

## Hemolysis production and resistance to fluoroquinolones among clinical isolates of *Escherichia coli* in Osogbo Metropolis, Southwest, Nigeria.

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**Abstract:** The activities of ampicillin, amoxicillin-clavulanic acid, gentamicin, tetracycline, nalidixic-acid, ciprofloxacin, pefloxacin and ofloxacin against 82 clinical isolates of *Escherichia coli* were determined by micro-dilution technique, according to NCCLS guidelines. Twenty two (27.3%) isolates were hemolytic, of which 16 (72.7%) were from urine samples, while 60 (72.7%) were non-hemolytic isolates. The percentage of resistance to quinolones - pefloxacin, ciprofloxacin, ofloxacin, nalidixic acid and amino-glycosides, among both hemolytic and non-hemolytic isolates were not significant ( $P>0.05$ ). However it is very significant for the penicillins- ampicillin, amoxicillin-clavulanic acid, tetracycline and Cotrimoxazole. ( $P<0.05$ ). Thus we conclude that susceptibility pattern to quinolones, nalidixic acid and aminoglycosides by *E. coli* is independent of the hemolysis factor since the level of resistance is not significantly different between the two isolates. While the susceptibility to the penicillins, tetracyclines and Cotrimoxazole is dependent on hemolysis factor since there is a significant difference between the sensitive hemolytic isolates and the non hemolytic isolates. [New York Science Journal. 2008;1(1):13-16]. (ISSN: 1554-0200).

**Keywords:** Hemolysis, Resistance, Fluoroquinolones, *Escherichia coli*, southwest, Nigeria.

### Introduction:

Resistance to fluoroquinolones in *Escherichia coli* is an increasing problem in Nigeria and other countries. [Daini, 2005]. Most *Escherichia coli* isolates are normal, benign residents in the intestines of animals. However, small percentages of *E. coli* are pathogenic and cause a variety of diseases ranging from diarrhea to septicemia. The properties which allow pathogenic *E. coli* to invade infect, and damage host cells are conferred by adhesins, toxins, and haemolysins. *E. coli* can produce several types of haemolysin, including an extracellular protein ( $\alpha$ -haemolysin), a cell-bound protein ( $\beta$ -haemolysin) and a haemolysin expressed by nalidixic acid-resistant mutants ( $\gamma$ -haemolysins). [Cavalier, 1984; Walton, 1969]. Several mechanisms are known to determine this resistance in *E. coli*, including mutations in the topoisomerase (II and IV) genes, and decreased accumulation because of outer membrane alterations and or the expression of efflux pumps. [Everett, 1996].

In uropathogenic *E. coli* and other isolates that cause extra intestinal disease, the alpha haemolysin, (hlyA) is a particularly important virulence factor [Beutin,1991] This haemolysin belongs to a family of proteins called repeat-in-toxins (RTX) because of the tandem arrays of a nine amino acid repeat with the consensus sequence [Weich,1988; Bauer, 1996]. While determining antimicrobial susceptibility in our clinical laboratory we have often observed that majority of *E. coli* strains resistant to quinolones were non-hemolytic. In this study therefore we intend, to establish the relationship of hemolysis with the levels of resistance of *E.coli* strains to quinolones and determine whether strains of *E.coli* resistant to quinolones that are hemolytic are from specific body fluid.

### Materials and methods.

#### **Bacterial strains**

Eighty-two isolates from, urine, cerebrospinal fluid, peritoneal fluid, blood culture, wound exudates were cultured for hemolysis testing from different patients referred to the clinical laboratory of the Ladoké Akintola University of Technology Teaching Hospital, Nigeria, from October 2004 to December 2005 were evaluated.

### Susceptibility testing

Bacterial identification to susceptibility testing was performed by micro-dilution, according to NCCLS guidelines. The following antimicrobial agents were studied: ampicillin, amoxicillin-clavulanic acid, gentamicin, tetracycline, nalidixic acid, ciprofloxacin, pefloxacin and ofloxacin. Organisms were considered resistant to the antimicrobial agents evaluated when the corresponding MICs were 16mg/ml for ampicillin, 16.2mg/ml for amoxicillin-clavulanic acid, 8mg/ml for gentamicin, tetracycline and pefloxacin, 32mg/ml nalidixic acid and 2mg/ml ciprofloxacin and ofloxacin. These breakpoints allowed comparison of susceptible versus non-susceptible (either intermediate or resistant) isolates, according to NCCLS guidelines. [NCCLS, 1997]

### Determination of hemolytic activity.

An organism was considered hemolytic when a clear halo was observed around isolated colonies after overnight incubation. The organisms were considered -haemolysin producers when hemolysis was observed on agar base containing sheep blood (5%, BioMérieux, Marcy l' Etoile, France) but not when containing human blood (5%).

### Statistical methods.

The statistical significance of differences in resistance to antimicrobial agents between hemolytic and non-hemolytic isolates was tested using the 2 test and (in the case of gentamicin) Fisher's exact test. Differences were considered significant when P was <0.05

### Results:

Among the eighty-two isolates tested for hemolysis 22 (26.8%) isolates produced hemolysis while 60 (73.2%) are non-hemolytic isolates. Of the hemolytic isolates 16 (72.1%) were isolated from the urine samples, 4 (18.2%) from stool samples and 2 (9.1%) from blood samples. The resistance pattern among both hemolytic and non-hemolytic isolates showed that 90.9% and 100% for ampicillin (P=0.06), 81.8% and 100% for amoxicillin-clavulanic acid (P=0.004), 9.1% and 11.7% for gentamicin (P=1.00), 81.8% and 98.3% for tetracycline (P=0.017), 31.8% and 40.0% for nalidixic acid (P=0.61), 31.8% and 24.0% for ciprofloxacin (P=0.61), 18.2% and 38.3% for pefloxacin (P=0.11) 50.0% and 60.0% for co-trimoxazole (P=0.46). Twenty two (26.8%) isolates were haemolytic on both sheep and human blood agar, suggesting that these organisms produce -haemolysins. When compared, the percentages of resistance of both isolates to quinolones- ciprofloxacin, pefloxacin, nalidixic acid, co-trimoxazole and gentamicin were not significant statistically (P=0.61, 0.11, 0.61, 0.46 and 1.0 respectively) While the percentage resistance of the two isolates to tetracycline, ampicillin and amoxicillin-clavulanic acid were statistically significant (P=0.017, 0.06 and 0.0042 respectively).

Table I: Showing the comparative resistance pattern of hemolytic and non-hemolytic isolates of Escherichia coli to various antimicrobial agents.

Drugs	Hemolytic isolates N=22 (26.8%)		Non-hemolytic isolates N=60 (73.2%)		P-value
	Sensitive (%)	Resistant (%)	Sensitive (%)	Resistant (%)	
Ampicillin	2 (9.1%)	20 (90.9%)	0 (0%)	60 (100%)	P=0.06
Amo-clacid	4 (18.2%)	18 (81.8%)	0 (0%)	60 (100%)	P=0.004
Gentamicin	20 (90.9%)	2 (9.1%)	53 (88.3%)	7 (11.7%)	P=1.00
Tetracycline	4 (18.2%)	18 (81.8%)	1 (1.7%)	59 (98.3%)	P=0.017
Nalidixic-acid	15 (68.2%)	7 (31.8%)	36 (60%)	24 (40%)	P=0.61
Ciprofloxacin	15 (68.2%)	7 (31.8%)	36 (60%)	24 (40%)	P=0.61
Pefloxacin	18 (81.8%)	4 (18.2%)	37 (61.7%)	23 (38.3%)	P=0.11
Cotrimoxazole	11 (50%)	11 (50%)	24 (40%)	36 (60%)	P=0.46

Amo-clavid = amoxicillin and clavulanic acid.

Table II: Showing the body fluid distribution of hemolytic isolates of *E. coli*.

Source of body fluid	No of samples with hemolysis.
Urine	16 (72.7%)
Blood	2 (9.1%)
Stool	4 (18.2%)
Cerebrospinal fluid	Nil
Peritoneal fluid	Nil
Wound swab	Nil

**Discussion:**

Although several workers have reported various different properties of virulence of *Escherichia coli* strains and their possible risk to health in humans [Wieler, 1998; Olowe, 2003] Hemolysis is one type of virulence factor that assist in the pathogenesis of *Escherichia coli* both in man and avian [Reingold, 1999]. Expression of cytotoxicity by potential pathogens of *Escherichia coli* was also believed to have been initiated by the presence of hlyA gene, which may be part of Pathogenicity island [Xin-Helai, 1999].

It is not known if resistance to quinolones and loss of haemolysis are caused by common or related mechanisms or if these phenotypes are derived from independent mutations. Most clinical isolates of *E. coli* resistant to quinolones are gyrA mutants [Everett, 1996]. The mechanisms responsible for the predominance of non-haemolytic *E. coli* strains among those expressing resistance to quinolones are unknown. Ciprofloxacin-resistant mutants (MIC of ciprofloxacin ranging from 0.03 to > 32 mg/L) derived in vitro from haemolytic-susceptible isolates still produced haemolysis. These mutants are not related to the previously described -haemolysin producers (selected in the presence of nalidixic acid) as they still haemolyse human erythrocytes which agrees with the previous work of Martinez [Martinez, 1998]. The relationship between haemolysin production and resistance to ofloxacin could not be evaluated, as all strains were susceptible to ofloxacin. Our data for tetracycline are similar to those obtained by other authors. But contrary to other authors our isolates showed a significant difference in resistance to Gentamicin among haemolytic and non-haemolytic isolates. In fact, the few gentamicin-resistant strains in our study were from both haemolytic and non-haemolytic. The only difference is that there are more Gentamicin resistance in non-haemolytic than haemolytic, and the degree of resistance is not significantly different  $P=1.0$ . It was initially reported that most clinical isolates of *E. coli* resistant to quinolones are gyrA mutants [Everett, 1996] and it is possible that altered super coiling of DNA in these mutants may affect the expression of genes involved in haemolysin production. Another possibility is the existence of pleiotropic mutations in quinolone-resistant *E. coli* strains that may interfere with the expression or activity of haemolysin, as has recently been reported for mutations affecting the genes involved in lipopolysaccharide synthesis. [Bauer, 1997] Our study shows that haemolysin is becoming pronounced among our clinical isolates especially among the gram negative organisms which have led to increasing development of resistant strains to the common antimicrobial agents that is currently available. Also the study shows that haemolysin is less frequently produced by quinolone-resistant isolates of *E. coli* when compared with the penicillin, tetracycline and co-trimoxazole even though the amino-glycosides have superior sensitivity, with marked reduction in the production of haemolysin compared with the quinolones. It is possible that resistance to quinolones may be indirectly attributable to the cost of the drug which has reduced the drug pressure, consequently decreasing bacterial virulence, this finding agrees with the work of Martinez [Martinez, 1999].

Further testing is required to fully determine hemolysis of *Escherichia coli* in various body fluids as linked with antimicrobial resistance with more emphasis on the current trends of resistance development to the quinolones.

The rates of quinolone resistance reported here are similar to those presented in other studies from Spain, [Daini, 2005; Martinez, 1999] and higher than those of a previous study in our institution, [Olowe et al unpublished communication] confirming the tendency to increased quinolone resistance during recent years. Hariharan et al. have shown that resistance to co-trimoxazole, neomycin and tetracycline in *E. coli* strains isolated from piglets with diarrhoea was less frequent among strains producing heat-labile enterotoxin (LT) and haemolysin than among those lacking both factors, while the resistance to gentamicin was more frequent in LT-haemolysin producers than among LT-haemolysin non-producers.

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