (Table 2). This can be explained by the fact that the non-pharmaceutical volunteers may have been previously administered azithromycin more frequently than the pharmaceutical workers. The resistance against this antibiotic may have developed in the non-pharmaceutical volunteers due to discontinuation of courses of the drugs during treatment, failure to maintain a proper dose interval, selection of the wrong antibiotic, taking these drugs without a prescription from a physician, frequent self-use of antibiotics, use of antibiotics prescribed by a quack or use of a substandard brand of an antibiotic. It is worthy to mention here that antibiotics are sold without a prescription; overuse and discontinuation of a prescribed dose are very common features in Bangladesh.

High exposure to different chemicals occurs in the grinding, sieving, compression, granulation, mixing, filling and packing steps during the manufacture of medicines in pharmaceutical industries[17]. Occupational exposure over a length of time, especially exposure to potent chemicals, such as hormones, antibiotics and anticancer agents, may cause the development of cancer, reproductive problems[3, 18], occupational asthma, musculoskeletal disorders and skin disorders[17, 19]. However, reports on occupational exposure to antibiotics are scarce worldwide, and in Bangladesh, such kind of scientific report is completely absent. The pharmaceutical sector in Bangladesh is booming and very prospective. However, no hard and fast rules or guidelines regarding occupational health hazards has been provided by the drug controlling authority of Bangladesh (DGDA) to the pharmaceutical industries.

Another important observation of our study is that the isolated bacteria were highly resistant to some antibiotics in both the pharmaceutical and non-pharmaceutical subjects. As shown in Table 2, the resistance profile of *Staphylococcus spp.* against amoxicillin, cloxacillin, kanamycin and cotrimoxazole was found to be higher than it usually should be in both groups of subjects. Similarly, it was found that *Pseudomonas spp.* was highly unresponsive to amoxicillin, cefixime, tetracycline and cotrimoxazole and that *E. coli* was highly resistant to doxycycline and cotrimoxazole in both groups of subjects. The observation indicates that the resistance developed by the specified bacteria to the antibiotics may not due to occupational exposure to the chemicals but rather because of several other causes of antibiotic resistance, such as treatment with substandard or counterfeit antibiotics[20, 21], easy availability of antibiotics without a prescription or professional control[22], incorrect selection of antibiotics, improper doses

Table 4. Comparison of the antibiotic resistance profiles of *E. coli* derived from stool of 20 pharmaceutical workers and 20 non-pharmaceutical healthy subjects. The sensitivity was tested in culture at 37°C for 24 hours by measuring the zone of inhibition against each antibiotic

Name of antibiotics (dose)	Pharma worker frequency (percentage)	Non-pharma volunteers frequency (percentage)	Chi-square test value	*p*-value
Amoxicillin (30 μg/disk) (n=40)				
Sensitive	0 (0)	14 (77.8)	22.909	<0.001***
Resistant	18 (100)	4 (22.2)		
Cloxacillin (5 μg/disk) (n=40)				
Sensitive	10 (52.6)	16 (88.9)	5.816	0.029*
Resistant	9 (47.4)	2 (11.1)		
Cephalothin (30 μg/disk) (n=40)				
Sensitive	10 (55.6)	18 (100)	10.286	0.003**
Resistant	8 (44.4)	0 (0)		
Cephradine (25 μg/disk) (n=40)				
Sensitive	6 (40)	14 (87.5)	7.630	0.009**
Resistant	9 (60)	2 (12.5)		
Cefuroxime (30 μg/disk) (n=40)				
Sensitive	10 (52.6)	16 (88.9)	5.816	0.029*
Resistant	9 (47.4)	2 (11.1)		
Cefixime (5 μg/disk) (n=40)				
Sensitive	12 (60)	18 (90)	4.800	0.065
Resistant	8 (40)	2 (10)		
Kanamycin (30 μg/disk) (n=40)				
Sensitive	0 (0)	20 (100)	34.000	<0.001***
Resistant	14 (100)	0 (0)		
Streptomycin (10 μg/disk) (n=40)				
Sensitive	6 (37.5)	14 (100)	13.125	<0.001***
Resistant	10 (62.5)	0 (0)		
Neomycin (30 μg/disk) (n=40)				
Sensitive	14 (70)	18 (100)	6.413	0.021*
Resistant	6 (30)	0 (0)		
Vancomycin (30 μg/disk) (n=40)				
Sensitive	0 (0)	14 (100)	34.000	<0.001***
Resistant	20 (100)	0 (0)		
Erythromycin (15 μg/disk) (n=40)				
Sensitive	2 (14.3)	12 (66.7)	8.780	0.005**
Resistant	12 (85.7)	6 (33.3)		
Azithromycin (15 μg/disk) (n=40)				
Sensitive	12 (60)	16 (80)	1.905	0.301
Resistant	8 (40)	4 (20)		
Ciprofloxacin (15 μg/disk) (n=40)				
Sensitive	4 (28.6)	10 (62.5)	3.453	0.081
Resistant	10 (71.4)	6 (37.5)		
Levofloxacin (5 μg/disk) (n=40)				
Sensitive	0 (0)	18 (100)	32.000	<0.001***
Resistant	14 (100)	0 (0)		
Tetracycline (30 μg/disk) (n=40)				
Sensitive	0 (0)	18 (90)	32.727	<0.001***
Resistant	20 (100)	2 (10)		
Doxycycline (30 μg/disk) (n=40)				
Sensitive	0 (0)	8 (40)	8.229	0.005**
Resistant	16 (100)	12 (60)		
Cotrimoxazole (25 μg/disk) (n=40)				
Sensitive	0 (0)	4 (25)	4.571	0.101
Resistant	16 (100)	12 (75)		
Chloramphenicol (30 μg/disk) (n=40)				
Sensitive	4 (20)	18 (100)	24.873	<0.001***
Resistant	16 (80)	0 (0)		

*p<0.05; **p<0.01; ***p<0.001.

Table 5. Impact of duration of work at the pharmaceutical companies on the development of antibiotic resistance among pharmaceutical workers

Name of the bacteria	Name of antibiotics (dose/disk)	Total no. of pharmaceutical workers who became resistant among entire group (n=20)	No. and % of workers with working durations of 1–5 years who became resistant (n=8)	No. and % of workers with working durations of 6–10 years who became resistant (n=7)	No. and % of workers with working durations of >11 years who became resistant (n=5)
Staphylococcus spp.	Amoxicillin (30 μg)	20	8 (100%)	7 (100%)	5 (100%)
	Cephalothin (30 μg)	12	4 (50%)	5 (71.4%)	4 (80%)
	Kanamycin (30 μg)	14	2 (25%)	7 (100%)	5 (100%)
	Streptomycin (10 μg)	18	7 (87.5%)	6 (86%)	5 (100%)
	Neomycin (30 μg)	14	5 (62.5%)	5 (71.4%)	4 (80%)
	Ciprofloxacin (15 μg)	12	1 (12.5%)	6 (85.7%)	5 (100%)
	Levofloxacin (5 μg)	18	6 (75%)	7 (100%)	5 (100%)
	Tetracycline (30 μg)	2	0 (0%)	1 (14%)	1 (20%)
Pseudomonas spp.	Cephradine (25 μg)	8	1 (12.5%)	3 (42.85%)	4 (80%)
	Kanamycin (30 μg)	6	0 (0%)	3 (42.85%)	3 (60%)
	Streptomycin (10 μg)	14	4 (50%)	6 (85.7%)	4 (80%)
	Neomycin (30 μg)	8	2 (25%)	3 (42.85%)	3 (60%)
	Vancomycin (30 μg)	8	2 (25%)	4 (57%)	2 (40%)
	Ciprofloxacin (15 μg)	8	1 (12.5%)	3 (42.85%)	4 (80%)
	Tetracycline (30 μg)	14	4 (50%)	5 (71.4%)	5 (100%)
	Doxycycline (30 μg)	14	3 (37.5%)	6 (85.7%)	5 (100%)
	Cotrimoxazole (25 μg)	18	7 (87.5%)	6 (85.7%)	5 (100%)
	Chloramphenicol (30 μg)	14	5 (62.5%)	5 (71.4%)	4 (80%)
Escherichia coli (E. coli)	Amoxicillin (30 μg)	18	6 (75%)	7 (100%)	5 (100%)
	Cloxacillin (5 μg)	9	2 (25%)	4 (57%)	3 (60%)
	Cephalothin (30 μg)	8	2 (25%)	3 (42.85%)	3 (60%)
	Cephradine (25 μg)	9	3 (37.5%)	2 (28.5%)	4 (80%)
	Cefuroxime (30 μg)	9	1 (12.5%)	3 (42.85%)	5 (100%)
	Kanamycin (30 μg)	14	4 (50%)	5 (71.4%)	5 (100%)
	Streptomycin (10 μg)	10	4 (50%)	3 (42.85%)	3 (60%)
	Neomycin (30 μg)	6	0 (0%)	3 (42.85%)	3 (60%)
	Vancomycin (30 μg)	20	8 (100%)	7 (100%)	5 (100%)
	Erythromycin (15 μg)	12	2 (25%)	5 (71.4%)	5 (100%)
	Levofloxacin (5 μg)	14	3 (37.5%)	6 (85.7%)	5 (100%)
	Tetracycline (30 μg)	20	8 (100%)	7 (100%)	5 (100%)
	Doxycycline (30 μg)	16	5 (62.5%)	6 (85.7%)	5 (100%)
	Chloramphenicol (30 μg)	16	4 (50%)	7 (100%)	5 (100%)

and discontinuing antibiotics before the end of the treatment course. This may be representative of the general feature of antibiotic resistance among the general population of Bangladesh. Antibiotics are the most commonly recommended drugs in general practice and in hospitals in Bangladesh[23]. In most cases, antibiotics are prescribed based on physical observation and asking questions to patients rather than clinical and microbiological investigations. Besides, most patients are treated by quacks and unqualified health workers who usually make mistakes in diagnosing diseases and in the selection of proper antibiotics. Sometimes they offer antibiotics for unnecessary cases. Moreover, manufacturing and dispensing of antibiotics are not strictly controlled. Substandard antibiotics are very common in Bangladesh. These antibiotics are administered by patients who receive prescription from quacks or who are self-medicating. Antibiotics are sold all over the country without a prescription. Therefore, self-medication of antibiotics is very common, and thus it plays a great role in the development of resistance against antibiotics.

Limitations of the study

Although 197 pharmaceutical companies are functionally operating in Bangladesh at present, we conducted our study in five randomly selected pharmaceutical companies. One limitation of this study may be that the data from these five companies may not represent the real features of all the pharmaceutical companies in Bangladesh. Also, the limited number of pharmaceutical workers may also be another limitation of this study.

Conclusions

The present study demonstrated that pharmaceutical workers involved in manufacturing antibiotic drugs are occupationally exposed to different antimicrobial chemicals and that this causes their MDR profiles to be higher compared with general people in Bangladesh. Expert handling of antibiotic chemicals and equipment and knowledge and training about personal hygiene and occupational health hazards associated with the handling of the chemicals can minimize the development of antimicrobial resistance. Dust in the production area should be strictly controlled by de-dusting and use of a proper ventilation system. Personal protection such as use of gloves and respirators is also very important to prevent bacterial resistance. Appropriate medical surveillance programs and regular physical examinations are needed to prevent morbidity and to minimize the lethality of diseases. Pharmaceutical companies in Bangladesh should set health-based occupational exposure limits (OELs) for pharmaceutical active ingredients and products as in-house risk management measures to prevent health hazards from chemical exposure according to the guidelines of OELs for the pharmaceutical industry[24–27]. In general, the bacterial resistance profiles of both the pharmaceutical workers and the non-pharmaceutical healthy volunteers were also alarming. Therefore, effective steps must be taken against the misuse of antibiotics to reduce MDR in the general population of Bangladesh as well.

Conflict of interest: The authors declare that they have no conflicts of interest.

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