



Antimicrobial susceptibility of enterohaemorrhagic *Escherichia coli* 0157:H7 isolated from diarrhoeal patients in Jos hospitals, Nigeria

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Received 19 May 2006, accepted 4 July 2006.

Abstract

A total of 26 enterohaemorrhagic *Escherichia coli* 0157:H7 isolates from diarrhoeal patients in Jos area were tested for susceptibility to 11 antimicrobial agents by the standard disc diffusion and tube dilution methods. All the isolates were susceptible to gentamycin, ciprofloxacin and chloramphenicol but resistant to cloxacillin. There was a high prevalence susceptibility of the isolates to tetracycline (92.3%) and cephalexin (80.8%) by disc diffusion method. The isolates were moderately susceptible to doxycycline (46.2%) and ampicillin (42.3%) by tube dilution method. A low prevalence of resistance by tube dilution method to ampicillin (42.3%), doxycycline (42.3%), erythromycin (42.3%), amoxicillin (42.3%) and cefuroxime (26.9%) was observed. The difference in susceptibility results between the two methods of susceptibility assessment was minor. The tube dilution method was found to better distinguish between sensitive and resistant strains of *E. coli* 0157:H7 to the chosen antibiotics.

Key words: Enterohaemorrhagic *Escherichia coli* 0157:H7, antibiogram, Jos.

Introduction

Escherichia coli is often regarded as a benign commensal of the intestinal tract in man. However, there are known pathogenic strains within this species that are associated with intestinal pathogenicity¹⁴. Currently there are four main strains of *Escherichia coli* well recognized to cause diarrhoeal disease in human⁴. These strains differ in pathogenesis, epidemiology and clinical syndrome¹⁴ and are grouped as non-shiga toxin producing *E. coli*. These are enteropathogenic *E. coli* (EPEC) which represents the first recognized diarrhoeagenic strain of *E. coli*, and is associated with children after the neonatal period¹⁰. Enterotoxigenic *E. coli* (ETEC) causes diarrhoea in adults and children in developing countries¹⁴. In adults, ETEC incidence frequently occurs where sanitary conditions are poor. Enteroinvasive *E. coli* (EIEC) causes disease that is indistinguishable from *Shigella* dysentery¹⁴. This strain is often non-motile and non-lactose fermenting like *Shigella* species and are generally identified to infect children in the developing countries⁴. Enterohemorrhagic *E. coli* (EHEC) is known as probable cause of chronic diarrhoea in HIV infected patients⁴.

An outbreak in the 1980's and sporadic cases of copious bloody diarrhoea and colitis in Oregon and Michigan 1982²², Nebraska nursing home²³ and others led to the discovery of enterohaemorrhagic *E. coli* (EHEC) serotype 0157:H7, a shiga-like toxin producing *E. coli* which causes disease ranging from mild diarrhoea to haemorrhagic colitis²². The disease may progress in some patients to haemolytic uremic syndrome (HUS) with its associated renal failure. Haemolytic uremic syndrome (HUS) is

a life threatening condition especially among children and the elderly. The death rate associated with HUS was reported to be 3-5% in USA⁶.

The role of EHEC disease has significantly been reported in the United Kingdom¹², North America⁴, Czechoslovakia and Germany²¹. In clinical practice, the ability to control and safely treat a disease is of major interest to clinicians, particularly those practicing in disease endemic areas such as developing countries. The post infection use of antibiotics is the primary means of combating infection. However, in the case of EHEC infection, some factors must be considered when treating patients with an antimicrobial/antidiarrhoeal agent. It was reported that treatment of this disease with antibiotics may precipitate kidney complication⁶. Thus lack of adequate information on the disease and on the susceptibility of this disease to antibiotics when used may result in inappropriate therapy and complication.

The aim of this research was to determine the susceptibility pattern of enterohaemorrhagic *E. coli* 0157:H7 isolated from diarrhoeal patients visiting three hospitals in Jos metropolis, Nigeria, to selected antibiotics; and also to determine whether this antibiogram could be adopted as biomarkers for this area and/or other geographical areas within the country.

Materials and Methods

Study population: A total of 850 diarrhoeal specimens collected from patients attending three selected hospitals in Jos and 200 control patients without diarrhoeal disease (visiting the same hospitals for illness other than gastroenteritis) were investigated.

Laboratory studies: The faecal specimens were cultured for common intestinal pathogens including *Salmonella* spp., *Shigella* spp., *Campylobacter* spp., *Vibrio* spp. and *Yersinia* spp. by standard bacteriological methods^{7,9}.

All colonies on MacConkey agar (MCA) plates suspected to be *E. coli* (lactose fermenter, non-mucoid, 2-3 mm diameter, circular, smooth and convex) were screened for *E. coli* 0157:H7 by subculturing onto Sorbitol MacConkey agar (Oxoid medium code CM 813) and incubated at 37°C for 18-24 hours¹⁵. Non-sorbitol fermenters on Sorbitol MacConkey agar (SMA) plates were tested with latex agglutination test kits (Oxoid Latex test kit code DR620)¹⁶ and immobilization test with antisera to *E. coli* 0157:H7 flagella antigen⁸ (H7 flagella antiserum from Difco laboratories, Detroit, Michigan). Isolates that did not ferment sorbitol within 24 hours, were latex agglutination test positive and immobilized in a column of semi-solid agar containing *E. coli* 0157:H7 flagella antiserum were confirmed as *E. coli* 0157:H7.

The following antibiotic discs were obtained and used: ampicillin (10 µg), chloramphenicol (30 µg), cephalexin (30 µg), ciprofloxacin (1 µg), erythromycin (15 µg), cloxacillin (1 µg), doxycycline (10 µg), gentamycin (10 µg), amoxycillin (10 µg), tetracycline (30 µg) and cefuroxime (30 µg). The choice of antibiotics/discs used was based on those commonly available in most chemists within the locality. Also they are groups of antibiotics recommended in previously published susceptibility profiles for their potential efficacy as antimicrobial agents on gastrointestinal infection^{3,5,13}. The antibiotic susceptibility patterns of *E. coli* 0157:H7 isolated were determined by disc diffusion test using iso-sensitest agar (Oxoid medium code CM471) according to standard procedures^{2,18,20}. Quality control strain used was *Staphylococcus aureus* NCTC 6571 to test iso-sensitest agar and susceptibility discs for proper zone sizes before it was used with clinical isolates. The interpretation of results was as susceptible, moderately susceptible or resistant based on zone diameters of inhibition described as international standard^{2,5,13,18,20}.

The standard broth dilution method employing an inoculum of 5x10³ cfu/ml to determine minimum inhibition concentration (MIC) and minimum bactericidal concentration (MBC) was used¹. Serial two-fold broth dilutions were performed in iso-sensitest broth (Oxoid medium code CM473). The MIC was defined as the lowest concentration of antibiotic at which no turbidity was observable; that is, the tube with the lowest concentration of antibiotic to show no growth of the organism.

Results

Susceptibility of enterohaemorrhagic *Escherichia coli* 0157:H7 to 11 antimicrobial agents by disc diffusion test method is shown in Table 1. All 26 isolates subjected to disc diffusion test method were susceptible to gentamycin (≥15 mm diameter), ciprofloxacin (≥11 mm diameter) and chloramphenicol (≥18 mm diameter) but were completely resistant to cloxacillin (≤10 mm diameter). The majority of isolates were also susceptible to tetracycline (92.3%) and cephalexin (80.8%). A number of isolates were defined "moderately susceptible" to cefuroxime (61.5%), erythromycin (46.2%), amoxycillin (46.2%), ampicillin (15.4%), doxycycline (11.5%), cephalexin (7.7%) and tetracycline (7.7%).

The susceptibility of *E. coli* 0157:H7 by tube (broth) dilution test method is shown in Table 2. All the 26 isolates had an end point of observable growth turbidity for ciprofloxacin at 0.2 µg/

Table 1. *In-vitro* susceptibility of 26 *E. coli* 0157:H7 isolates to 11 antimicrobial agents: disc diffusion method.

Antimicrobial agent	Sensitive n(%)	Moderately sensitive n(%)	Resistant n(%)
Ampicillin	11(42.3)	4(15.4)	11(42.3)
Amoxycillin	3(11.5)	12(46.2)	11(42.3)
Cloxacillin	0(0.0)	0(0.0)	26(100.0)
Cephalexin	21(80.8)	2(7.7)	3(11.5)
Cefuroxime	3(11.5)	16(61.5)	7(26.9)
Doxycycline	12(46.2)	3(11.5)	11(42.3)
Tetracycline	24(92.3)	2(7.7)	0(0.0)
Chloramphenicol	26(100.0)	0(0.0)	0(0.0)
Gentamycin	26(100.0)	0(0.0)	0(0.0)
Erythromycin	3(11.5)	12(46.2)	11(42.3)
Ciprofloxacin	26(100.0)	0(0.0)	0(0.0)

n = sample number

ml, gentamycin 0.2 µg/ml and chloramphenicol 0.8 µg/ml and were considered very effective against the isolates. However, some isolates were not exclusively susceptible to some antibiotics based on the standard criteria^{5,17,19} and had the following percentage susceptibility pattern: ampicillin (42.3%), amoxycillin (11.5%), erythromycin (11.5%) and cefuroxime (11.5%). In both test methods, the isolates were resistant to cloxacillin (100.0%). The susceptible isolates had MIC value of <0.2 µg/ml for gentamycin, 0.2 µg/ml for ciprofloxacin, 0.8 µg/ml for tetracycline, 1.6 µg/ml for cephalexin and 6.4 µg/ml for ampicillin. The mean MIC value for chloramphenicol was 0.8 µg/ml and 6.4 µg/ml for amoxycillin. More than 80% of the isolates were not susceptible to 0.5 µg/ml erythromycin and 3.2 µg/ml cefuroxime. In the case of doxycycline and ampicillin, over 50% of isolates were not susceptible at 0.8 µg/ml and 6.4 µg/ml respectively (Table 2).

Table 2. *In vitro* susceptibility of 26 *E. coli* 0157:H7 isolates to 11 antimicrobial agents: tube (broth) dilution method (MIC).

Antimicrobial agent	B (ml)	Mean MIC (µg/ml)	Susceptible n(%)	Moderately susceptible n(%)	Resistant n(%)
Ampicillin	8	6.4	11(42.3)	4(15.4)	11(42.3)
Amoxycillin	8	6.4	3(11.5)	12(46.2)	11(42.3)
Cloxacillin	8*	-	0(0.0)	0(0.0)	26(100.0)
Cephalexin	2	1.6	24(92.3)	2(7.7)	0(0.0)
Cefuroxime	4	3.2	3(11.5)	16(61.5)	7(26.9)
Doxycycline	1*	0.8	12(46.2)	3(11.5)	11(42.3)
Chloramphenicol	8	0.8	26(100.0)	0(0.0)	0(0.0)
Gentamycin	1	0.2	26(100.0)	0(0.0)	0(0.0)
Erythromycin	0.5	0.4	3(11.5)	12(46.2)	11(42.3)
Tetracycline	1	0.8	24(92.3)	2(7.7)	0(0.0)
Ciprofloxacin	1	0.2	26(100.0)	0(0.0)	0(0.0)

MIC Minimum inhibition concentration (µg/ml), B Break point concentration µg/ml used to define susceptibility²⁵. *Recommended by Collins *et al.*⁵, n Sample number.

Discussion

The antimicrobial agents chosen for this study were those known to be effective in controlling bacteria capable of causing gastrointestinal infection. The susceptibility profile for *E. coli* 0157:H7 showed marked susceptibility to protein synthesis inhibitors such as ciprofloxacin (quinolones), chloramphenicol and an aminoglycoside gentamycin. Susceptibility to the aminopenicillin (ampicillin and amoxycillin) was inconsistent in the two test methods. The organisms were susceptible to tetracycline and cephalexin by tube dilution method, 'moderately' susceptible to doxycycline and ampicillin in the same tube dilution test method, but resistant to these antibiotics in the disc diffusion

test method. It was observed that the tube dilution test method is useful where quantitative results are clinically relevant. The penicillinase resistant penicillin studied (cloxacillin) was not useful in the elimination of *E. coli* 0157:H7. The organism was resistant to this antibiotic by both test methods.

The lactam stable cephalosporin antibiotics (cephalexin and cefuroxime) differ in their susceptibility tests on the organism. The organism was susceptible to the older cephalosporin (cephalexin) by tube dilution test method at 0.2 µg/ml concentration, but not the third generation cephalosporin (cefuroxime) by the same tube dilution test method. Similar results were observed with the tetracycline analogue. The long-acting compound (doxycycline) was ineffective by disc diffusion test method, while the short-acting group (tetracycline) was effective against 92.3% of the organism isolates by both methods. Reasons for the discrepancies within these groups of antibiotics could not be explained.

E. coli 0157:H7 is a newly emerging enteropathogenic bacterium, first identified in Centre for Disease Control (CDC) in USA in 1975 and was only recognized as an enteropathogen in 1982²². Therefore, this demands a thorough study on the disease before application of chemotherapeutic treatment. To date, there has not been any accepted antimicrobial agent to treat the disease. It was reported that most people recover from *E. coli* 0157:H7 infection without antibiotic or other specific treatment in 5-10 days^{6, 17, 24}. However, about 2-7% of infected individuals in USA are known to go on to develop HUS, a severe life threatening complication⁴. To date, the use of antibiotics is questionable since they are not yet proven to be useful or harmful¹¹.

Although *in vitro* susceptibility tests can be useful in guiding antimicrobial therapy, the individuals' response or complication to such chemotherapeutic agents must also be considered. In the case of *E. coli* 0157:H7 infection, making a selection of choice chemotherapeutic regimen requires consideration of some factors pending international research approval for types of antimicrobial agents and/or their combination with other therapeutic agent(s) for their relevance in treatment. These factors include age of patient, presence of immunocompromising disease or therapy, necessity of surgical drainage, adequacy of antimicrobial dosage and susceptibility of these bacteria to the chosen antimicrobial agent(s).

In general, it is best to choose an antimicrobial agent to which the organism is sensitive as shown in the *in vitro* tests. Among the *in vitro* susceptibility tests, MIC values are useful in correlating urine, blood and tissue concentration of the antimicrobial agents in patients, especially when the aminoglycoside antibiotics which have a narrow margin between their therapeutic and their toxic concentrations are used.

In the present study, substantial agreement was observed between the disc diffusion and tube dilution methods for four antimicrobial agents (gentamycin, ciprofloxacin, chloramphenicol and tetracycline) to which all isolates were susceptible. More isolates showed susceptibility for cephalexin by tube dilution test method while this agent was less effective by disc diffusion test method. The tube dilution test method was found to distinguish between sensitive and resistant of *E. coli* 0157:H7 to the chosen antibiotics. Therefore, this method is recommended as an acceptable alternative to the disc diffusion test method in hospitals which may desire quantitative susceptibility test result.

It is known that *E. coli* 0157:H7 and *Shigella* species cannot be differentiated early in the clinical course on the basis of clinical findings or simple laboratory tests. Therefore, withholding antibiotic therapy until the cause of the diarrhoea is known may have more risks than benefits in places where the prevalence of *Shigella* and *E. coli* infections is similar and strains of *E. coli* other than 0157:H7 are common.

The decision to use or not to use antibiotics in the treatment of *E. coli* 0157:H7 is left in the hand of the clinicians. However, in this study in Jos area, Nigeria, the effective antibiotics against the disease are ciprofloxacin, gentamycin, chloramphenicol, tetracycline and cephalexin. Further studies need to be done to determine how to control and treat the disease.

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