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Antibiotic-resistant *Escherichia coli* in a government piggery farm in Owerri, Nigeria

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ABSTRACT

The acquisition of antibiotic-resistant commensal *Escherichia coli* was examined in a cohort of newborn pigs. Faecal samples were collected weekly from young pigs over a 2 month period and screened for *E. coli* resistant to ampicillin, nalidixic acid, gentamicin and ciprofloxacin at concentrations of 16, 8, 8 and 8 mg/l, respectively. *E. coli* viable counts were also performed on the samples. All the pigs acquired gentamicin-, nalidixic acid- and ampicillin-resistant *E. coli*, while 80% acquired ciprofloxacin-resistant *E. coli* during the study. Sixty-five per cent of samples were resistant to at least one of the four antibiotics. The prevalence of the antibiotics declined significantly with age and time ($p<0.05$). Total counts and antibiotic resistant counts declined with age ($p<0.05$), with the rate of decline in antibiotic resistant counts being greater than that for total counts ($p<0.05$). The cohort pigs rapidly acquired antibiotic-resistant bacteria shortly after birth and the carriage of resistant bacteria was associated with age.

Keywords: antibiotics resistance, *Escherichia coli*, new-born pigs, faecal samples.

INTRODUCTION

Exposure to antibiotics increases the level of resistance observed in the normal commensal flora of both animals and humans (van den Bogaard and Stobberingh, 2000). The principal area of concern has been the increasing emergence of antibiotic resistance phenotype in both clinically relevant strains and normal commensal microbiota (Chee-Sanford et al., 2001). Carriage of resistance by commensals has therefore been proposed as an indication of the burden of antibiotic resistance that may be present in a population, (van den Bogaard and Stobberingh, 2000; Caprioli et al., 2000), with low-level carriage suggested as a suitable public health goal, (van den Bogaard, 1997). The acquisition of resistance by commensal bacteria is a serious concern, because intestinal flora acts as potential reservoir pools of resistance genes which may transfer to pathogenic bacteria within the host (Summers, 2002). Higher prevalence of commensal flora is also known to contribute to the general increase and dissemination of bacterial resistance worldwide (Summers, 2002).

Current strategies to monitor the presence of antibiotic-resistant bacteria in food animals target mainly resistance in clinical specimens and involve only periodic cross-sectional evaluations of resistance in faecal flora on a larger scale (Caprioli et al., 2000; Department of Health, 2000; Gunn and low, 2003). However, such surveys do not provide any information about the dynamics of antibiotic resistance in the normal flora. When resistant bacteria are acquired during the life-time of a food animal and whether such acquisitions are transient or not, are fundamental issues that need to be explored.

In the developed world, 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 when the animals are infected, and only the infected ones are treated. However, even in the absence of heavy use of antibiotics it is important to identify and monitor the dynamics of antibiotic resistance particularly of commensal organisms. This will provide information on resistance trends including emerging antibiotic resistance which are essential in clinical practice (John and Fishman, 1997).

This study was undertaken to investigate the acquisition of antibiotic resistant commensal *Escherichia coli* in newborn pigs in the absence of extensive use of antibiotics for both prophylaxis and growth promotion.

MATERIALS AND METHODS

The study area was piggery section of Songhai Redemption Farms, Nekede, Owerri, which is a farm established by Imo State Government. Imo State is situated in the Southern rain forest vegetation belt of Nigeria. It lies between latitude 5^o and 6^{o}3^{1}N and longitude 6^{o}15^1 and 7^{o}34^{1}E. The area is dominated by plains 200 m above sea level (Ofomata, 1975). It has an annual rainfall of about 1700 mm to 2500 mm, which is concentrated almost entirely between March and October. Average relative humidity is about 80% with up to 90% occurring during the rainy season. The mean daily maximum air temperature range from 28 to 35 ^{o}C, while the mean daily minimum range from 19 to 24 ^{o}C. (Okoli et al., 2004).

Piglets (n = 200) born into Songhai Redemption Farm during the period of May to July 2006 were enrolled in the study. Individual per-rectal faecal samples were collected at weekly intervals for a period of 10 weeks. Antibiotic treatments of the individual animals were recorded.

Faecal samples were kept at 4 ^{o}C and screened within 48 hours of collection on violet red bile agar (VRB agar) containing antibiotics (Sigma) at the following breakpoint concentrations (based on the requirement to detect the potential for resistance within a veterinary context): ampicillin, 16 mg/l; gentamicin, 8 mg/l; nalidixic acid, 8 mg/l and ciprofloxacin, 8 mg/l. Antibiotics were selected based on the antibiotics frequently used in farm animals in Nigeria. Samples were diluted 1:10 in Maximum Recovery Diluent (MRD) containing peptone, 1 g and sodium chloride, 8.5 g in one litre of deionized water. Ten microlitre of the diluted samples was spread onto non-selective (antibiotic-free) and antibiotic-containing plates and incubated overnight at 44 ^{o}C. Characteristic *E. coli* colonies were recorded. *E. coli* NCTC 10418 was used to confirm the activity of antibiotic-containing plates. A serial dilution in MRD up to 10^{-5} was prepared using the remaining samples. Total and antibiotic-resistant counts were determined by duplicate 0.1 ml spread plates for all dilutions on unsupplemented VRB agar and agar containing the respective antibiotics at break-point concentrations (as above). Positive colonies were counted after incubation for 24 hours at 44 ^{o}C. Viable counts as colony forming units per g (cfu/g) were calculated as: plate count × 10 × dilution factor.

RESULTS AND DISCUSSION

Sixty-five per cent of all samples (n = 1308) carried *E. coli* resistant to at least one of the four antibiotics tested. Gentamicin-resistant *E. coli* (Gen^R*E.coli*) was detected in 63%, nalidixic acid-resistant *E. coli* (Nal^R*E.coli*) in 63.5%, ciprofloxacin-resistant *E. coli* (Cip^R*E.coli*) in 61.5% and ampicillin-resistant *E. coli* (Amp^R*E.coli*) in 73.5% of samples. Gen^R*E.coli*, Nal^R*E.coli* and Amp^R*E.coli* were isolated from all the pigs at least once during the survey while Cip^R*E.coli* was detected in 80% of the pigs. There were three treatments of fluoroquinolone and β-lactam agents during the study. In all cases pigs had already exhibited resistance to nalidixic acid or ampicillin prior to treatment with the respective agent, or resistance was detected on the same day that the drug was administered. This is in accordance with the work of Chikwendu et al. (2008) who observed 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 pattern s were also observed.

The prevalence of antibiotic-resistance *E. coli* changed with age and time (Figure 1). The prevalence of Gen^{R}*E.coli* declined progressively with age though there was a higher prevalence at 5th and 6th weeks of age. There was significant difference in the prevalence in relation to age $P<0.05$ (Duncan multiple range test). Similarly, the prevalence of nalidixic acid, ciprofloxacin and ampicillin-resistant *E. coli* in the cohort are shown in Figure 2. Total counts declined as pigs aged ($P<0.05$). Antibiotic-resistance also declined with age but was lower than the total counts.

Overall, viable counts analysis demonstrated that while total *E. coli* counts do decline with age, the rate of decline of the antibiotic-resistance *E. coli* counts were significantly greater, with the proportion of total counts that were antibiotic resistant also declining rapidly with age. These results indicate that pigs preferentially lost resistance relative to susceptible bacteria as they aged.

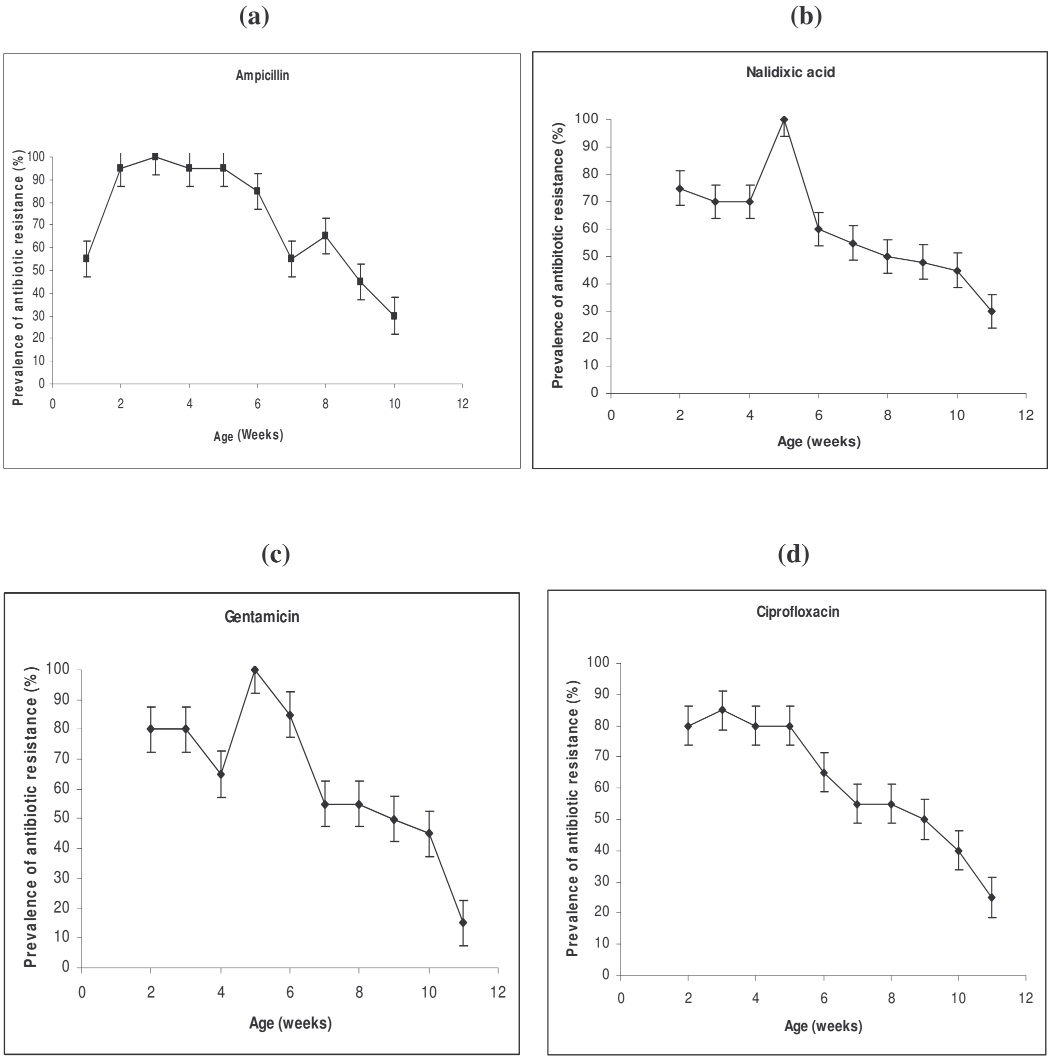

Figure 1: Prevalence of cohort newborn pigs carrying antibiotic-resistant *E. coli* as a function age for Amp^{R}*E.coli* (a), Nal^{R}*E.coli* (b) Gen^{R}*E.coli* (c) Cip^{R}*E.coli* (d). Error bars represents standard error of means.

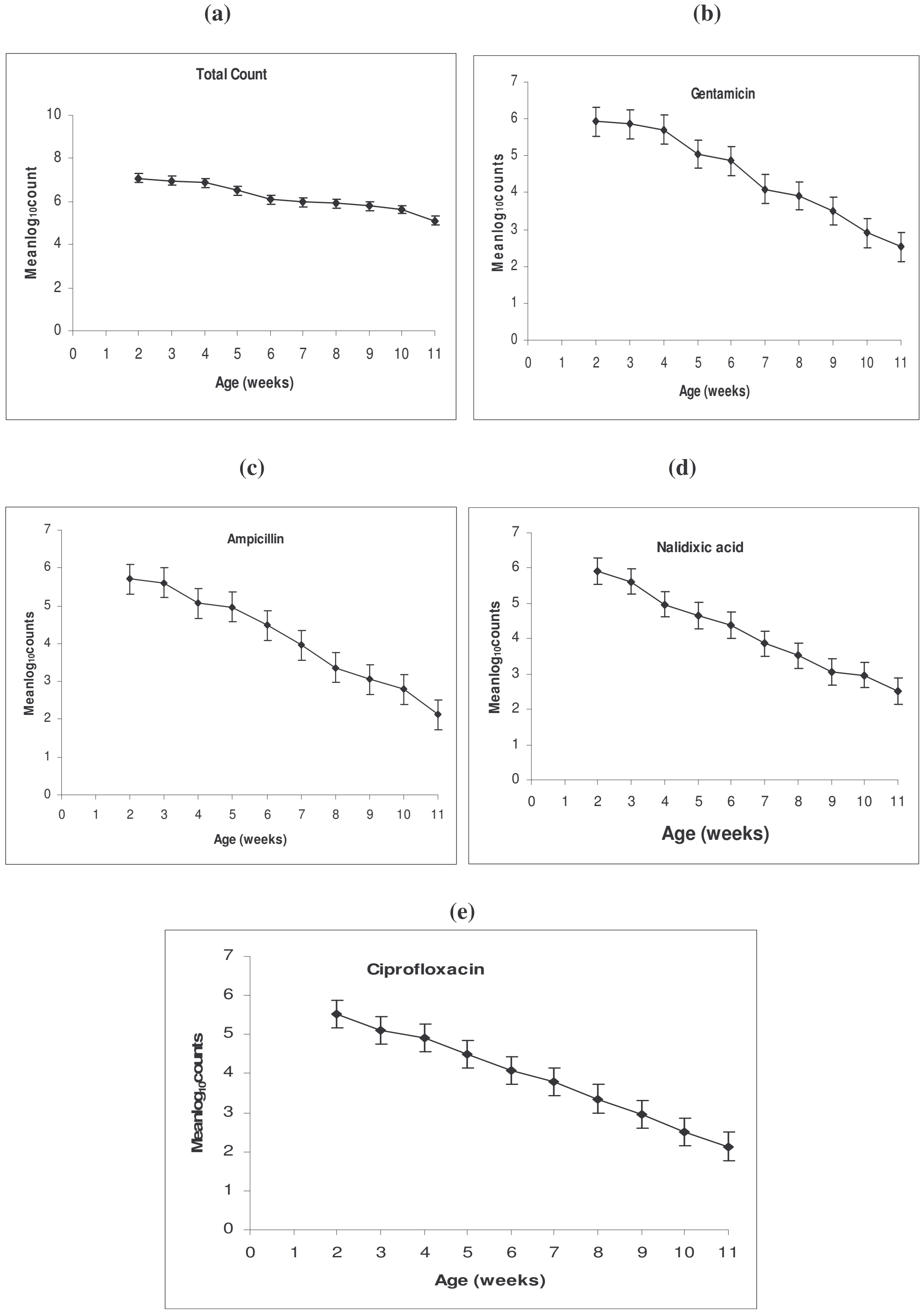

Figure 2: Change in the mean log-transformed viable *E. coli* counts of duplicate samples as a function of pig age for total counts (a), Gen^R*E.coli* (b), Amp^R*E.coli* (c), Nal^R*E.coli* (d) and Cip^R*E.coli* (e). Error bars indicate standard error of means.

In conclusion, it has been demonstrated that pigs were rapidly colonized by antibiotic-resistant *E. coli* shortly after birth. The prevalence of antibiotic-resistance *E. coli* within the cohort declined over the study period. Age-related changes within an animal had a significant negative effect upon gut carriage of resistant bacteria by cohort pigs.

The *E. coli* strains exhibited high resistance to some common antibiotics used by humans. This is of public health concern. The results also indicate that non-pathogenic *E. coli* may represent a reservoir of antibiotic-resistance genes that could be transferred to pathogenic organisms.

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