

# Starch paper technique is easy to detect beta lactamase detection from cases of diarrheagenic *Escherichia coli* in Osogbo

Olowe OA<sup>1\*</sup>, Eniola KIT<sup>2</sup>, Olowe RA<sup>3</sup>, Olayemi AB<sup>2</sup>

<sup>1</sup>Department of Medical Microbiology and Parasitology, P.M.B.4400. Ladoke Akintola University of Technology, College of Health Sciences, Osogbo Osun State, Nigeria; <sup>2</sup>Department of Microbiology, Faculty of Sciences, University of Ilorin, Kwara State, Nigeria; <sup>3</sup>Department of Biology, Federal University of Technology, Akure Ondo State, Nigeria

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## Abstract

Out of two hundred and twenty isolates recruited for childhood diarrhea cases, were reported in other studies. We selected eighty-two *Escherichia coli* samples that were positive for beta-lactamase through paper techniques, using Odugbemi techniques and non beta-lactamase producing strains as control, relative resistance pattern was observed for both. The resistance pattern of *Escherichia coli* to all the antibacterial tested for among the beta-lactamase, showed highest resistance to penicillin 100%, ampicillin with 98.8% and ceftriaxone showed the highest susceptibility of 65% and resistance of 20.7% respectively. The distribution pattern of *Escherichia coli* was highest in age group 1 – 3 years among the beta-lactamase producing *Escherichia coli* strains with 42.7% and lowest within the age group 5 – 7 with 3.7%. There was no significant difference from the results of beta-lactamase producing strains of *Escherichia coli* to non beta-lactamase producing *Escherichia coli* strains,  $P < 0.05$ . From the results beta-lactamase proliferation in our clinical samples is becoming a concern to antimicrobials usage. [Life Science Journal. 2007; 4(4): 72 – 74] (ISSN: 1097 – 8135).

**Keywords:** beta lactamase; prevalence pattern; paper technique; southwest Nigeria

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## 1 Introduction

Diarrhoeal diseases are part of the main social problems in Osogbo, Nigeria, as in other developing countries in the tropics (Olowe *et al*, 2003). However, the major pathogen most commonly associated with children diarrhoea is *Escherichia coli*. *Escherichia coli* is one of the main causes of nosocomial infections in humans. *E. coli* is also a common inhabitant of the human and animal gut and is considered an indicator of fecal contamination in food (Laura *et al*, 2002). Despite the fact that antibiotics were initially developed for the treatment of infectious disease in people, multidrug resistance to these antibiotics are becoming of global concern. In Nigeria, few reports have documented the prevalence of beta-lactamase producing microorganism especially the gram-negative bacilli. Hence the existence of the  $\beta$ -lactamases presented a distinct survival advantage to the bacteria (Brook *et al*, 1995). This widespread use of antibiotics could be associated

with the selection of antibiotic resistance mechanisms in pathogenic and non-pathogenic strains of *Escherichia coli* (Odukoya *et al*, 1997; Livermore *et al*, 1998). This has given rise to misuse of antimicrobial agents with the emergence of multi-drug resistance pathogens (Pitout *et al*, 1998; Agbolahour *et al*, 1982). This study is a report of our first twelve months experience of the pattern and susceptibility profile of beta-lactamase producing *Escherichia coli* from cases of diarrhea using starch paper method at Ladoke Akintola University Teaching Hospital Osogbo, Southwest Nigeria.

## 2 Materials and Methods

The study was carried out in Lautech college of Health Sciences Teaching Hospital; samples were collected from stool samples. All the samples were aseptically processed in the laboratory, according to standard microbiological procedures. Biochemical tests were carried out on the isolates using conventional microbiological methods according to Cowan and Steel (1985). Antibiotic susceptibility

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\*Corresponding author. Email: olowekunle@yahoo.com

by the disc diffusion method was performed on all bacterial isolates, following NCCLS standard using McFarland standard. The diameters of the zone of inhibition were measured using meter rule and interpretation chart was used to determine the resistance pattern of the isolates (Odukoya *et al*, 1997) The detection of the beta-lactamase was carried out using the starch paper technique according to Odugbemi starch paper technique (Odukoya *et al*, 1997, 1995).

### 3 Results

In Table 1, a consideration of the age distribution showed *Escherichia coli* was more prevalent in children aged 1 to 3 years (42.7%). The prevalence rate in males and females were 25 and 10 respectively within this age group. Whereas in age over 5 years old, the prevalence pattern was found to be lowest with percentage rate of (3.7%), male accounting for 1 and female 2 respectively. Table 2 showed the results of overall susceptibility profile of beta-lactamase producing *E. coli* from clinical isolates. Out of the 82 beta-lactamases that were positive by starch paper method, (98.8%) were resistant to Ampicillin, while resistance to penicillin G was (100%), Gentamicin (35.4%), Cephalexine with (24.4%) and tetracycline (94%). Results of antibiotic susceptibility with respect to Non-β-lactamase production were shown in Table 3. Highest resistance was seen with Ampicillin, with 10 out of the 18 isolates showing resistance (55.6%), for Cloxacillin 16 out of 18 showed resistance (88.9%), and Penicillin had 14 out of the total 18 that showed resistance (77.8%).

**Table 1.** Age distribution of isolated *E.coli* that are positive to beta-lactamase

Age	Male	Female	Total	Rate (%)
< 1	12	13	25	30.4
1 – 3	10	25	35	42.7
3 – 5	6	13	19	23.2
> 5	1	2	3	3.7
Total	29	53	82	100

### 4 Discussion

Beta-lactamase producing gram negative bacilli is becoming a problem in the tropics, yet the epidemiology is not well charted, the aetiopathogenesis is not fully understood and treatment is becoming more difficult due to high prevalent with high multidrug resistant strains. This study was

**Table 2.** Susceptibility pattern of isolated *E. coli* to different antibiotics and percentage resistance

Antibiotics	Isolated (n)	Sensitivity (%)	Resistance n (%)
Ampicillin	82	1	81 (98)
Penicillin	82	0	82 (100)
Amoxicillin	82	7	75 (91)
Cephalexine	82	60	20 (24.4)
Ceftriaxone	82	65	17 (20.7)
Gentamicin	82	53	29 (35.4)
Erytromycin	82	59	23 (28.0)
Streptomycin	82	40	42 (51.0)
Tetracycline	82	5	77 (94.0)
Chloramphenicol	82	54	28 (34.0)

**Table 3.** Susceptibility pattern of non-beta-lactamase producing *E. coli* to different antibiotics and percentage resistance

Antibiotics	Isolated (n)	Sensitivity (%)	Resistance n (%)
Ampicillin	18	8	10 (55.6)
Penicillin	18	4	14 (77.6)
Amoxicillin	18	2	16 (88.9)
Cephalexine	18	18	0 (0)
Ceftriaxone	18	16	2 (11.1)
Gentamicin	18	11	7 (38.9)
Erytromycin	18	14	4 (22.2)
Streptomycin	18	8	10 (55.6)
Tetracycline	18	3	15 (83.3)
Chloramphenicol	18	14	4 (22.2)

designed to highlight the current antimicrobial susceptibility pattern of beta-lactamase producing *Escherichia coli* strains in this area, from cases of diarrhea especially from childhood. Over 93% of *Escherichia coli* respectively were found to be β-lactamase producers. The results of isolates to be positive for beta-lactamase production is in line with the previous reported incident of extended spectrum beta-lactamase prevalence of *Escherichia coli* as reported by Odugbemi *et al* (1995). Beta-lactamase production by *Escherichia coli* is by recognized mechanism of resistance to β-lactam antibiotics, such as penicillin and Ampicillin (Marchese *et al*, 1998) . High rate of resistance was also observed in non-β-lactamase producer. Resistance to penicillin may be due to lack of penicillin receptors or inaccessibility receptors because of permeability barriers of bacterial outer membranes (Odugbemi *et al*, 1995). Another possibility is the failure of activation of autotypic enzymes in the cell wall, which can result

in inhibition without killing bacteria, may also contribute to high resistance rate as reported by Brook *et al* (1995). The resistance pattern exhibited by the bacterial isolates of commonly used antimicrobial agent in our environment is a reflection of misuse as a result of poor antibiotic prescription policy and off - the - counter availability of these agents. The third generation cephalosporins, and fluoroquinolones, which are rather expensive, are not yet prone to such abuse. These can provide viable alternative treatment of resistant infection (Machese *et al*, 1998). From these findings,  $\beta$ -lactamase producing *Escherichia coli* was found to have highest resistance pattern to most antimicrobial drugs available in this area. But the resistance pattern of non-beta-lactamase was seen to exhibit some degree of resistance but not as predominant as beta-lactamase ( $P < 0.05$ ). This result is first of its kind from cases of childhood diarrhea testing for beta-lactamase production using starch paper techniques.

## 5 Conclusion

Results shows that there was high indication of presence of the beta-lactamase found in the isolates used for the test and of the percentage of resistance to commonly used antibiotics. The method also is a probability that isolates may actually harbor plasmids, which may actually cause the ability for resistance among the isolates. In light of these findings, we suggest prompt antibiotics susceptibility test in addition to test for  $\beta$ -lactamase production. This will aid in mentoring multi-drug results pattern of the beta-lactamase in our environment.

## References

1. Olowe OA, Olayemi AB, Eniola KIT, Adeyeba OA. Aetiologic agents of diarrhoea in children under 5 years of age in Osogbo, Osun state. African J of Clinical and Exp. Microbiology 2003; 4(2): 62 – 6.
2. Laura B, Myriam Z, Yolanda S, Fernanda RL, Carmen T.  $\beta$ -lactamases in ampicillin- resistant *Escherichia coli* isolates from foods, humans, and healthy animals. AAC 2002; 46: 3156 – 63.
3. Brooks GF, Rutel JS, Ornston LM. Jawetz, Melnick and Adelberg's Medical Microbiology 20th ed. 1995; 14: 191.
4. Olukoya DV, Olasupo NA. Drug resistance and plasmid profile of diarrhoeagenic bacterial isolated in Nigeria (1988 – 1996). Nig Ot J Hosp Med 1997; 7: 29 – 32.
5. Livermore DM. Beta-Lactamase-mediated resistance and opportunities for its control. J Antimicrob Agents 1997; 41(suppl. D): 25 – 41.
6. Pitout JD, Thomson KS, Hanson ND, Ehrhardt AF, Moland ES, Sanders CC.  $\beta$ -Lactamases responsible for resistance to expanded-spectrum cephalosporins in *Klebsiella pneumoniae*, *Escherichia coli*, and *Proteus mirabilis* isolates recovered in South Africa. Antimicrob Agents Chemother 1998; 42: 1350 – 4.
7. Cowan ST. Cowan and Steel Manual for the Identification of Medical Bacterial, Cambridge University Press, London and New York. 1985, 54 – 120.
8. Odugbemi T, Hafiz S, Mc entergert MG. Penicillinase-producing Neisseria gonorrhoea: detection by starch paper techniques. British Medical Journal 1977; 2: 500.
9. Odugbemi T, Animashaun T, Kesha K, Oduyebo O. Une etude dela sensibility antimicrobienne *in vitro* disolate bacteria bacieriens cliniques a Lagos au Nigeria. In Medicine Digest  $\beta$ -lactamasesurvey (African team) 1995; Vol.xxi-suppl No 41: 39 – 54.
10. Abraham EP, Chain E. An enzymes from bacteria able to destroy penicillin. Nature 1940; 146: 837.
11. Marchese A, Arlet G, Schito GC, Lagrange PH, Philippon A. Characterization of FOX-3, an AmpC-type plasmid-mediated beta-lactamase from an Italian isolate of *Klebsiella oxytoca*. Antimicrob Agents Chemother 1998; 42: 464 – 7.
12. Sunde M, Sorum H. Characterization of integrons in *Escherichia coli* of the normal intestinal flora of swine. Microb Drug Resist 1999; 5: 279 – 87.
13. Agbolahor DE, Odugbemi TO. *In Vitro* Antimicrobial susceptibility of Yersinia enterocolitica isolates in Lagos. Nigeria Nig Med J 1982; 12(2): 125 – 79.