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Congruence between the drug resistance pattern of *Escherichia coli* and *Proteus* spp. isolated from humans and those from wild animals

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As human populations grow and transform landscapes, contact with wildlife concomitantly increases. Disease emergence has been an important consequence of these contacts, with many of emerging infectious diseases in humans arising from wildlife reservoirs. Drug resistance is a very important dimension of disease emergence and tracing the source is a veritable containment strategy. *Escherichia coli* and *Proteus* spp. have important roles in the increasing cases and dissemination of antibiotic resistance, potentially acquiring resistance determinants and acting as reservoirs for resistance genes. This study was conducted to determine the similarity or otherwise of the drug resistance pattern of *Escherichia coli* and *Proteus* spp. isolated from humans and those from wild animals (a source with only minimal, hypothetical or no antibiotic exposure). The human samples were obtained from two human groups: persons not on antibiotics and humans on antibiotics. Animal samples were taken from rats (*Rattus* spp.), grasscutters (*Thryonomys swinderianus*), squirrels (*Xerus erythropus*) antelopes (*Tragelephus scriptus*), rabbits (*Oryctolagus cuniculus*), and farm lizards (*Agama* spp.). Organisms were isolated and identified based on basic microbiological methods and subjected to antibiotic disc diffusion tests and electrophoretic plasmid analysis. Results show that *E. coli* strains isolated from persons on antibiotics were resistant to ampicillin (46.62%), augmentin (39.72%), clarithomycin (5.16%); while resistance to antibiotics by *E. coli* isolated from those that are not on antibiotics were ampicillin (27.27%), augmentin (20.00%), cetriaxone (18.18%) and nitrofurantoin (14.55%). Also, *E. coli* strains isolated from wild life were resistant to ampicillin (85.71%) and clarithomycin (14.29%). *Proteus* spp. isolated from human sources were susceptible to all test antibiotics except ampicillin; while those from wildlife were resistant to ampicillin (54.17%), chlorampenicol (16.67%), nitrofurantoin (8.33%), clarithomycin (8.33%), ofloxacin (4.17%), augmentin (4.17%) and pefloxacin (4.17%). The correlation matrices determined at p (≤ 0.05), revealed high resistance correlation among the different genera of bacteria isolated from one source to those from the other sources. Resistance plasmid analysis revealed the presence of 23 kb plasmid DNA in organisms obtained from the different sources. This suggests the possibility of bidirectional resistant gene transmission at the human–wildlife interface, indicating congruence between the drug resistance pattern of *E. coli* and *Proteus* spp. isolated from humans and wild animals. This calls for a holistic and forward-looking approach that will take the complex interconnections among species into full account, recognizing the important link between humans, animals and the environment in a bid to contain antibiotic resistance.

Key words: Drug resistance, humans, wild animals, *Escherichia coli*, *Proteus* spp., resistance plasmids.

INTRODUCTION

As human populations grow and transform landscapes, contact with wildlife concomitantly increases. Human modification of the environment is seen as the primary driver of the emergence of zoonotic diseases, through providing the opportunity for direct and indirect contact between humans and (sympatric) wildlife and increasing pathogen exposure and transmission potential (Mayer, 2000; Deem and Karesh, 2001; Pesapane et al., 2013). Disease emergence has been an important consequence of this escalation in interaction, with the majority of emerging infectious diseases in humans arising from wildlife reservoirs (Jones and Petel, 2008). These changes can induce immediate as well as long-term effects on pathogen transmission dynamics, modifying genetic and biological characteristics, biophysical elements, ecological dynamics, and socioeconomic, as well as host(s)–pathogen interactions (Smolinski and Hamburg, 2003).

Although controversy continues to surround the origin of many infectious diseases (Pearce-Duvet, 2006), many of humanity's most serious infections seem to have been the result of increasingly close and frequent contact with a new array of potentially zoonotic pathogens from animals intentionally domesticated for human use. Indeed, some of the most devastating and persistent human pathogens can be traced to zoonotic origins.

While environmental fecal waste may be an important source of pathogen exposure for both wildlife and humans, we still have a limited understanding of the complex process of pathogen spillover between wildlife and humans. The relative infrequency of pathogen spillover events limits our ability to evaluate the complexity of interacting and cascading factors driving this process (Goldberg et al., 2008).

Similarly, the genus *Proteus*, (an opportunistic infectious agent) which occur worldwide have been isolated from domestic dogs and cats around the world (Turkyilmaz, 2008; August, 1988). As opportunistic pathogens, they produce infections in humans only when they leave the intestinal tract. They are found in urinary tract infections, bacteremia, pneumonia and local lesions in debilitated patients or those receiving intravenous infusion. *Proteus* spp. are also alleged in food poisoning (Al-Mutairi, 2011).

E. coli and *Proteus* spp. have important roles in the increasing cases and dissemination of antibiotic resistance, potentially acquiring resistance determinants and acting as reservoirs for resistance genes (Reuben et al., 2013). Determining the resistance profile of these two organisms isolated from two different and diverse sources is the subject of this study. The study was aimed at comparing the resistance patterns of these two organisms from humans (presumably exposed to antibiotic selective pressure) and those from wild animals (with at most conjectural exposure to antibiotics).

MATERIALS AND METHODS

Study population and sample collection

Human population aged 10 and above, and Wild animals (such as squirrels, farm lizards, grass cutters, rabbits, antelopes and rats) were analyzed for drug resistant bacterial load. The evaluation was carried out from January, 2011 to March, 2013. Stool samples were collected from human subjects after informed consents were obtained from both the individuals and the hospital authority. Rectal and intestinal swabs were collected from dead or sacrificed wild animals caught with traps or bought from hunters. The clinical human samples were collected from Bishop Shanahan Hospital, Nsukka and the wild life samples were obtained from Okutu, Okpuje, Ede-oballa, Allor uno, Opi, Opi agu and Obollo communities in Enugu state, Nigeria. Human samples were obtained from two human groups, designated as Human not on antibiotics and Human on antibiotics. Human not on antibiotics represented persons who had not used antibiotics for 3 months prior to sample collection while Human on antibiotics represented groups that have had antibiotics therapy within 3 months.

Isolation and identification of bacteria

Each sample was directly inoculated onto separate nutrient and MacConkey agar plates. The inoculated plates were incubated for 24 h at 37^{o}C. The colonies obtained were isolated and further purified until pure cultures were obtained. Colonies obtained from MacConkey agar were classified as either lactose fermenters or non-lactose fermenters based on the pigmentation. All the isolates were Gram stained and examined microscopically. Biochemical tests were carried out based on the Gram reactions. Among the tests carried out were sugar fermentation, catalase, indole, oxidase, coagulase, H_2S and motility test. CHROMagar ™ (orientation) was used to further confirm the identity of the isolates.

Antibiotic susceptibility testing and plasmid profiling

Resistance to commonly used antibiotics was determined using the Kirby-Bauer disc diffusion method with the following Gram negative susceptibility disc: ciprofloxacin (CPX) (10 µg/ml), gentamicin (GEN) (10 µg/ml), ofloxacin (OFX) (10 µg/ml), augmentin (AUG) (30 µg/ml), pefloxacin (PEF) (10 µg/ml), clarithromycin (CMN) (30 µg/ml), chloramphenicol (CMP) (10 µg/ml), ampicillin (AMP) (30 µg/ml), nitrofurantoin (NIT) (100 µg/ml) and ceftriaxone (CTN) (30 µg/ml) (Poltes Med. Lab. Enugu, Nigeria). Isolates were scored as susceptible or resistant based on the interpretive criteria of the Clinical and Laboratory Standards Institute (CLSI) 2008. *Escherichia coli* (ATCC 25922) was used as reference (control) strain. Antibiotic resistant strains were subjected to plasmid profiling using the agarose gel electrophoretic separation of DNA materials (Kraft et al., 1988; Kado and Liu, 1981).

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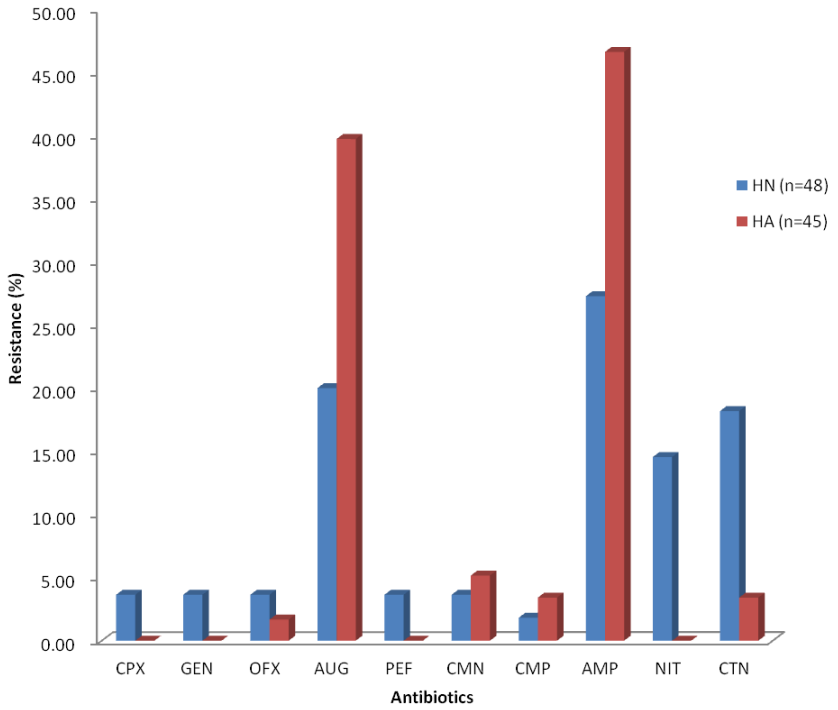

Figure 1. Antibiotic resistance pattern of *E. coli* isolated from the two human groups. CPX = Ciprofloxacin; GEN = Gentamycin; OFX = Ofloxacin; AUG = Augumentin; PEF = Pefloxacin; CMN = Clarithromycin; CMP = Chloramphenicol; AMP = Ampicillin; NIT = Nitrofurantoin , CTN = Ceftriaxone. HN=Human not on Antibiotics. HA= Human on Antibiotics.

Data analysis

Data obtained were subjected to analysis of variance (ANOVA) and Pearson Correlation Analysis using the Statistical Package for Social Sciences (SPSS 16.0) Inc. (444N Michigan USA). Correlation was carried out as a measure of the number of test isolates of a genius from a particular source that are resistant to the test drugs in relation to the isolates from same and the other sources.

RESULTS

Antibiotic sensitivity

E. coli isolated from persons on antibiotics showed resistance to ampicillin (46.62%), augmentin (39.72%), clarithomycin (5.16%); while resistance to antibiotics by *E. coli* isolated from those persons that are not on antibiotics were against ampicillin (27.27%), augmentin (20.00%), cetriaxone (18.18%) and nitrofurantoin (14.55%) (Figure 1).

All members of the genus *Proteus* isolated were susceptible to all the test antibiotics, except the *Proteus* sp. isolated from one individual on antibiotics that exhibited resistance to ampicillin.

The microbial resistance pattern exhibited by each of the microbial genera against the test antibiotics is shown in Table 1.

Table 1. Percentage distribution of antibiotic resistance among *E. coli* and *Proteus* spp. from wild life (WL) Percentage Resistance.

Antibiotics	*E. coli*	*Proteus* spp.
	WL(n=6)	WL(n=13)
CPX	0.00	0.00
GEN	0.00	0.00
OFX	0.00	4.17
AUG	0.00	4.17
PEF	0.00	4.17
CMN	14.29	8.33
CMP	0.00	16.67
AMP	85.71	54.17
NIT	0.00	8.33
CTN	0.00	0.00

CPX = Ciprofloxacin; GEN = gentamycin; OFX = ofloxacin; AUG = Augumentin; PEF = pefloxacin; CMN = clarithromycin; CMP = chloramphenicol; AMP = ampicillin; NIT = nitrofurantoin , CTN = ceftriaxone. 0.00 = No resistance that is all susceptible.

The organisms were most resistant to AMP, CMN and AUG, while the least resistance was demonstrated against

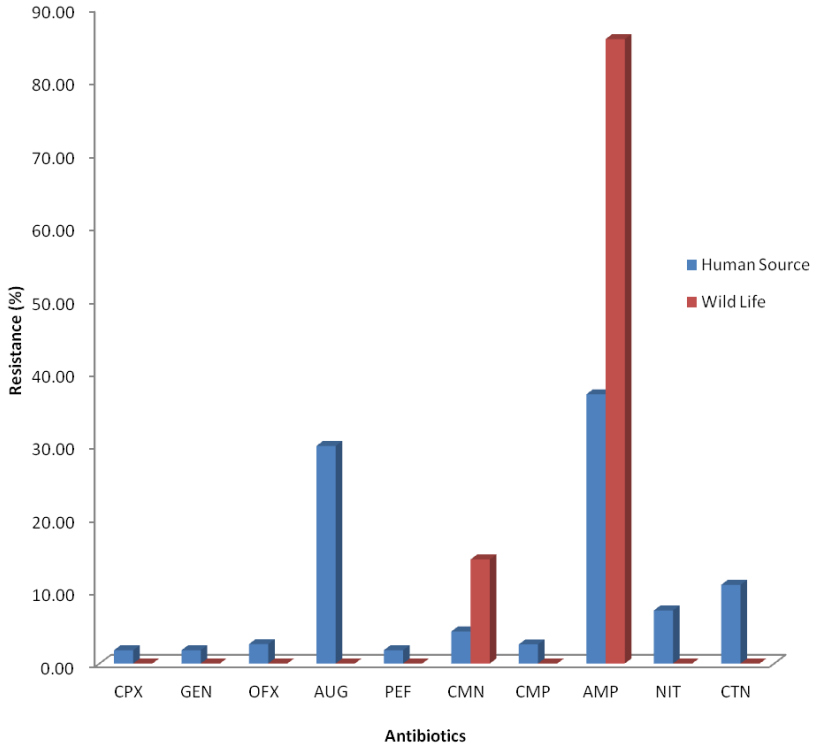

Figure 2. Antibiotic resistance pattern of *E. coli* isolated from the human and wild life sources. CPX = Ciprofloxacin; GEN = gentamycin; OFX = ofloxacin; AUG = Augumentin; PEF = pefloxacin; CMN = clarithromycin; CMP = chloramphenicol; AMP = ampicillin; NIT = nitrofurantoin, CTN = ceftriaxone. HN = Human not on antibiotics; HA = human on antibiotics.

PEF, CTN and CPX. Strains of *E. coli* isolated from wild life were more resistant to AMP (85.71%) than CMN (14.29%). They were susceptible to every other test antibiotic. In the same vein, *E. coli* isolated from Human sources also exhibited resistance against AMP (36.95%), AUG (29.86%) and CTN (10.80%). The comparative resistance pattern is shown in the Figure 2.

Species of *Proteus* isolated from human sources were susceptible to all antibiotics except AMP. Those isolated from wildlife were resistant to AMP (54.17%), CMP (16.67%), NIT (8.33%), CMN (8.33%), OFX (4.17%), AUG (4.17%) and PEF (4.17%) (Figure 3).

Correlation of resistance

A further analysis using correlation matrix showed that a strong direct relationship existed in the resistance pattern of the *E. coli* (0.704) regardless of the source of the isolate while the lowest correlate existed among *E. coli* and *Proteus* spp isolated from HN and HA (0.543) respectively.

In general, the analysis showed that there existed a high correlation in the resistance pattern of all the microorganisms obtained from both sources.

The correlates also examined the relationship between isolates from wildlife and human on antibiotics, as well as wild life and human not on antibiotics. Significance of correlation on the matrices was determined at p (≤0.05).

From the correlation matrix, high correlation coefficient was observed among the different genera of bacteria isolated from one source to those from different sources. For instance, the correlation matrix between isolates from persons on antibiotics and those from wild life revealed a correlate of 0.986 between *Proteus* (from those on antibiotics) and *E. coli* (from wildlife). In the same vein, a correlation coefficient of 0.948 existed between wildlife isolates of *E.coli* and *Proteus*.

Plasmid profile

The plasmid isolation and electrophoretic separation revealed the presence of plasmid DNA in some of the isolates. The plasmid DNAs were isolated from both resistant and susceptible organisms. Agarose gel electrophoresis of plasmid DNA (plates not shown) revealed the presence of relatively large plasmids in few of the test organisms.

The molecular weights of most of the plasmids were

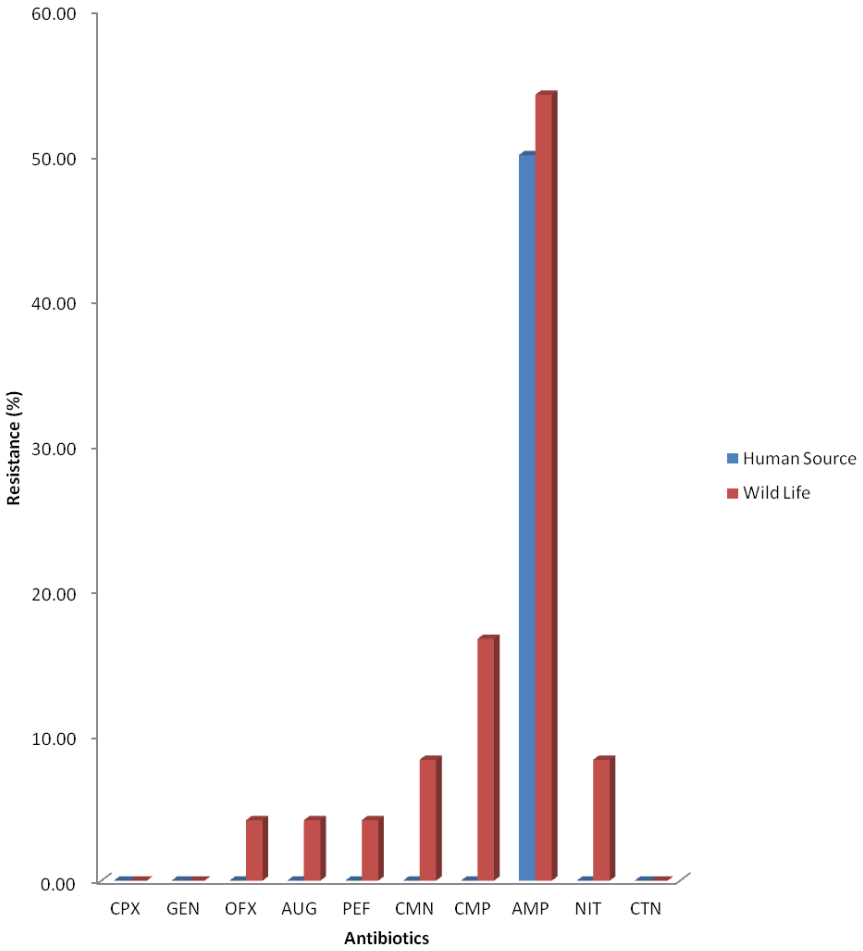

Figure 3. Antibiotic resistance pattern of *Proteus* isolated from human and wildlife sources. CPX = Ciprofloxacin; GEN = gentamycin; OFX = ofloxacin; AUG = Augumentin; PEF = pefloxacin; CMN = clarithromycin; CMP = chloramphenicol; AMP = ampicillin; NIT = nitrofurantoin, CTN = ceftriaxone. HN = Human not on antibiotics. HA = human on antibiotics.

Table 2. Distribution of the plasmid DNAs in *E. coli* and *Proteus* spp.

Bacteria Isolates	Number of Isolates tested	Number with plasmids	Antibiotic Profile	Mol. wt. of plasmid (kb)
E. coli	10	3	MDR & MDS	23.1
Proteus	10	4	MDR & MDS	23.1

MDR, Multi drug resistant; MDS, multi drug sensitive.

around 23.1 kb. The distribution of the plasmid DNA is shown in Table 2.

DISCUSSION

In this study, *E. coli* and *Proteus* were isolated and studied for the congruence in resistance pattern exhibited by both against commonly used antibiotics. Human and Wildlife isolates of *E. coli* and *Proteus* were examined for resistance to some antibacterial agents and by extension their potentials as reservoirs of multidrug resistance traits. These enterics were selected in the study because of their ubiquity and the diversity of the ecological environments of their hosts. Particularly, wildlife samples were chosen because it is expected that they have

negligible, if any, previous exposure to antibiotics.

E. coli was predominantly isolated from all the samples examined in the study, while the recovery rate of *Proteus* was relatively low. Both genera of bacteria have been previously reported to be commensals in humans except where they become opportunistic pathogens as a result of immunological challenges on the host(s) or other factors (Costa et al., 2013). In some cases they are found in the blood system where they cause blood sepsis and in urinary and genital tracts where they cause varying degrees of urinogenital disorders (Aibinu et al., 2003; Kruthi, 2006). Because these bacteria are found in large numbers in intestinal tract, they are transmitted most often via the faecal/oral route.

Tests of the ability of the *E. coli* and *Proteus* to resist commonly used antibiotics, using the disc diffusion test, revealed no observable significant difference in the resistance pattern between organisms isolated from persons on antibiotics and those that are not. This indicates that the ability of these organisms to resist some antibiotics is not only a function of previous exposure to antibiotics. It may also suggest a possible robust two-way transmission and colonization of these human groups by these organisms. Increasing microbiological and clinical evidences reveal that resistant bacteria or resistance determinants may be passed from animals to humans resulting in infections that are more difficult to treat (Sayah et al., 2004). The fact that ecological overlap increases the risks of microbial exchange between humans and wildlife gives credence to this. Infectious agents transmitted between humans and wildlife pose a risk to both human and animal. Human behavior such as hunting modifies and enhances this risk (Goldberg et al., 2007). Hunting (which made sample collection in this study easy) is a common feature in the study areas. This will definitely make it possible for wildlife borne organisms to be transferred to people through the food chain or direct contact and subsequent colonization, proliferation and development of difficult –to-treat or even untreatable diseases (Barbosa and Levy, 2000).

Typically, *E. coli* has been said to exhibit different resistance patterns (Reuben et al., 2013) without experiencing significant selection pressure (Goldberg et al., 2007). In Nigeria, data has shown that the prevalence of resistance to most drugs tested against *E. coli* isolates from apparently healthy students is within high range and has increased from 1986 to 1998. The observed increase in prevalence of resistance to beta-lactams and aminoglycosides were statistically significant (Okeke et al., 2000). The combined effects of fast growth rates to large densities of cells, genetic processes of mutation and selection, and the ability to exchange genes, account for the extraordinary rates of adaptation and evolution that can be observed in the bacteria. For these reasons *E. coli* adaptation (resistance) to the antibiotic environment seems to take place very rapidly. The results of this study support this impression.

The difference in resistance between wildlife and human bacterial isolates was analyzed statistically to determine whether it was systematic or random. Being systematic implies that the mechanisms are similar if not the same (Mach and Grimes, 1982). High resistance correlations noticed among some bacteria of different genera isolated from the two sources are indicative of systematic rather than random variation. These results strongly suggest that the antibiotic resistance patterns in these bacterial groups are similar, perhaps showing similar mechanisms for the development of this resistance with a difference only in the rate. Specifically, this points to the presence of a common or closely related genetic trait as responsible for resistance among these bacteria. Support for this is found in the molecular weights of the plasmids isolated from the test organisms that were obtained from different ecological sources. Large plasmids in bacteria are important in public health perspective because they mediate more than one mechanism of resistance among their host bacteria. For example, Gram negative efflux pump genes are widely distributed and associated with large plasmids (Byarugaba, 2010). Their common presence in these isolates is indicative of congruence between the drug resistant pattern of *E. coli* clones and *Proteus* spp. isolated humans and wild animals.

In light of these, it is necessary to seriously consider strategies to prevent the emergence and dissemination of antimicrobial resistant bacteria and develop a more holistic and forward-looking approach that will take the complex interconnections among species into full account, recognizing the important link between humans, animals and the environment. This is our recommendation.

Conflict of interests

The authors did not declare any conflict of interest.

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