Infection and Drug Resistance

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ORIGINAL RESEARCH

Antibiograms of Gut Flora of Poultry Farms Workers Reveal Higher Resistance Levels as Compared to Non-Workers

Muhammad Qamar Saeed[1], Mubashar Aziz[1], Raja Kamran Afzal[2], Aamir Hussain[3], Hamid Manzoor[4], Sumaira Rasul[4], Tahira Ruby[5], Ahmed Akrem[5], Samrah Masud[5], Adnan Arshad[1], Ayesha Latif[1], Muhammad Yousif[1], Hamdan Aali[1], Menahil Fatima[1], Muhammad Mujahid Bhutta[1], Aleem Ahmed Khan[5]

[1]Dr. Ghulam Nabi Chaudhary Laboratory of Microbial Technologies, Department of Microbiology and Molecular Genetics, Institute of Pure and Applied Biology, Bahauddin Zakariya University, Multan, Pakistan; [2]Department of Microbiology, CMH Institute of Medical Sciences, Multan, Pakistan; [3]Institute of Basic Medical Sciences, Khyber Medical University, Peshawar, Pakistan; [4]Institute of Molecular Biology and Biotechnology, Bahauddin Zakariya University Multan, Multan, Pakistan; [5]Institute of Pure and Applied Biology, Bahauddin Zakariya University Multan, Multan, Pakistan

Correspondence: Muhammad Qamar Saeed, Dr. Ghulam Nabi Chaudhary Laboratory of Microbial Technologies, Department of Microbiology, Institute of Pure and Applied Biology, Bahauddin Zakariya University Multan, Pakistan, Tel +92-333-0231222, Email mqamarsaeed@bzu.edu.pk

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Introduction: Antibiotics are being used in humans and animals for treatment and control of bacterial infections. Excessive use of antibiotics in the production of poultry is a popular practice, but it poses serious health issues by transferring resistance from farm to humans via food or direct exposure.

Study Objective: The objective of this study was to carry out a comparison of the resistance and sensitivity profile of isolated isolates from sewage of toilets that were in use of workers inside the farm and from sewage of household toilets.

Methodology: In this study, a total of 320 sewage samples were collected. The antibiotic susceptibility profile was checked by Kirby-Bauer disc diffusion method, and the statistical analysis was carried out by MS excel. Chi-square test was performed to determine whether the antibiograms from two sample types were statistically different from each other or not.

Results: From 320 sewage samples, a total of 296 bacterial isolates were isolated among which the leading bacterium was *E. coli*. The proportion of resistance, ESBL production and MDR was significantly higher in bacteria isolated from sewage of toilets under use of poultry farm workers as compared to the sewage from domestic use toilets.

Conclusion: Resistance significantly increased in the bacteria isolated from toilets under use of poultry farm workers as compared to the ones isolated from control sewage samples.

Keywords: *E. coli*, antibiotic resistance, MDR, sewage, human microbiota

Introduction

Poultry industry is one the leading food providers in the world.[1] Intensive farming, however, necessitates use of antibiotics as prophylactics and growth promoters. This use is contributing to the development and spread of antibiotic resistance in the environment. Environmental dynamics, ultimately, lead to transfer of resistance determinants into bugs of public health importance. Although, antibiotic resistance is an inevitable outcome of natural selection; excessive use of antibiotics has exacerbated the problem.[2] Zoonotic infections caused by MDR bugs are now challenging the health care establishments as increased morbidity and mortality is being reported worldwide.[3]

Poultry farms are hotspots of highest concern where resistance emerges, persists and spreads into the environment.[4,5] Antibiotics are being used in poultry not only for therapeutic purposes but also to protect them from environmental stresses, such as stocking density, and as growth promoters.[6] Therefore, the use of antibiotics in poultry farm has increased in order to cope with the challenges of intensive farming and thus increasing the production of poultry.[7] The

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incorporation of antibiotics in farms has developed antibiotic resistant strains through genetic and/or environmental processes.[8] The efficacy of currently available antimicrobial agents is diminishing because of these resistant strains causing infections.[9]

Studies showed that in Egypt, excessive use of antibiotics to increase poultry production has increased drug resistance in enteric pathogens including *E. coli* and has made the treatment of these infections difficult.[10] Researchers believe that most of the antibiotics that animals consume are excreted out of the animal body in the form of feces without any metabolism. In this way it becomes part of soil and by rinsing off water from such surfaces, these residues come into the water sources. Thus, veterinary antimicrobials threaten the environment as well as human health.[11]

Most commonly used antibiotics in agricultural field and veterinary are lincosamides, sulphonamides, aminoglycosides, macrolides, quinolones and β-lactams.[12,13] It has been observed that *E. coli* isolated from poultry has increased resistance to ampicillin and tetracyclines, and lower resistance to gentamicin and co-trimoxazole.[14] Similarly, salmonella exhibit higher resistance to sulfisoxazole and tetracyclines while lower resistance to cefoxitin.[15] In Jamaica, *E. coli* was found to be resistant against kanamycin and nalidixic acid and was sensitive to gentamicin.[16] Frequent interaction between animals and humans is a potent cause of resistance transfer from poultry to poultry attendants. As there is an interaction between animals and humans, so resistance can transfer from poultry to poultry farm attendants.[17]

Our study aims to investigate the resistance profile in gut flora of poultry farm workers in an attempt to see whether their working environment has an effect on normal commensal microbes. For this, we took sewage samples from toilets present within the farm area and under exclusive use of workers. We compared resistance profiles of the isolates with those from toilets in the households. Statistically significant differences in resistance profiles were noted, indicating that their work environment has an influence on the fecal microbiota.

Methodology

Sampling

A total of 320 sewage samples were taken from toilets that were in use of poultry farm workers and from the sewage of toilets that were for domestic use (control sewage). Sampling sites were randomly selected. We collected 160 samples from toilets within poultry farm establishments and 160 from domestic toilets. In rural areas, toilets are usually linked to "gutters" to store and decompose fecal material as it is flushed from toilets. Samples were collected those gutters in sterile containers. The sampling was basically carried out to collect the human fecal material from sewage. Containers were kept in ice until further processing. All the samples were processed within 8 hours of collection.

Culturing Characteristics and Identification

The samples were directly spread on MacConkey and blood agar plates in parallel followed by incubation at 37°C for 24 h. The colonies were identified by different biochemical tests and further confirmed by commercial kits such as rapID (Oxoid and Remel).[18]

Antimicrobial Susceptibility Testing

Kirby-Bauer disc diffusion method was used to check the susceptibility of bacterial isolates to different antibiotic classes. The inhibition zones were observed and classified according to Clinical Laboratory Standards Institute (CLSI) standards.

ESBL Production

Combined disc test was used to check the production of ESBL. Cefepime, cefotaxime, ceftazidime and cefpodoxime discs were. The inhibition zones around the discs were measured and results were predicted by use of CARBA plus calculator (Mast-Group UK, Cat No. D73C).

Statistical Analysis

Microsoft Excel was used to carry out the statistical analysis. Mean values and percentages were calculated. Chi-square test was performed and p-value was calculated accordingly.[19] It was hypothesized that resistance patterns of the samples collected from household toilets represented normal baseline resistance values. Therefore, if working environment had no influence on fecal microbiota of workers; Chi-square test would give us insignificant value. P-value was calculated and P-value <0.05 was considered statistically significant, which meant that work environment had a role in shaping resistance profiles of fecal microbes.

Results

A total of 320 sewage samples were taken from toilets that were in use of poultry farm workers and from the control sewage.

Isolation of Bacteria and Antibiotic Sensitivity and Resistance

Among total 320 sewage samples, 296 bacterial isolates were recovered. In these 296 isolates, 146 bacterial isolates were obtained from toilets that were in use of poultry farm workers and 150 bacterial isolates were recovered from control sewage (toilets for domestic use). In both sample types, *E. coli* was the leading species. Toilets under workers' use yielded 102 (69.8%) *E. coli* isolates, while 105 (70%) *E. coli* were recovered from domestic toilets. We also recovered *Salmonella* spp.,*Enterococcus* spp., *Shigella* spp. and *Enterobacter* spp. from sewage samples but isolate counts were very low (Table 1).

We applied 14 different antibiotics belonging to various classes on these isolates. For all antibiotics applied, we found higher number and percentage of resistant bugs recovered from workers' toilets (Table 1).

Table 1 Resistance Profile of Isolated Isolates to Different Classes of Antibiotics

Bacteria		chl	ctx	te	fep	cip	lvx	ofx	sxt	cro	cn	caz	cxm	mrp	amc
E. coli	A n = 102	54	22	20	23	53	74	45	46	50	51	20	31	39	37
	B n = 105	33	16	13	20	44	62	36	18	42	22	12	16	24	32
Salmonella	A n = 11	7	9	8	6	10	8	10	4	9	2	9	4	5	4
	B n = 12	3	2	1	0	2	4	4	0	0	0	3	0	0	0
Enterococcus spp.	A n = 12	3	1	6	3	1	7	5	3	4	6	2	2	2	4
	B n = 9	0	0	2	0	0	1	1	0	0	2	0	0	0	0
Shigella	A n = 12	4	5	6	6	7	10	9	2	0	2	0	2	3	3
	B n = 14	2	0	1	2	0	5	1	0	0	0	0	0	0	0
Enterobacter spp.	A n = 9	8	7	6	7	9	7	7	8	8	3	8	0	6	6
	B n = 10	3	4	4	1	4	2	3	3	2	1	5	0	1	2
Total n=296	A n = 146	76	44	46	45	80	106	76	68	71	64	39	39	55	54
	B n =150	41	22	21	22	50	74	45	21	32	25	20	16	25	34

Notes: A, Sewage from toilets under workers use; B, Sewage from toilets of domestic use.

Abbreviations: chl, Chloramphenicol; ctx, Cefotaxime; te, Tetracycline; fep, Cefepime; cip, Ciprofloxacin; lvx, Levofloxacin; ofx, Ofloxacin; sxt, Trimethoprim-Sulfamethoxazole; cro, Ceftriaxone; cn, Gentamycin; caz, Ceftazidime; cxm, Cefuroxime; mrp, Meropenem; amc, Amoxicillin-clavulanic Acid.

Comparison of Antimicrobial Sensitivity and Resistance of *E. coli*

The leading strain isolated from sewage samples was *E. coli*. Data of Kirby-Bauer disc diffusion suggests that the sensitivity of this bacterium to antibiotics isolated from sewage of toilets for domestic use was higher than the isolates isolated from sewage of toilets that were under use of poultry farm workers (Table 1). Therefore, we decided to run chi-square test to see whether the difference between resistance profiles was statistically significant or not (Table 2). We found that the differences were statistically significant in all cases except two (amoxiclav and cefepime).

Production of ESBL and MDR Isolates

The total number of isolates isolated from sewage of toilets under workers use that were producing ESBL were 20 (13.6%) and among 102 isolates of *E. coli* 11 (10.7%) were producers of ESBL. While isolates from control sewage samples, 11 (7.3%) were producing ESBL and among 105 *E. coli* isolates, 5 (4.7%) were producers of ESBL. The MDR isolates isolated from sewage samples of toilets under workers use were 37 (25.3%) and MDR isolates isolated from control sewage samples were 24 (16%) (Table 3).

Table 2 Comparison of Sensitive and Resistant Profile of Isolated Isolates

No. of Sensitive and Resistant Isolates				For Chi-Square Analysis, No. of *E. coli* Isolates Were Normalized to 105			
Antibiotics	**Pattern**	***E. coli* from Sewage of Toilets Under Workers Use (n = 102)**	***E. coli* from Control Sewage Samples (n = 105)**	***E. coli* from Sewage of Toilets Under Workers Use**	***E. coli* from Control Sewage Samples**	**Chi-Square**	**P-value**
LVX	S	28	43	29	43	7.7	0.005
	R	74	62	76	62		
AMC	S	65	73	67	73	1.6	0.5
	R	37	32	38	32		
CTX	S	80	89	82	89	3.6	0.05
	R	22	16	23	16		
CIP	S	49	61	50	61	4.7	0.05
	R	53	44	55	44		
FEP	S	79	85	81	85	1.0	0.5
	R	23	20	24	20		
OFX	S	57	69	59	69	4.2	0.05
	R	45	36	46	36		
TE	S	82	92	84	92	5.6	0.025
	R	20	13	21	13		
CRO	S	52	63	54	63	3.2	0.1
	R	50	42	51	42		

Abbreviations: CTX, Cefotaxime; TE, Tetracycline; FEP, Cefepime; CIP, Ciprofloxacin; OFX, Ofloxacin; CRO, Ceftriaxone; AMC, Amoxicillin-clavulanic Acid.

Table 3 Association of Antibiotic Exposure to Production of ESBL and MDR

Variables	ESBL Production		Ratio of Odds	MDR		Ratio of Odds
	Yes	No		Yes	No	
Sewage from toilets under workers use	20	126	2.22 (1.0–4.9) P = 0.04	37	109	1.78 (1.00–3.16) P = 0.04
Sewage from toilets of domestic use	10	140		24	126	

Abbreviations: ESBL, extended spectrum β-lactamases; MDR, multidrug resistance.

Discussion

The use of antibiotics is primarily aimed at improving animal health and increasing meat production. But it has serious consequences for human health specifically in the situations where same type of antibiotics are being used for humans.[20] Usually hospitals are considered hotspots of resistance and sewage management is advocated for them. We show that farm environment carries risks not only for the workers but also for extended environment, as resistant bugs are carried through sewage into ecological chain. Use of antibiotics develops resistance not only in the bacteria in the farm but also in the endogenous microflora of the individuals that are exposed to that environment.[21] At the time of slaughtering, the resistant isolates from the gut of poultry animals may contaminate the meat.[22] Also, the eggs become contaminated during laying with the resistant isolates. In this way the humans are affected with the resistant isolates either directly via exposure or indirectly via food.[23] The resistant isolates may colonize the gut of humans and they can also transfer the resistance genes to the normal microbiota of the gut.

In this study, we compared the bacterial isolates isolated from the sewage lines of toilets that were under use of poultry farm workers and from the sewage lines of toilets from the households. This study was carried out to check the effects of antibiotic usage in the poultry farm on the gut bacteria of workers and farm handlers. Results indicated the higher proportion of resistant bacteria isolated from sewage of toilets that were under use of poultry farm workers as compared to the bacteria isolated from control sewage. The possibility of this acquired resistance is through the excessive usage of antibiotics inside the poultry farm.

Leading species isolated from all the sewage samples was *E. coli*. The proportion of resistant *E. coli* was higher in samples isolated from sewage of toilets that were under use of poultry farm workers as compared to those isolated from control sewage. MDR *E. coli* were found in both type of samples, but results showed significant difference in overall MDR isolates isolated from sewage of toilets under farm workers use and household toilets. MDR isolates isolated from sewage of toilets that were under use of poultry farm workers were higher as compared to the sewage of household toilets. This indicate that direct exposure of workers to the inside environment of poultry farm may develop multidrug resistance in the gut microflora of the workers.

The type of antibiotics being used inside the poultry farm had a significant effect on the resistance profile of isolated bacteria. This indicates that the resistance is being transferred directly or indirectly between the isolates.

The matter of serious concern regarding antibiotic resistance is the production of ESBLs. The isolates that were isolated from sewage of toilets used by poultry farm workers and handlers had a higher production of ESBLs due to which they were posing higher level of resistance to different classes of antibiotics. The incidents of increasing ESBL production in the bacteria may limit therapeutic options and are a serious threat for environment, humans, and the health care workers.

Conclusion

This study focuses on the fact that the use of antimicrobial agents inside the poultry farm has effects on the individuals who are exposed to this environment. The resistance profile of the bacteria isolated from control sewage is lower as compared to the isolates isolated from sewage of toilets that were under use of poultry farm workers. This indicates that the resistance is being transferred from farms to humans either directly or indirectly. This practice is reducing the efficacy of therapeutics to treat life threatening infections. Thus, it is necessary to develop some other ways to increase production of poultry rather than use of antibiotics and to minimize direct exposure of workers to the inside environment of poultry farm. Additionally, protocols must be developed to keep farm sewage from entering into normal sewerage lines without treatment.

Ethical Approval

Ethical approval was not required as samples were not collected from individual persons but from toilet sewages under use of workers and non-workers.

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Disclosure

All authors declare no conflicts of interest in this work.

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