International Scholars Journals

African Journal of Virology Research ISSN 3421-7347 Vol. 2 (2), pp. 142-147, February, 2008. Available online at www.internationalscholarsjournals.org © International Scholars Journals

Full Length Research Paper

Antibiotic resistance profile of *Escherichia coli* from clinically healthy pigs and their commercial farm environments

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Accepted 11 January, 2008

The study was conducted to determine the antibiotic resistance profile of *Escherichia coli* isolated from clinically healthy pigs and their commercial farm environments. Differential and selective media were used to isolate a total of 142 *E. coli* strains from 202 samples. These were tested against 16 antibiotics using the disc diffusion method. The isolates showed high resistance rates to Cefuroxime (89.4%), Nitrofurantoin (89.4%), Tetracycline (74.6%), Ceftazidime (73.9%), Cefotaxime (72.5%) and Cephalexin (53.5%). Rates of resistance to Septrin and Chloramphenicol were moderate (12.7 to 39.4%), while low rates were recorded for Gentamycin (0.09%), Ciprofloxacine (0.08%), Perfloxacine (0.05%), Augumentine (0.06%), Nalidixic acid (0.07%), Streptomycin (0.05%) and Ofloxacine (0.05%). A total of 78 resistance patterns were identified. The high rates of resistance, as well as the large number of resistant patterns recorded in the absence of the use of antibiotics for growth promotion or as prophylactics suggested that antibiotics are not the only selective factors for antibiotic resistance.

Key words: *Escherichia coli*, antibiotics, resistance, clinically healthy, prophylaxis

INTRODUCTION

The widespread use of antibiotics in animals has raised several concerns related to human and animal health. The principal area of concern according to Chee-Sanford et al. (2001) has been the increasing emergence of antibiotic resistance phenotypes in both clinically relevant strains and normal commensal microbiota. Higher prevalence of commensal flora is also known to contribute to the general increase and dissemination of bacterial resistance worldwide (Summers, 2002) and can be a source of resistance genes for respiratory pathogens such as *Streptococcus pneumoniae* (Dowson et al., 1994) and intestinal pathogens like *Shigella* and *Salmonella* (Hunter et al., 1992).

It is well known that species of Enteriobacteriaceae are widely distributed in nature, occurring in the intestinal tract of man and animals, as well as in water and soil (Holt et al., 1994) . *Escherichia coli* are a prominent member of this group. Hartl and Dykhuzien (1984) defined *E. coli* as an important commensal pathogen that inhabits the gastrointestinal tracts of humans and animals, and according to Neu (1992) it is regarded as an important source of antimicrobial resistance determinants for other human and animal pathogens. Smith (1975; 1977) has also reported transfer efficiencies between *E. coli* strains and between *E. coli* and *Salmonella* spp. within the rumen, although only following a brief period of starvation. The above assertion supports the role of commensal *E. coli* as a reservoir of resistance genes and its ability to maintain a continuous flow of resistance genes in an animal, even after the target pathogens have been destroyed, especially in animals that are admini-stered antibiotics as growth promoters.

In the developed world, the extensive use of antibiotics in agriculture, especially for prophylactic and growth promoting purposes, has generated much debate as to whether this practice contributes significantly to increased frequencies and dissemination of resistance genes into other ecosystems. In developing countries like Nigeria, antibiotics are used only when necessary, especially if the animals fall sick, and only the sick ones are treated in

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Table 1. Frequency of antimicrobial resistance of porcine *E. coli* strains to the test antibiotics.

	No. (%) of resistant strains per sample type						
Antibiotic S*	**Rectal swabs n = 121**	**Feed samples n = 6**	**Water samples n = 2**	**Soil sample n = 1**	**Slurry samples n = 10**	**Wall swabs n = 2**	**Total isolates n = 142**
C X M	107 (88.4)	6 (100)	2 (100)	1 (100)	10 (100)	1 (50)	127 (89.4)
N	109 (90.0)	6 (100)	2 (100)	1 (100)	8 (80)	1 (50)	127 (89.4)
P N	103 (85.1)	4 (66.7)	2 (100)	0 (0)	5 (50)	2 (100)	116 (81.7)
T	88 (72.7)	5 (83.3)	2 (100)	1 (100)	9 (90)	1 (50)	106 (74.6)
C A Z	90 (74.3)	5 (83.3)	2 (100)	0 (0)	7 (70)	1 (50)	105 (73.9)
C T X	89 (73.6)	4 (66.7)	2 (100)	0 (0)	7 (70)	1 (50)	103 (72.5)
C O X	67 (55.4)	2 (33.3)	0 (0)	0 (0)	6 (60)	1 (50)	76 (53.5)
S X T	43 (35.5)	1 (16.7)	2 (100)	1 (100)	9 (90)	0 (0)	56 (39.4)
C	14 (11.6)	0 (0)	2 (100)	0 (0)	2 (20)	0 (0)	18 (12.7)
C N	11 (0.09)	1 (16.7)	0 (0)	0 (0)	1 (10)	0 (0)	13 (0.09)
C P X	9 (0.07)	0 (0)	0 (0)	0 (0)	2 (20)	0 (0)	11 (0.08)
N A	8 (0.06)	0 (0)	0 (0)	0 (0)	2 (20)	0 (0)	10 (0.07)
A U	7 (0.05)	0 (0)	0 (0)	0 (0)	1 (10)	0 (0)	8 (0.06)
P E F	5 (0.04)	0 (0)	0 (0)	0 (0)	2 (20)	0 (0)	7 (0.05)
S	5 (0.04)	0 (0)	1 (50)	0 (0)	1 (10)	0 (0)	7 (0.05)
O F X	5 (0.04)	0 (0)	1 (50)	0 (0)	1 (10)	0 (0)	7 (0.05)

CXM–Cefuroxime, N–Nitrofurantoin, PN-Ampicillin, T-Tetracycline, CAZ-Ceftazidime, CTX- Cefotaxime, COX-Cephalexin, SXT-Co-trimozazole, C-Chloramphenicol, CN-Gentamycin, CPX-Ciprofloxacin, NA-Nalidixic Acid, AU-Augumentin, PEF-Perfloxacine, S-Streptomycin, OFX-Ofloxacin.

such cases. However, even in the absence of heavy use of antibiotics it is important to identify and monitor susceptibility profiles of bacterial isolates, particularly of commensal organisms. This, according to John and Fishman (1997), will provide information on resistance trends including emerging antibiotic resistance which are essential for clinical practice.

This work was therefore undertaken to investigate the antibiotic resistance profile of *E. coli* isolates from clinically healthy pigs and their commercial farm environments, in the absence of extensive use of antibiotics for both prophylaxis and growth promotion.

MATERIALS AND METHODS

Sampling sites

The sampling sites were located in and around the state capital and were all commercial farms where the animals are reared for food. Sampling sites A, B and C have just one farm hand each. Sampling sites C and D have their own source of water on the farms. Sampling site A is supplied water by the university water tanker, while sampling site B has to purchase water from commercial water peddlers. Animal wastes on sampling site D are also properly disposed by channeling into a properly constituted tank and its environment is therefore clean, unlike the other sites where they are thrown all around the farms in bulk form. Animals on sampling site D were also fed and washed twice a day.

The study was also carried out between the months of December and April, a time when the atmospheric temperatures were usually around 37 - 40^{o}C.

(a) Sampling site A (SS A) is the university piggery and has a total of 40 pigs. These include the West African Dwarf (WAD) and Large White species. Others include student research animals, which have never been administered antibiotics. On this farm, antibiotics are administered only for therapeutic purposes.

(b) Sampling site B (SS B) is privately owned and has a total of 100 pigs, including piglets. Here antibiotics are neither administered prophylactically nor used as growth promoters. Antibiotics are used only for therapeutic purposes and administered only on sick pigs.

(c) Sampling site C (SS C) is privately owned and has a total of 70 pigs. Again, here antibiotics are used only for therapeutic purposes and only on sick animals.

(d) Sampling site D (SS D) has about 130 pigs, including sows and piglets and is owned and managed by the state government. At weaning, vitamin packs in which are incorporated small quantities of antibiotics are added to the animals' feed as growth promoters. Generally antibiotics are administered only for therapeutic purposes but on signs of infection, the whole herd rather than just the affected animals are treated.

Sampling

A total of 202 samples were collected comprising 158 rectal swabs, 8 wall swabs, 8 feed samples, 5 water samples, 6 soil samples, 11 slurry samples and 6 swabs from farm attendant's hands.

MacConkey agar (Lab M) was utilized for primary isolation, followed by streaking of suspected colonies on eosin – methylene blue agar (Lab M). Green metallic sheen colonies positive for *E. coli* were then subjected to confirmatory biochemical tests (IMViC) for the identification of *E. coli* (Cheesebrough, 2000) . The *E. coli* colonies were then subjected to antibiotic sensitivity testing by the disc diffusion method (Bauer et al., 1966).

Locally produced commercial antimicrobial discs (Optun Lab) for 16 antibiotics were used in the study and included: Chloramphenicol (30 µg), Nitrofurantoin (200 µg), Co- Trimozazole (25 µg), Tetracycline (25 µg), Cephalexin (15 µg), Ofloxacin (10 µg), Ciprofloxacin (10 µg), Gentamycin (10 µg), Ampicillin (30 µg), Per-

Table 2. Resistance rates for *E. coli* strains from pigs that have never been exposed to antibiotics and pigs that have been exposed.

Antibiotics	Number (%) of resistant strains	
	Antibiotic-exposed (n = 87)	Non-antibiotic expose (n = 34)
Cefuroxime	79(90.8)	28 (82.4)
Nitrofurantoin	80(91.9)	29 (85.3)
Ampicillin	76(87.3)	27 (79.4)
Tetracycline	72(82.8)	16 (47.1)
Ceftazidime	65(74.7)	25 (73.5)
Cefotaxime	67(77.0)	22 (64.7)
Cephalexin	48(55.1)	19 (55.9)
Co-Trimozazole	37(42.5)	6 (17.6)
Chloramphenicol	11(12.6)	3 (8.8)
Gentamycin	7(8.0)	4 (11.8)
Ciprofloxacin	6(6.9)	3 (8.8)
Nalidixic Acid	6(6.9)	2. (5.9)
Augumentin	5. (5.7)	2 (5.9)
Perfloxacine	3(3.4)	2 (5.9)
Streptomycin	3. (3.4)	2 (5.9)
Ofloxacin	2(2.3)	3 (8.8)

floxacine (10 µg), Augumentine (30 µg), Streptomycin (30 µg), Nalidixic Acid (30 µg), Cefuroxime (30 µg), Ceftazidime (30 µg), and Cefotaxime (30 µg). Plates were incubated at 35^{o}C.

Zones of inhibition were interpreted as resistant or sensitive using the interpretative chart of the zone sizes of the Kirby – Bauer sensitivity test method (Cheesbrough, 2000).

Statistical analyses

Comparative resistance rates for *E. coli* strains from pigs that have been exposed to antibiotics and those pigs that have never been exposed to antibiotics were statistically analyzed by paired comparisons. Comparative rates among the four sampling sites were analyzed by ANOVA. These tests are as described by Eason et al. (1989) and Kelly and Onyeka (1992), respectively. Results were considered significant at 99% confidence level.

RESULTS

A total of 142 *E. coli* strains were isolated from 202 swine rectal swabs and environmental samples analysed. This translates to 70.3% of all samples being positive for *E. coli*. The *E. coli* strains displayed high rates of resistance to Cefuroxime, Nitrofurantoin, Ampicillin, Tetracycline, Ceftazidime, Cefotaxime and Cephalexin (89.4 to 53.5%). Rates of resistance to Septrin and Chloramphenicol were moderate (39.4 to 12.7%), while low rates (0.09 to 0.05%) were recorded for Gentamycin, Ciprofloxacine, Perfloxacine, Augumentine, Nalidixic acid, Streptomycin and Ofloxacine (Table 1).

Resistance rates for *E. coli* strains isolated from pigs that have never been administered antibiotics when compared to resistance rates from pigs that have been administered antibiotics show similar resistance trends for the same antibiotics for both groups of animals (Table 2). Table 3 shows comparative rates of resistance among the four sampling sites.

Statistical analysis showed no difference in resistance between those animals that have never been exposed and those that have been exposed to antibiotics. On the other hand, statistical analysis on comparative resistance rates between the four sampling sites showed significant differences between them. Results were considered significant at 99% confidence level.

A total of 78 resistance patterns were identified among the *E. coli* strains with N+T+COX+PN+CTX+CXM+CAZ being the predominant pattern (Table 4). 16 *E. coli* strains reported this pattern, while 4 *E. coli* strains were found resistant to all 16 antibiotics under study and one *E. coli* strain was not resistant to any antibiotic.

In all, 134 (94.2%) *E. coli* strains were resistant to more than two antibiotics, the largest block being 33 strains (24.6%) that were resistant to seven antibiotics (Figure 1).

DISCUSSION

The *E. coli* strains isolated exhibited high rates of resistance against the following antibiotics: Cefuroxime, Nitrofurantoine, Ampicillin, Tetracycline, Ceftazidime, Cefotaxime and Cephalexin. This high resistance level is in spite of the fact that the farms under study do not have histories of supplementing animal feed with antibiotics as growth promoters, nor are antibiotics used prophylactically. It is therefore probable that other factors and pressures have brought about this high incidence of resistance in the absence of antibiotic exposure.

Many factors apart from antibiotic exposure can contribute to the development of antibiotic resistance in bacterial isolates. Major among these are environmental stress. Heat stress in swine, according to Moro et al. (2000), increased propulsion of intestinal content causing a reduction in transit time, which, in turn, increases shedding of resistant *E. coli*. Overcrowding in holding pens (Molitoris et al., 1987) also increases the percenttage of antimicrobial resistant enteric bacteria shed into the environment by pigs. Other factors that may disturb gastrointestinal microflora are starvation or other dietary changes, fear and other conditions like cold (Moon et al., 1979; Savage, 1982; Tannock, 1983; Moro et al., 1998). Three of the sampling sites A, B and C had overcrowded pens, with adult pigs being as many as 8 in a pen. On SS C the grower pigs were as many as 40 in a pen. The animals were fed once a day in all the sites, but

at SS D they were fed twice a day. Sometimes when the farms ran out of animal feed, the animals had nothing to eat. These practices can bring about starvation conditions.

Transfer efficiencies have been reported between *E. coli* strains, and *E. coli* and *Salmonella* spp. following a period of starvation (Smith, 1975; 1977) . Another effect of starvation on the rumen is a decrease in acidity and an increase in total *E. coli* population (Duncan et al., 2000).

Table 3. Comparative rates of resistance among the four sampling sites.

Anibiotics	Resistance rates (%) per sampling site.			
	SS A n = 13	SS B n = 65	SS C n = 54	SS D n = 10
Cefuroxime	12 (92.3)	55 (84.6)	50 (92.6)	10 (100)
Nitrofurantoin	12 (92.3)	59 (90.8)	46 (85.2)	10 (100)
Ampicillin	12 (92.3)	54 (83.1)	42 (77.8)	8 (80)
Tetracycline	8 (61.5)	46 (70.8)	42 (77.8)	10 (100)
Ceftazidime	10 (76.9)	45 (69.2)	40 (74.1)	10 (100)
Cefotaxime	11 (91.7)	48 (73.8)	36 (66.7)	8 (80)
Cephalexin	8 (61.5)	38 (58.5)	30 (55.6)	0 (0)
Co-Trimozazole	3 (23.1)	40 (61.5)	9 (16.7)	4 (40)
Chloramphenicol	3 (23.1)	9 (13.8)	5 (9.3)	1 (10)
Gentamycin	3 (23.1)	8 (12.3)	2 (3.7)	0 (0)
Ciprofloxacin	3 (23.1)	4 (6.2)	4 (7.4)	0 (0)
Nalidixic Acid	3 (23.1)	1 (1.5)	6 (11.1)	0 (0)
Augumentin	3 (23.1)	2 (3.1)	3 (5.6)	0 (0)
Perfloxacine	3 (23.1)	0 (0)	3 (5.6)	1 (10)
Streptomycin	3 (23.1)	1 (1.5)	3 (5.6)	0 (0)
Ofloxacin	4 (30.8)	2 (3.1)	1 (1.9)	1 (10)

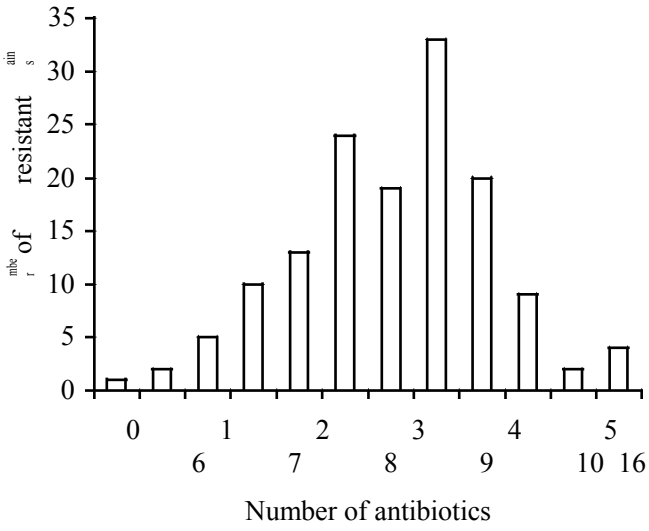

Figure 1. Multiple antibiotic resistance among *E. coli* strains.

than one dominating factor. Results of the statistical analysis between antibiotic-exposed and non-antibiotic exposed animals suggested that antibiotic exposure is not the only determining factor for resistance. In a similar study conducted by Chah et al. (2003) on non-clinical *E. coli* strains from chicken, including the local species where antibiotics are rarely used, a high number of resistance patterns were also observed.

Martinez and Chez (1990), proposed that *E. coli* strains resistant (not intermediately resistant) to at least two of the following antimicrobials tetracycline, sulfamethoxazole, trimethoprim, streptomycin and chloramphenicol be designated multi – drug resistant, based on the fact that these resistance traits are very often found together on transferable elements e.g. Tn21 and related transposons. According to that proposal, forty – five (45) or 31.6% of the 142 isolated *E. coli* strains can be said to be multi – drug resistant, since they were all resistant to sulfamethoxazole, trimethoprim and tetracycline.

Supplying the public pork meat from farms with such high levels of antibiotic resistance among resident flora has grave implications for public health for the following reasons. The *E. coli* strains exhibited high resistance to some fairly common antibiotics used by humans and even some newer third generation antibiotics. Also non-pathogenic porcine *E. coli* may represent a reservoir of antibiotic genes that could be transferred to both pathogenic organisms and other ecosystems.

Statistical analysis of the resistance rates of those animals that have been exposed to antibiotics compared to strains that have never been exposed to antibiotics was not significant at 99% probability, probably because antibiotics were not the dominant selective factor here for resistance. Both groups of animals have been exposed to almost the same environmental conditions and stress. On the other hand, analysis of the resistance rates between the four sampling sites was significant at the same level, showing a marked difference between them. The sampling site D where most of the swabs and samples taken did not yield any isolates would have brought about this difference. One can therefore state in conclusion that crude management practices, which imposed stress rather than antibiotic exposure, have contributed to the high rates of resistance observed in the porcine *E. coli* strains isolated in all four sampling sites. There were also

Table 4. Antibiotic resistance patterns of porcine E. coli isolates.

Resistance patterns	Number	Resistance patterns	Number
PN	1	T+COX+PN+CTX+CXM+CAZ	1
CXM	1	N+SXT+T+PN+CXM+CAZ	1
SXT+P.N	1	N+T+COX+PN+CXM+CAZ	1
T+PN	1	N+T+COX+PN+CTX+CXM+CAZ	16
N+CXM	3	N+SXT+T+PN+CTX+CXM+CAZ	7
N+CXM+CAZ	3	N+T+COX+CPX+PN+CTX+CXM	1
N+PN+CXM	2	N+COX+OFX+PN+CTX+CXM+CAZ	1
N+SXT+CXM	1	N+SXT+T+CN+PN+CXM+CAZ	1
N+CTX+CXM	1	N+SXT+T+COX+CPX+PN+CXM	1
N+T+PN	1	N+T+COX+PN+S+CXM+CAZ	1
CTX+CXM+CAZ	1	C+N+SXT+T+PN+CTX+CXM	1
T+PN+CXM	1	N+SXT+T+COX+PN+CTX+CXM	1
N+PN+CXM+CAZ	3	N+T+PEF+CTX+CXM+CAZ	1
N+T+CXM+CAZ	2	N+SXT+COX+PN+CTX+CXM+CAZ	1
N+PN+CTX+CXM	1	N+COX+CN+PN+CTX+CXM	1
T+COX+PN+CXM	1	N+SXT+T+COX+PN+CTX+CXM+CAZ	9
N+COX+CTX+CXM	1	C+N+SXT+T+PN+CTX+CXM+CAZ	3
COX+CN+CTX+CAZ	1	C+N+SXT+T+COX+PN+CTX+CAZ	1
N+COX+CXM+CAZ	1	N+SXT+T+COX+CPX+PN+CXM+CAZ	1
N+CN+CTX+CAZ	1	N+SXT+T+COX+PN+NA+CTX+CXM+CAZ	1
T+PN+CTX+CXM	1	N+T+COX+CN+PN+CTX+CXM+CAZ	1
N+COX+PN+CXM	1	N+T+COX+PN+S+CTX+CXM+CAZ	1
N+COX+PN+CXM+CAZ	3	C+N+T+COX+PN+CTX+CXM+CAZ	1
N+COX+CTX+CXM+CAZ	2	T+COX+PN+PEF+NA+CTX+CXM+CAZ	1
COX+PN+CTX+CXM+CAZ	1	N+T+COX+OFX+PN+CTX+CXM+CAZ	1
N+T+CTX+CXM+CAZ	3	N+T+COX+CPX+NA+CTX+CXM+CAZ	1
N+PN+CTX+CXM+CAZ	2	C+N+SXT+T+COX+PN+CTX+CXM+CAZ	5
N+T+PN+CTX+CXM	3	N+SXT+T+COX+PN+NA+CTX+CXM+CAZ	1
N+T+COX+PN+CXM	1	N+SXT+T+COX+OFX+CN+PN+CTX+CXM	1
N+SXT+PN+CXM+CAZ	1	C+N+SXT+T+COX+CPX+PN+CTX+CXM	1
N+SXT+T+PN+CXM	1	C+N+SXT+T+COX+OFX+PN+CTX+CXM+CAZ	1
T+COX+PN+PEF+CXM	1	C+N+SXT+T+COX+CPX+PN+CTX+CXM+CAZ	1
T+COX+PN+CTX+CXM	1	C+N+SXT+T+COX+PN+OFX+CPX+CN+PEF+AU+S+NA+CTX+CXM+CAZ	4
SXT+T+PN+CTX+CAZ	1		
N+T+PN+CXM+CAZ	2		
N+AU+CTX+CXM+CAZ	1		
N+SXT+PN+CTX	1		
N+T+PN+CTX+CXM+CAZ	6		
N+T+COX+PN+CTX+CXM	4		
N+COX+CN+PN+CTX+CXM	1		
C+N+T+PN+CTX+CXM	1		
N+CN+PN+CTX+CXM+CAZ	1		
N+COX+PN+CTX+CXM+CAZ	1		
N+T+COX+CN+CTX+CAZ	1		
N+SXT+XT+PN+CTX+CXM	1		

* CXM–Cefuroxime, N–Nitrofurantoin, PN-Ampicillin, T-Tetracycline, CAZ-Ceftazidime, CTX- Cefotaxime, COX-Cephalexin, SXT-Co-trimozazole, C-Chloramphenicol, CN-Gentamycin, CPX-Ciprofloxacin, NA-Nalidixic Acid, AU-Augumentin, PEF-Perfloxacine, S-Streptomycin, OFX-Ofloxacin.

high rates of isolation of *E. coli* from the sampling sites. The *E. coli* strains exhibited high resistance to some fairly common antibiotics used by humans and even some newer third generation antibiotics. The results also indi-

cate that non-pathogenic porcine *E. coli* may represent a reservoir of antibiotic genes that could be transferred to both pathogenic organisms and other ecosystems.

ACKNOWLEDGEMENTS

The authors wish to express their gratitude to the entire laboratory staff of the Imo State Environmental Protection Agency (ISEPA), as well as that of the Department of Microbiology, Federal University of Technology Owerri Imo State Nigeria for their assistance. They also wish to acknowledge the support of the management of the different commercial piggeries sampled.

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