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Research Article

SURVEILLANCE OF ANTIMICROBIAL RESISTANCE IN PIGS USING *ESCHERICHIA COLI* FAECAL ISOLATES

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ABSTRACT
Resistant and multi drug resistant bacteria in food animals threaten food security and public health worldwide. Thus, periodic surveillance of antibiotic resistance among bacteria isolated from food animals is recommended. Hence, 50 *Escherichia* coli strains isolated from 50 pig faecal samples, each from one pig farm in Ashanti region, Ghana were used as indicators of resistance in pigs using eight antibiotics.

72% and 42% of the isolates were resistant and multi drug resistant respectively. Resistance to streptomycin (22%) was highest, followed by amoxicillin (20%), sulphamethoxazole-trimethoprim (10%), tetracycline (8%) and doxycycline (6%); these closely reflected the antibiotic usage patterns of the farmers. Most strains showed multi drug resistance to amoxicillin and streptomycin (26%) and amoxicillin, streptomycin and tetracycline (24%). Multidrug resistance to both tetracycline and sulphamethoxazole-trimethoprim (16%), tetracycline and doxycycline (12%) and streptomycin and norfloxacin (12%) were substantial. Cross resistance to antibiotics not used on the farms (tetracycline, doxycycline, amoxicillin and ciprofloxacin) was observed. The selection of resistant and multidrug resistant phenotypes in pigs is influenced by antibiotic use by pig farmers and could result in resistant food-borne bacterial infections to consumers, farmers and abattoir workers alongside environmental contamination with resistant genes. Prudent antibiotic use and better farm waste handling practices are advised to safeguard food security and public health.

Keywords: *Enterobacteriaceae, Bacterial Resistance, Antibiotics, Pig Farmers, Ashanti Region, Escherichia Coli*

INTRODUCTION
The resistance of bacteria to antibiotics is making headlines around the world as an increasing cause of morbidity and mortality. In the EU alone, it is estimated that 25 000 people die annually from infections caused by antibiotic resistant bacteria (WHO, 2011). Antibiotic resistance has become so extensive that in some cases, there have been limited therapeutic options for multi drug resistant pathogens (Gilchrist *et al.*, 2007; The Lancet Infectious Disease Commission, 2013).

The immeasurable benefits of antibiotics have been checked with the emergence of resistance among almost all strains of microorganisms (Levy and Marshall, 2004). The inefficacy and consequent uselessness of our current antibiotic arsenals results in protracted hospitalisation, resort to expensive antibiotic alternatives, increased healthcare costs, emergence of new strains of pathogenic microorganisms and their attendant infectious diseases, increased morbidity and mortality. Coupled with the reduced interest of pharmaceutical companies to invest in the research and development of new antibiotic agents, the development of multidrug and extra drug resistant bacteria are cogent reasons for taking a serious look at the threats posed by resistant bacteria (Gilchrist *et al.*, 2007; WHO, 2011).

Antibiotics have become important in veterinary medicine and in animal food production as growth promoters, prophylactic, metaphylactic and therapeutic agents (Chee-Sanford *et al.*, 2009). Though initially produced for clinical use, it is now estimated that the largest use of antibiotics worldwide occurs in the production of food animals (Silbergeld *et al.*, 2008).

The use of antibiotics in food animal production has resulted in the selection of resistant bacteria both in exposed farmers during antibiotic administration and in intestinal commensals of farm animals (Levy, 1978; Ojeniyi, 1989). Studies have proved the transfer of resistant bacterial clones from food animals to farmers, birds, rodents, manure amended soils, crops, ground and surface waters (Levy *et al.*, 1976; Mutalib *et al.*, 1992; van den Bogaard *et al.*, 2001). Moreover, resistant bacteria have been shown to contaminate food animal carcasses during slaughter, posing a threat to abattoir workers and consumers; the death of two patients from a *Salmonella Typhimurium* DT104 clone infected pork in Denmark is a paradigm (Nijsten *et al.*, 1994: Molbak *et al.*, 1999).Consequently, the prevalence of resistance among bacteria, especially *Enterobacteriaceae,* in food animals is both an environmental hazard and a food safety and public health threat; from farmers and abattoir workers to consumers.

As a result, periodic surveillance studies in food animals to evaluate the burden of Antibiotic resistance using selected indicator species has been recommended by the WHO (2011) as a measure to pre-empt food-borne zoonosis and resistant bacterial infections transmission. Due to the constant exposure of *Enterobacteriaceae* to administered antibiotics, members like *Escherichia coli* and *Enterococcus faecium* have been suggested as indicator strains in surveillance studies for assessing the levels of resistance to antibiotics among bacteria in food animals (Lester *et al.*, 1990; WHO, 2011). Therefore, fifty pig farms within thirty nine widely distributed settlements with well organised and recognised pig farms within the Ashanti region, Ghana, were selected to study the antibiotic resistance levels of *Escherichia coli* isolated from pig faeces.

MATERIALS AND METHODS

Study Area and Sampling

The study was conducted in thirty nine pig rearing settlements in the Ashanti region, Ghana, which has a total land surface area of 24 389 km^2. These settlements are officially recognised by the Ashanti regional Veterinary Department due to their higher density of pig farms and well established pig farmers' associations. The farms were distributed within five districts within the Ashanti Region of Ghana: Ejisu-Juaben district (12 settlements), Atwima Nwabiagya district (12 settlements), Bosomtwe and Atwima Kwanwoma districts (5 towns) and Kwabre East districts (10 towns). Ashanti region was chosen due to proximity to the university.

The farms visited were those enlisted by the pig farmers' associations in the various districts. However, farms were carefully chosen from villages and town within the districts to ensure a fair representation of the district. Farms in which the owners were absent or unwilling to undertake the study were not included. Farms that were within proximity of 100 meters were not selected. The farms were mostly situated at the outskirts and farming allocations of the towns and except for minor instances, were not more than a kilometre away from residential areas.

A total of fifty farms were selected for the study. .

Faecal Collection, Isolation and Identification of Enterobacteria Isolates

50fresh pig faeces were collected from 50 pig farms (one from each farm) from 39 settlements in the Ashanti region between October 2012 and May, 2013 in sample tubes and transported on ice to the university. 10g of each sample was diluted to 10^{-1} in 100mL of 0.9% saline containing 20%w/v glycerol and immediately stored at -20°C till analysed. *Escherichia coli* colonies were respectively detected, isolated and confirmed using already described methods (Nijsten *et al.*, 1994; van den Bogaard *et al.*, 2001) after thawing samples. Only one *Escherichia coli* strain was isolated per sample, representing one farm, and used in the sensitivity tests. A mini questionnaire asking farmers about the types of antibiotics used within the last one year was used to collect information on the antibiotics used by the farmers.

Antibiotic Sensitivity Testing

Antibiotic sensitivity testing discs (Gentamicin (Gen)-10µg, Streptomycin-10µg, Norfloxacin (Nor)-10µg, Ciprofloxacin (Cip)-5µg, Tetracycline (Tet)-30µg, Doxycycline (Dox)-30µg, Amoxycillin (Amo)-10µg and Sulphamethoxazole/Trimethoprim (SXT)-25µg) from Oxoid (Oxoid, Basingstoke, UK) and a semi-automated multi disc dispenser (Oxoid, Basingstoke, UK) were used to determine the sensitivities of the *Escherichia coli* isolates according to described standards (CLSI, 2012). Positive controls were set up for every batch of plates tested using *Pseudomonas aeruginosa* ATCC 27853 and *E. coli* ATCC 25922. All tests were carried out in triplicates to ensure reproducibility.

The zones of inhibition produced by the antibiotics were measured thrice and the average was compared with the CLSI tables to determine the susceptibility levels of the various bacterial isolates. Strains with resistance to more than two antibiotics were classified as multi drug resistant.

Data Analysis

The number and percentages of resistant isolated to amoxicillin, ciprofloxacin, norfloxacin, gentamicin, streptomycin, tetracycline, doxycycline and Sulphamethoxazole-trimethoprim were analysed with Microsoft Excel© 2010 (Microsoft Corporation, Microsoft office package, 2010, USA). Susceptible and intermediate resistant strains were left out of the analysis.

Ethical Approval and Informed Consent

Ethical exemption and study approval were obtained from the Faculty of Pharmacy of the Kwame Nkrumah University of Science and Technology. Informed consent was not required though the approval of the regional and district Veterinary offices and the farmers were obtained before faecal collection began.

RESULTS

A total of 50 non-repetitive *E. coli* strains were isolated from all the 50 collected faecal samples. 36 (72%) of these isolates showed resistance to at least one antibiotic. The highest levels of resistance were against amoxicillin (20%) and streptomycin (22%) followed by sulphamethoxazole-trimethoprim (10) and tetracycline (8) (table 1)

Antibiotics commonly used in the pig farms within the last one year were oxytetracycline and chlortetracycline (58.00%), penicillin-streptomycin combinations (44.00%), sulphadimidine (28.00%), enrofloxacin and norfloxacin (10.00%), tylosin (6.00%), erythromycin (4.00%), trimethoprim (2.00%) and gentamicin (2.00%) in a descending order.

58.33% of the resistant strains and 42% of all strains were multi drug resistant. Multidrug resistance were especially seen in amoxicillin, streptomycin, tetracycline and doxycycline combinations, in streptomycin and norfloxacin combinations and tetracycline and sulphamethoxazole-trimethoprim combinations (table 2 and figure 1)

Table 1: Percentage of *E. coli* isolates resistant to the tested antibiotics

Antibiotics	Number of resistant strains (n=36) (%)	Percentage (%) of isolates (n=50)
Amoxicillin (Amo)	10 (27.78)	20
Ciprofloxacin (Cip)	2 (5.26)	4
Norfloxacin (Nor)	1 (2.78)	2
Gentamicin (Gen)	0 (0)	0
Streptomycin (Stp)	11 (30.56)	22
Tetracycline (Tet)	4 (11.11)	8
Doxycycline (Dox)	3 (8.33)	6
Sulphamethoxazole-Trimethoprim (SXT)	5 (13.89)	10

Table 2: Spectrum of tested antibiotics to which *E. coli* isolates showed multi drug resistance

Antibiotics spectrum	Number of multi drug resistant strains (n=36) (%)	Percentage (%) of isolated strains (n=50)
Amo, Cip, Stp, Tet and SXT	3 (8.33)	6
Amo, Gen, Stp and Tet	2 (5.26)	4
Amo and Stp	13 (36.11)	26
Amo, Stp and Tet	12 (33.33)	24
Amo, Stp, Dox and Tet	7 (19.44)	14
Amo, Stp, Dox, SXT and Tet	3 (8.33)	6
Cip, Stp and Tet	3 (8.33)	6
Cip, Nor, Stp and SXT	2 (5.26)	4
Nor, Gen, Stp and Dox	2 (5.26)	4
Stp and Dox	3 (8.33)	6
Stp and Tet	3 (8.33)	6
Stp and Nor	6 (16.67)	12
Stp and Gen	2 (5.26)	4
Tet and Dox	6 (16.67)	12
Tet and SXT	8 (22.22)	16

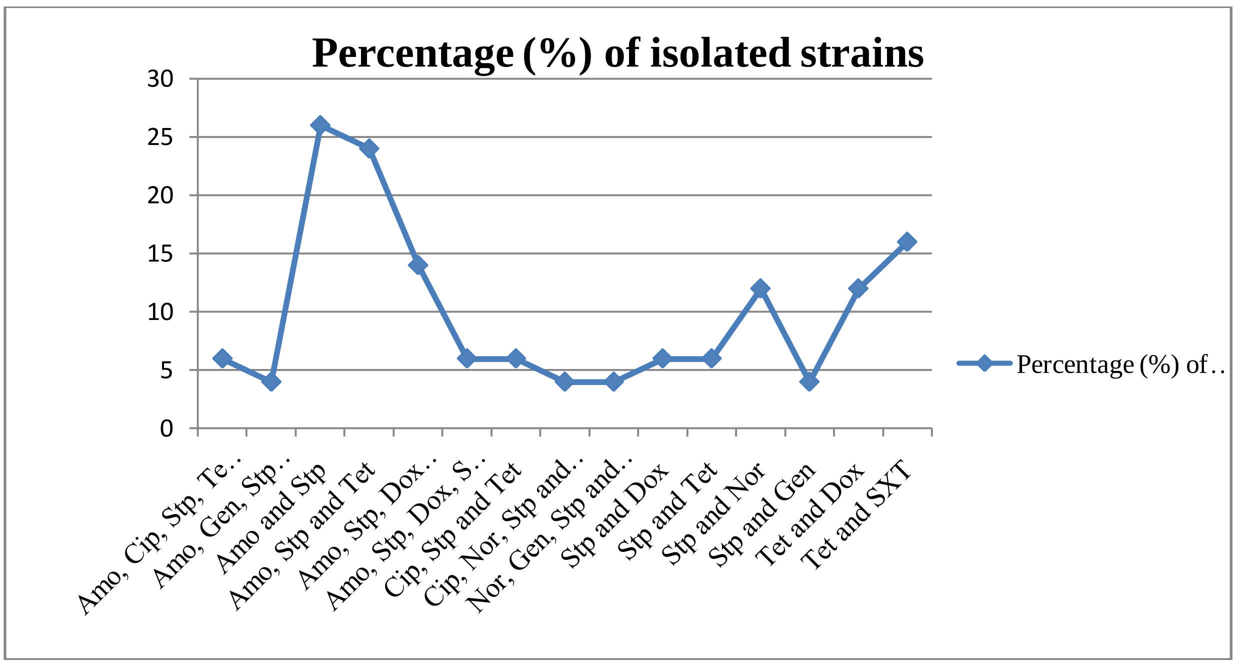

Figure 1: Spectrum of antibiotics to which isolated *E. coli* strains show multi drug resistance

Figure 1 shows the antibiotic combinations to which the isolated *E. coli* strains are multi drug resistant. Most strains are resistant to both amoxicillin (Amo) and streptomycin (Stp) and to amoxicillin, streptomycin and tetracycline (Tet). The strains show multi drug resistance to antibiotics commonly used by farmers.

DISCUSSION

The percentages of strains showing resistance to the various antibiotics tested obviously mirrors the antibiotics used by the farmers (table 1) and lends support to recommendations to proscribe the use of similar and same antibiotics in both veterinary and clinical medicine (Teuber, 2001; Silbergeld *et al.*, 2008). The effect of using similar antibiotics in both veterinary and clinical medicine can be seen in table

1 where resistance to amoxicillin was common among several strains (20%); however, amoxicillin is not used by the farmers though penicillin, in combination with streptomycin, is used extensively. The same trend exists in the case of tetracycline and doxycycline which was not common among the antibiotics used by farmers though oxytetracycline was the commonest antibiotic used.This possible paradigm of cross resistance, however, was not seen with streptomycin and gentamicin, antibiotics of the same class (aminoglycosides). The combinations of drugs to which the multi drug resistant strains were resistant to is a further evidence of the selection pressure provided by veterinary antibiotics to enteric bacteria (Levy *et al.,* 1976; Teuber, 2001; van den Bogaard *et al.,* 2001); table 1 and 2 and figure 1show that most strains were resistant or multi drug resistant to antibiotics in common use by the pig farmers.

Whereas the study did not analyse bacterial isolates from farmers and the farm environments, the profiles herein seen do provide an indication of resistance burden of bacteria in farmers and the farms' environments as has already been shown in previous studies in livestock farms (Levy *et al.,* 1976; Mutalib *et al.,* 1992; Baloda *et al.,* 2001). Consequently, farmers, rodents, birds, manure, ground and surface waters can serve as conduits to transfer these resistant phenotypes into human communities (Mutalib *et al.,* 1992; van den Bogaard *et al.,* 2001; Silbergeld *et al.,* 2008). Furthermore, the levels of resistance and multidrug resistance observed in the *E. coli* strains could pose potential hazards to food safety and subsequently, to public health through contaminated pork as was reported in Denmark (Molbak *et al.,* 1999).

In an epidemiological study of non-human antibiotic usage in Accra, Ghana, by Donkor and colleagues (2012), higher resistance (>65%) and multi drug resistance (>70%) were observed among *E. coli* isolates from livestock to gentamicin and tetracycline and all antibiotics tested respectively than observed in this study. In a similar study by Ojo and peers (2012) in Nigeria, *E. coli* faecal isolates from free range fowls showed higher resistance to ciprofloxacin (34.6%), norfloxacin (46.2%), tetracycline (76.9%) and streptomycin (80.8%). Zhu and peers (2013) have proved that there is an abundant diversity of resistance genes to several antibiotics, both used and unused in manure from pig farms in China. Consequently, antibiotic resistance in food animals is a potential source of resistance genes and untreatable food-borne infections. In a recent study (Osei, unpublished data) among pig farms in Ashanti region, it was observed that pig faeces were dumped at random around farm environments with little or no treatment, exposing resistant bacteria and their genes to environmental bacterial populations (Nijsten *et al.,* 1994).

Hence, the bactericidal activity of current antibiotics can only be maintained for the equal good of humanity through prudent antibiotic use in veterinary medicine.

In conclusion, the presence of resistant and multi drug resistant *E. coli* isolated from pigs' faeces, reflecting the antibiotic usage patterns of pig farmers, is a potential threat to food security, the environment and public health. Husbandry practices that limit the use of antibiotics in pig farms are necessary. Also, there is the need to proscribe antibiotics used in clinical medicine in veterinary medicine.

Competing Interests

The author declares that he has no competing interests and the sponsors had no role or whatsoever in the preparation of the manuscript, data collection and analysis and decision to publish.

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