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# Drug Resistant *Proteus mirabilis* and *Proteus vulgaris* Isolated from Rats Captured from Some Poultry Houses in Ibadan, Oyo State, Nigeria and their Public Health Importance

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**ABSTRACT**

*Proteus* species especially *Proteus mirabilis* and *Proteus vulgaris* are zoonotic pathogens often associated with drug resistance traits. They are of public health importance with zoonotic status. They have been globally associated with humans and poultry infections. Multidrug resistant strains of these organisms are routinely isolated from organs samples from carcasses of birds submitted for bacteriological diagnostic process in Nigeria with little or no information on their access route to poultry.

The uncontrolled close association of rats with poultry and other materials involved with poultry production in Nigeria, informed screening of 22 *Proteus mirabilis* and 1 *Proteus vulgaris* isolates from poultry houses rats, identified by standard methods. The isolates were further confirmed with Oxoid Microbact GNB 24E® (MB24E) and accompanying computer software package (Oxoid Microbact®) 2000 version 2.03 and by 16S ribosomal RNA PCR identification procedure. Their susceptibilities to 10 commonly used antibiotics using standard methods. Subsequently, the fluoroquinolone resistant isolates were PCR screened for point mutation at the gyrA of the quinolone resistant determining region. All the 23 isolates were multi-drug resistant, with 100% resistance to 6/10 of the antibiotics examined including: ceftazidime, amikacin, sulfamethoxazole, chloramphenicol, ampicillin and streptomycin. One of the 9, high fluoroquinolone resistant isolates MICs ranges 64µg/mL - >128µg/mL displayed 6 point mutations. This work identified rats as the possible source of multidrug resistant *Proteus* species for poultry in Nigeria. It also exposes the potential public health risk of the rats transmission of drug resistant factors through the pathogens to humans involved with poultry production in the study area.

**Keywords:** *Proteus mirabilis*; *Proteus vulgaris*; rats; zoonotic; public health; poultry

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## INTRODUCTION

*Proteus mirabilis and Proteus vulgaris* are members of the family enterobacteriaceae of medical importance (Mobley and Belas, 1995). *Proteus mirabilis* particularly, is a well-known zoonotic human pathogen of great public health importance found in soil, water, intestinal tracts of mammals, human inclusive (Mobley, 1995; Rozalski *et al*., 1997; Siddiquee *et al*., 2014). They have also been isolated from poultry meat (Kim *et al*., 2005; Wong *et al.*, 2013). The ability of the organism to harbour both plasmids and integron mediated antimicrobial resistant determinant is one of the indicators of their public health threats in terms of possible transmission of antibiotic resistance factors to other pathogens (Hall and Collis, 1998; Bush and Jacoby, 2010). For instance, it has been reported that some extended-spectrum β-lactamases (ESBL) and AmpC β-lactamases producing *Proteus mirabilis* could cause clonal spread resulting in intra- hospital, regional and even nationwide outbreaks (D'Andrea *et al*., 2011; Nakano *et al*., 2012).

*Proteus mirabilis* are usually associated with urinary tract infections, as opportunistic pathogens in wounds, burns and within the respiratory tracts (Deighton *et al*., 1992; Senior *et al*., 1995; Saito *et al.*, 2007). They have also been implicted in a lot of community and hospital acquired infections such as intra- abdominal and blood stream infection (O'Hara *et al*., 2000; Endimiani *et al.*, 2005).

Until the 1990s, when incidence of progressive increase in the resistance of the *Proteus* species to fluoroquinolones and broad spectrum cephalosporins began, its wild types were known to be susceptible to the antimicrobial agents (Hernandez *et al*., 2000; Kim *et al.*, 2004; Endimiani *et al.*, 2005). Since then, in addition to *Proteus mirabilis* producing extended beta lactamase (ESBLs) or the AmpC-type cephalosporinase, there are also reports of relative increase in prevalence of carbapenemases producers (Spanu *et al*., 2002; Endimiani *et al.*, 2005; Tsakris *et al.*,2007; Empel *et al.*, 2008;

Luzzaro *et al.,* 2009; Cohen-Nahum *et al*., 2010; D'Andrea *et al.,* 2011). Another public health concern regarding this organism is the development of resistance of the organism to broad spectrum fluoroquinolone which is usually a good treatment options for drug resistant *Proteus mirabilis*. There are reports also of increase in incidence of low susceptibility or resistance to some fluiroquinolones by the organism (de Champs *et al*., 2000; Hernandez *et al.,* 2000).

Rodents such as rats are often associated with infrastructural damage and eating or spoiling/contaminating of stored feed and products. They are of public health significance in terms of transmission of reservoir and vector for zoonotic pathogens and possibility as means of antibiotic resistant agents transmissions are often underestimated or even ignored (Meerburg and Kijlstra, 2007). This observation of Meerburg and Kijlstra (2007), is true in the area where the current work was carried out. Whereas, rats can transmit bacteria through feces, urine, and hair remnants (Padula *et al*., 2000; Meerburg *et al.,* 2006). Also, it has been reported that rat population at poultry farms can be a major reservoir of pathogenic bacteria which can transmit bacteria in the environment, food and animals and possibly to human (Rose *et al.,* 2000). It is therefore important to consider screening of rodents like rats for pathogenic zoonotic bacteria such as *Proteus mirabilis* and *Proteus vulgaris* to determine the risk of their transmission to poultry birds and products as well as to human. The current work thus screened 22 *Proteus mirabilis* and *1 Proteus vulgaris* isolated from some rats captured in some poultry houses in Ibadan, Oyo State, Nigeria, for their antibiotics susceptibilities to 10 commonly used antibiotics for food animals and humans in the study area. We subsequently screened for point mutation of the quinolone resistant determining region of the fluoroquinolone resistant isolates through PCR assay. The public health implication of the findings in terms of zoonotic disease and antibiotic resistant transmission was discussed.

## MATERIALS AND METHODS

### Bacteria Isolates

The 22 *Proteus mirabilis* and 1 *Proteus vulgaris* used for this study were recovered from oral/rectal swabs from rats captured in some commercial poultry houses located in the suburb areas of Ibadan, Oyo State Nigeria. They were identified as *Proteus mirabilis* and *Proteus vulgaris* based on standard morphological and biochemical, bacteriological procedures (Barrow and Felthams, 2004; Garcia and Isenberg, 2007). Their identities were further confirmed with Oxoid Microbact GNB 24E® (MB24E) and accompanying computer software package (Oxoid Microbact®) 2000 version 2.03 according to the manufacturers procedures as well as through *16S ribosomal RNA* PCR identification procedure.

### 16S RNA Identification of the *Proteus mirabilis and Proteus vulgaris*

The *16S ribosomal RNA* identification of the 23 isolates were performed as previously described by Weisburg *et al*., (1991) as modified. Chromosomal DNAs were produced from the 23 isolates by heating the LB broth cultures at 99ºC for 15minutes. A 100µl of the boiled isolates were mixed with equal volume of PCR grade water, 1 µl of the mixture was used as DNA template in a 50 µl reaction. The DNA was amplified using QS PCR reagents (New England Bio labs) using 1µM of fD2= 5'AGATTTGATCATGGCTCAG3' and rP1 = 5'ACGGCTACCTTGTTACGACTT3', including 10 µl QS buffer, 1 µl dNTPs, 0.25 µl fD1,0.25 µl rP1, 0.5 µl QS enzyme, 10 µl QS enhancer and 27 µl PCR water. The PCR programme involved: 98ºC for 30 seconds and 35 cycles of 98ºC for 10 seconds, 55ºC for 30 seconds, 72ºC for 1minutes 15 seconds and 72ºC for 7 minutes.

The amplified products were purified with Qiagen (QIA quick purification kit) based on the manufacturer's protocol and sequenced at Iowa State University DNA sequencing facilities (Ames, IA, USA). The identities of the sequenced products were analysed by using BLASTN 2.2.31+ as described by Zhang *et al.,* (2000).

### Determination of Resistance to Kanamycin, ampicillin, chloramphenicol, streptomycin, sulfamethoxazole, levofloxacin, ceftazidime, ceftriaxone, cefepime and amikacin

The isolates were grown aerobically in breakpoint concentrations of 32µg/mL each for kanamycin, ceftazidime, ceftriaxone, amikacin, ampicillin, and cefepime; at 64 µg/mL for streptomycin, 16 µg/mL for chloramphenicol, sulfamethoxazole at 1,024µg/mL and 8µg/mL for levofloxacin (all from SIGMA- ALDRICH) according to standard method (CLSI, 2009). Resistance was ascribed if flocculent growth was observed after 16h of aerobic growth at 37ºC.

### Analysis of the Quinolone resistant Determinant Region(QRDR) for the Levofloxacin resistant isolates

The MIC of the 9/23(45%) isolates that were resistant to 8µl/mL of levofloxacin were determined by standard method according to the CLSI procedure (CLSI, 2009).

The high fluoroquinolone resistant isolates were subsequently screened for point mutation through the amplification of the gyrA QRDR and DNA sequencing of the PCR product. It was carried out as previously described (Ogunleye *et al*., 2011).

A 560base pair region of gyrA of the crude boiled DNA was amplified with a universal forward and reverse oligonucleotide primers QRDR F=5´ATGAGCGACCTTGCGAGAAATACACCG3´ and QRDR R=5´TTCCATCAGCGCCCTTCAATGCTGAT GTCTTC3´ using QS polymerase reagents in a 50µl reactions containing 10 µl QS buffer, 1 µl dNTPs, 0.25 µl QRDRF, 0.25 µl QRDRR, 0.5 µl QS enzyme, 10 µl QS enhancer and 27 µl PCR water. The PCR protocol used involved: initial denaturation at 98ºC for 30seconds, and 35cycles of 98ºC for 10 seconds, 55ºC for 30 seconds, 72ºC for 1minute 15secondsand 72ºC for 7minutes.The amplified products were resolved with precast E- gel in an Electrophoresis unit (Life Technologies).

The amplified products were purified with Qiagen (QIAquick purification kit) and sequenced at Iowa State University DNA sequencing facilities (Ames, IA, USA).

## RESULTS

Twenty-two of the isolates were identified as *Proteus mirabilis* and 1 as *Proteus vulgaris* based on the conventional bacteriological analysis and were further confirmed with Oxoid Microbact GNB 24E® (MB24E) as well as with the 16s ribosomal RNA analysis (Table 1).

The 23 *Proteus* isolates were multidrug resistant. They exhibited 100% resistance to 6 of the anibiotics, namely: ceftazidime, amikacin, sulfamethoxazole, chloramphenicol, ampicillin and streptomycin. They also had 95.6 %( 22/23) resistance to kanamycin, 91 % ( 21/23) to ceftriaxone, 86.95% (20/23) to cefepime and the lowest percentage resistance of 39.1% for levofloxacin.

As shown in table 2, the 9 levofloxacin resistant to 8µg/mL of levofloxacin showed a high level of resistance with the minimum inhibitory concentrations ranges between 64 µg/mL to >128 µg/mL. One of the isolates (A15nlf) showed 6 point mutation at an MIC of 64 µg/mL. Plate 1 shows the gel picture of some the *Proteus* species amplified by 16s ribosomal PCR screening.

**Table 1:**
Drug resistance profiles of *Proteus mirabilis* and *Proteus vulgaris* from rat

| Isolate | 16s RNA identity | source | Ceftaz | Ceftria | Amik | cefep | Levo | sulf | Chloram | Kan | amp | strep |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A26nlf | *Pm* | Rat | R | R | R | S | S | R | R | R | R | R |
| A21nlf | *Pm* | Rat | R | R | R | S | S | R | R | R | R | R |
| A28nlf | *Pm* | Rat | R | R | R | R | S | R | R | R | R | R |
| A5nlf | *Pm* | Rat | R | R | R | R | S | R | R | R | R | R |
| B2anlf | *Pm* | Rat | R | R | R | R | R | R | R | R | R | R |
| A16nlf | *Pm* | Rat | R | R | R | R | S | R | R | R | R | R |
| B23nlf | *Pm* | Rat | R | R | R | R | S | R | R | R | R | R |
| B64nlf | *Pm* | Rat | R | R | R | R | S | R | R | R | R | R |
| A7nlf | *Pm* | Rat | R | R | R | R | S | R | R | R | R | R |
| A22lf | *Pm* | Rat | R | R | R | R | R | R | R | R | R | R |
| B4nlf | *Pm* | Rat | R | S | R | R | S | R | R | R | R | R |
| A15nlf | *Pm* | Rat | R | R | R | R | R | R | R | R | R | R |
| A48nlf | *Pm* | Rat | R | R | R | R | S | R | R | R | R | R |
| B6nlf | *Pm* | Rat | R | S | R | R | S | R | R | R | R | R |
| Ul5 | *Pm* | Rat | R | R | R | R | R | R | R | R | R | R |
| Ul3 | *Pm* | Rat | R | R | R | R | R | R | R | R | R | R |
| B63nlf | *Pm* | Rat | R | R | R | R | S | R | R | R | R | R |
| B19nlf | *Pm* | Rat | R | R | R | R | R | R | R | R | R | R |
| B14lf | *Pm* | Rat | R | R | R | R | S | R | R | S | R | R |
| A17lf | *Pm* | Rat | R | R | R | R | S | R | R | R | R | R |
| A6lf | *Pm* | Rat | R | R | R | R | R | R | R | R | R | R |
| Ul4 | *Pm* | Rat | R | R | R | R | R | R | R | R | R | R |
| B27nlf2 | *Pv* | Rat | R | R | R | S | S | R | R | R | R | R |

Pm= *Proteus mirabilis* Pv= *Proteus vulgaris;* Ceftaz=ceftazidime; ceftria= ceftriaxone; amik= amikacin; cefep= cefepime; levo=levofloxacin; sulf= sulfamethoxazole, kan= kanamycin; amp= ampicillin; strep= streptomycin

**Table 2:**
Minimum inhibitory concentrations and point mutation of *Proteus mirabilis* from rat

| Isolate | 16s rRNA identity | No of point mutation | levo(8µg/mL | Levo MIC |
|---|---|---|---|---|
| A15nlf | *P mirab* | 6 | R | 64µg/mL |
| B2anlf | *P mirab* | Nil | R | 128µg/mL |
| A22lf | *P mirab* | Nil | R | 64µg/mL |
| B19lf | *P mirab* | Nil | R | >128µg/mL |
| A26 nlf | *P mirab* | Nil | R | 64µg/mL |
| Ul4 | *P mirab* | Nil | R | > 128µg/mL |
| B2lf | *P mirab* | Nil | R | 64µg/mL |
| Ul3 | *P mirab* | Nil | R | >128µg/mL |
| Ul5 | *P mirab* | Nil | R | >128µg/mL |

P mirab= *Proteus mirabilis*; R= resistant; levo= levofloxacin; 16s RNA= 16s Ribosomal RNA

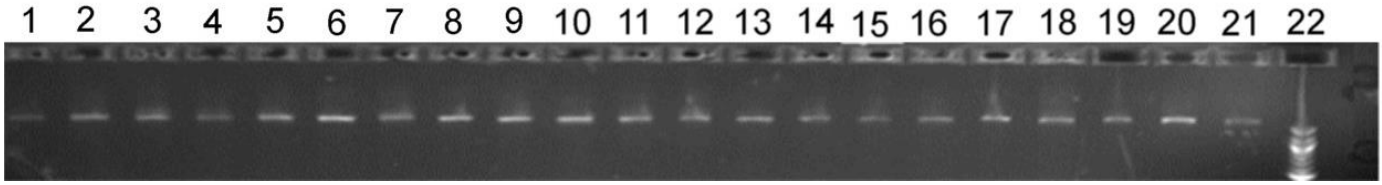


**Plate 1:**
Gel picture of 16s ribosomal RNA amplification.
Lane 1-20 were loaded with 20 of the *Proteus mirabilis* isolate, lane 21 contained *Proteus vulgaris* isolate and lane 22 was loaded with 1kb DNA ladder.

## DISCUSSION

*Proteus* species are of great public health importance, based on their established involvements in human diseases, as agents of food animal infections and contaminant of food animal products (Kim *et al*., 2005; Wong *et al.*, 2013; Siddiquee *et al.*, 2014). They are also increasingly identified with increase prevalence of antibiotic resistance, particularly to important antibiotics like fluoroquinolones and cephalosporin group of drugs, which are usually the treatment options for life threatening infections both in humans and animals from most parts of the world, Nigeria inclusive (Bush and Jacoby. 2010; D'Andrea *et al.*, 2011; Nakano *et al*., 2012). *Proteus* species occasionally, are responsible for embryonic death, yolk sac infections and mortalities in young chickens, turkeys and ducks (Baruah *et al.*, 2001). *Proteus mirabilis* among other enterobacteria have been reported in poultry and poultry products in Bangladesh (Barua *et al*., 2013; Siddiquee *et al.*, 2014); Croatia (Tonkic *et al.*,2010) and Brazil (Lima-Filho *et al.*, 2013).

In Nigeria, 2/20(10%) multidrug resistant *Proteus mirabilis* with 100% resistance to tetracycline and ampicillin, but sensitive to ofloxacin, ciprofloxacin and gentamicin were isolated from poultry feed samples screened bacteriologically at Calabar, Eastern part of Nigeria (Okonkwo *et al.*, 2010). Also *Proteus vulgaris* accounted for 0.7% from a total of 2000 organ samples comprising 400 each from the bone marrow, heart, liver, lungs and spleen of sick chickens collected from Jos, Northern, Nigeria (Dashe *et al*., 2013). Likewise, multidrug resistant *Proteus* species and *Pseudomonas aeruginos*a are also commonly isolated from organs of birds presented for postmortem examinations in a Veterinary Teaching hospital located in the town where the current work was carried out (Unpublished data). The isolation of the multidrug resistant *Proteus mirabilis* and *Proteus vulgaris* from rats captured in poultry houses in the study area gives insight to rats being the possible sources of the multidrug resistant *Proteus* species often encountered during postmortem examinations of carcasses from sick birds in the Veterinary Teaching hospital in the area, since rodents are well acknowledged reservoirs and vectors of bacteria agents of animal and human (Gratz, 1994).

The profiles of the antibiotic resistance exhibited by the isolates from rats are much more of public health significance. Unlike the multidrug resistant *Proteus* species (based on their resistance to 3 or more antibiotics), earlier isolated by Okonkwo *et al.*, (2010) from poultry feeds from the Eastern part of Nigeria, that were sensitive to fluoroquinolones and cephalosporins, 100% of the *Proteus* species in the current study were resistant to ceftazidime as well as amikacin, 91% to ceftriazone, 86.95% to cefepime and 39.1% to levofloxacin. The 9 fluoroquinolone resistant isolates also displayed a high level of fluroquinolone resistance with MICs ranges from 64µg/mL - >128 µg/mL, and one having 6 point mutation. The antibiotic resistant patterns exhibited by these bacteria isolates shows that they constitute potential health threat as possible agents of spreading not only zoonotic pathogen, but also as agents for spreading antibiotic resistant agents for different groups of antibiotics like aminoglycosides, fluoroquinolones as well as cephalosporin group of drugs to poultry and human poultry handlers. It is therefore important, that rats control in the study area should be taking more seriously, not just based on the basis of their well accepted roles in the destruction of poultry pen and poultry production appliances, but also from the point of view of their role in disease transmissions to human and animals.

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