EXTENDED-SPECTRUM Beta-LACTAMASE PRODUCTION AMONG AMPICILLIN-RESISTANT E. coli STRAINS FROM CHICKEN IN ENUGU STATE, NIGERIA

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ABSTRACT

One hundred and seventy-two ampicillin-resistant E. coli strains isolated from commercial chickens in Enugu State, Nigeria, were screened for beta-lactamase production using the broth method with nitrocefin® as the chromogenic cephalosporin to detect enzyme production. Beta-lactamase producing strains were further examined for extended-spectrum beta-lactamase (ESBL) production using the Oxoid combination discs method. One hundred and seventy (98.8%) of the 172 ampicillin-resistant E. coli strains produced beta-lactamase enzyme. Sixteen (9.4%) beta-lactamase producers were phenotypically confirmed to produce ESBLs. Six of the ESBL producing strains were only detected with ceftazidime versus ceftazidime/clavulanate combination while ten of the ESBL producers were detected with cefotaxime versus cefotaxime/clavulanate combination. Chicken may serve as a reservoir of ESBL-producing E. coli strains which could be transferred to man and other animals.

Key word: Ampicillin-resistant, Escherichia coli, beta-lactamase, extended-spectrum

INTRODUCTION

Beta-lactam antibiotics account for approximately 50% of global antibiotic consumption and this heavy usage has exerted considerable selection for resistance to β-lactam antibacterial agents among Gram-negative bacteria (21). These enzymes undermined the utilization of ampicillin and first and second-generation cephalosporins in the chemotherapy of infections caused by Gram-negative bacteria. To overcome the problems posed by the β-lactamase enzymes, third- and fourth-generation (extended-spectrum) cephalosporins were developed. Unfortunately, members of the Family Enterobacteriaceae have developed resistance to these extended-spectrum cephalosporins via production of extended-spectrum β-lactamases (ESBLs) (6,9). Apart from the ESBL enzymes, resistance to expanded-spectrum cephalosporins in enterobacteria has also been found to be mediated by AmpC β-lactamases (20). These enzymes are resistant to cephalosporin/clavulanate combination.

Human clinical strains of Escherichia coli producing ESBLs have been described from different parts of the world (5,8,16, 21). In Nigeria, these enzymes have been reported in Enterobacter species from human patients in Lagos (1).

Escherichia coli strains resistant to extended-spectrum cephalosporins have been isolated from calves (2) and dogs (23). This study was undertaken to assess ampicillin-resistant E. coli strains isolated from chickens for β-lactamase and extended-spectrum β-lactamase production.

MATERIALS AND METHODS

Bacterial strains

One hundred and seventy-two ampicillin-resistant Escherichia coli strains isolated from commercial chickens (broilers and layers) in Enugu State, Nigeria were used in the study.

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Detection of beta-lactamase production

Test *E. coli* strains were screened for β-lactamase production using the broth method (14). Two colonies of each of the test bacteria were picked from an over night nutrient agar culture plate and inoculated into 1 ml of sterile nutrient broth. Inoculated broths were appropriately labeled and incubated at 37°C for 18 hours. After incubation, four drops of nitrocefin® solution (Calbiochem, Germany), prepared as directed by the manufacturer, were added to the broth culture and observed for colour change within 30 minutes. Nitrocefin® is a chromogenic cephalosporin that changes colour from yellow to red on hydrolysis. Beta-lactamase production was inferred when the broth turned red within 30 minutes of addition of reagent.

Detection of extended-spectrum beta-lactamase (ESBL) expression by *Escherichia coli* strains

This was done by initially screening for susceptibility to two extended-spectrum cephalosporins (ceftazidime and cefotaxime; Oxoid, England). Presumptive ESBL-producers were subjected to Phenotypic Confirmatory test for ESBL production.

Susceptibility test

Beta-lactamase producing *E. coli* strains were screened for susceptibility to ceftazidime (30 µg) and cefotaxime (30 µg), using the standard disc diffusion method in accordance with the National Committee for Clinical Laboratory Standards guidelines (18). Test bacteria were grown on Nutrient agar for 18 hours at 37°C and colonies of the bacteria were suspended in sterile normal saline and the inoculum density adjusted to 0.5 McFarland turbidity standards. Surfaces of Mueller-Hinton agar were flooded with the standardized bacterial suspension and excess fluid drained into a discard pot containing Isol® disinfectant. With a disc dispenser (Oxoid, Basingstoke, England), ceftazidime (CAZ-30 µg) and cefotaxime (CTX-30 µg) were placed on the inoculated plates. The plates were incubated at 37°C for 24 hours. After incubation the diameters of zones of inhibition around each antibacterial disc were measured with a metre rule. Each test was performed in triplicate and susceptibility test was presented in Table 1.

Extended-spectrum beta-lactamase production

The distribution of the mean inhibition zone diameters around the two cephalosporin discs (ceftazidime-30 µg and cefotaxime-30 µg) for the 170 β-lactamase producing *E. coli* strains is presented in Fig. 1. As shown in the figure, the zone sizes ranged from 18 mm to 40 mm for ceftazidime and 18 mm to 45 mm for cefotaxime. Using NCCLS (18) breakpoints for susceptibility, 16 (9.4%) of the test strains were resistant to ceftazidime while 14 (8.2%) were resistant to cefotaxime. Of the 170 beta-lactamase producing strains, 52 (30.6%) were presumptively identified as ESBL-producers with cephalosporin alone. However, when both discs were taken into consideration, 76 (44.7%) of the strains were predicted as ESBL producers.

RESULTS

Beta-lactamase production

Using the nitrocefin® solution, beta-lactamase production was detected in 170 (98.8%) of 172 ampicillin-resistant *Escherichia coli* strains screened.

Extended-spectrum beta-lactamase production

Phenotypic confirmation of ESBL production

Presumptive ESBL-producing *E. coli* strains were tested by the Oxoid Combination Disc method (3, 14) to confirm ESBL production. This test was done in accordance with standard recommendations (18). Two pairs of combination discs (ceftazidime-30 µg versus ceftazidime/clavulanate-30/10 µg and cefotaxime-30 µg versus cefotaxime/clavulanate-30/10 µg) were used for the test. These discs were placed on the surface of Mueller-Hinton agar inoculated with the standardized inoculum of the test bacteria. After 24 hours incubation at 37°C the inhibition zones produced were measured. Each test was performed in duplicate and mean zone size recorded to the nearest whole millimetre. An *E. coli* strain was interpreted as ESBL-producers if there was a difference in zone size of ≥ 5 mm between the combination disc compared to that of the cephalosporin alone.
16 (9.4%) of the 170 beta-lactamase producing *E. coli* strains examined were ESBL producers. Out of the 76 possible ESBL producing strains, 24 (31.6%) were unaffected by at least one of the test cephalosporin/clavulanate discs as evidenced by zero zone difference between the cephalosporin versus cephalosporin/clavulanate combination discs.

The proportion of the beta-lactamase producing *E. coli* strains presumptively identified as ESBL-producer and phenotypically confirmed as ESBL-producers with either ceftazidime versus ceftazidime/clavulanate, cefotaxime versus cefotaxime/clavulanate or both combination discs is presented on Table 1. The inhibition zones produced around combination discs by ESBL-producing *E. coli* strains are shown in Figs. 3 and 4.

**DISCUSSION**

Antibacterial preparations containing β-lactams, particularly ampicillin, are widely used for prophylaxis and chemotherapy of avian bacterial infections in Nigeria (4). Heavy usage of β-lactam antibacterials exerts considerable selection for resistance to this class of antibacterial agents (15). In this study 98.8% of

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**Table 1. Phenotypes of ampicillin-resistant *E. coli* strains isolated from chicken in Enugu State (N=170)**

<table>
<thead>
<tr>
<th>Phenotype</th>
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<tr>
<td></td>
<td>Ceftazidime&lt;sup&gt;1&lt;/sup&gt;</td>
<td>Cefotaxime&lt;sup&gt;2&lt;/sup&gt;</td>
<td>Both discs&lt;sup&gt;3&lt;/sup&gt;</td>
</tr>
<tr>
<td>Cephalosporin resistance&lt;sup&gt;a&lt;/sup&gt;</td>
<td>10 (5.9)*</td>
<td>8 (4.7)</td>
<td>6 (3.5)</td>
</tr>
<tr>
<td>Presumptive ESBL-producer&lt;sup&gt;b&lt;/sup&gt;</td>
<td>14 (8.2)</td>
<td>24 (14.1)</td>
<td>38 (22.4)</td>
</tr>
<tr>
<td>Confirmed ESBL-producer&lt;sup&gt;c&lt;/sup&gt;</td>
<td>6 (3.5)</td>
<td>10 (5.9)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Cephalosporin/clavulanate resistance&lt;sup&gt;d&lt;/sup&gt;</td>
<td>4 (2.4)</td>
<td>18 (10.6)</td>
<td>2 (1.2)</td>
</tr>
</tbody>
</table>

* = Figures in parenthesis represent percentage of beta-lactamase producing *E. coli* strains examined

a = Ceftazidime resistance (zone size ≤ 20 mm); cefotaxime resistance (zone size ≤ 22 mm)
b = Strain with zone size ≤ 22 mm with ceftazidime or zone size ≤ 27 mm with cefotaxime
c = Strain with cephalosporin/clavulanate vs plain cephalosporin zone difference ≥ 5 mm
d = Strains with cephalosporin/clavulanate vs plain cephalosporin zone difference of 0mm

1 = Phenotype detected with ceftazidime but not with cefotaxime
2 = Phenotype detected with cefotaxime but not with ceftazidime
3 = Phenotype detected with ceftazidime and also with cefotaxime
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The 172 ampicillin-resistant *E. coli* strains tested were beta-lactamase producers, while only 2 (1.2%) were non-producers. This observation supports previous reports (11,12) that resistance to β-lactams in *E. coli* is mainly due to production of beta-lactamases. These enzymes hydrolyze the cyclic amide bond in the β-lactam molecule leading to the formation of penicilloic acid or cephalosporoic acid (7). These products are unable to bind to penicillin-binding proteins (PBPs) located on the bacterial cytoplasmic membrane and therefore cannot inhibit

**Figure 2.** Inhibition zone differences produced by test cephalosporins alone and in combination with clavulanate.

**Figure 3.** Extended-spectrum beta-lactamase producing *E. coli* strain. Confirmed with ceftazidime-clavulanate (a) versus ceftazidime alone (b).

**Figure 4.** Extended-spectrum beta-lactamase producing *E. coli* strain. Confirmed with cefotaxime-clavulanate (c) versus cefotaxime alone (d).
bacterial cell wall formation. However, it has been pointed out other mechanisms mediating resistance to β-lactams include porin deficiency, modification of PBPs, and permeability barrier and efflux pumps (19). Resistance to ampicillin among the 2 non-β-lactamase producing E. coli strains may be due to one of the above resistance mechanisms.

Although 52 (30.6%) and 62 (36.5%) of the β-lactamase producing E. coli strains were predicted as ESBL producers by ceftazidime and cefotaxime respectively, only 16 (9.4%) were phenotypically confirmed as ESBL producers. This is similar to the observations of previous workers (5,8). This underscores the need for phenotypic confirmation of ESBL production using Oxoid combination Disc method or other sensitive methods. In the present study, none of ESBL-producing strains confirmed with ceftazidime were identified as such with cefotaxime and vice versa. This observation supports previous reports that E. coli strains elaborate various types of ESBL enzymes with different substrate profiles (10).

In the present study a greater proportion of the ESBL-producing strains were detected with cefotaxime alone versus ceftazidime/clavulanate discs than with ceftazidime versus ceftazidime/clavulanate discs. This finding is contrary to the observations of other investigators (8,10,17). However, the finding in this study that when both pairs of the combination discs were taken into consideration, a higher proportion of ESBL-producing strains were detected than the rate recorded for either pair taken separately is similar to the report of M’Zali et al. (17) in which 86%, 65.5% and 93% of ESBL-producing members of the family Enterobacteriaceae were detected by discs containing ceftazidime, cefotaxime and both agents taken into consideration respectively. Optimal substrate profile varies from one ESBL-producer to another (22). This may explain the differences in the ESBL detection rates by the different cephalosporins. The overall 9.4% detection rate of ESBL production among the 170 lactamase producing E. coli strains examined may not represent the actual prevalence of ESBL among the E. coli isolates studied. This is because some of the ESBL producers might have escaped detection by the Oxoid combination discs used in this study. It is therefore possible that the true prevalence of extended-spectrum beta-lactamase producers among E. coli strains in the poultry population in the state may be higher than the value recorded in this study. Hence, inclusion of a wide variety of cephalosporins (such as cepodoxime, aztreonam, ceftriaxone, cephalothin etc) in the screening panel could possibly increase the rate of detection of ESBL-producing E. coli strains. Although the occurrence of a wide variety of β-lactamas in South Africa was believed to reflect over use of the newer extended spectrum cephalosporins in medical practice in that country (21), in Nigeria, these agents are not used in veterinary practice. Thus, the presence of ESBL producing E. coli strains in poultry in the country may not be related to overuse of these agents. However, ampicillin is widely used in poultry production in the country (4) and this agent may provide a selective pressure favouring the emergence of E. coli strains that produce ESBL enzymes.

Results of this study have shown that ESBL producing E. coli strains are present in the chicken population in Nigeria. Chicken may therefore serve as reservoir of ESBL-producing E. coli strains, which could be transferred to humans and other animals.

**REFERENCES**