Phenotypic detection of AmpC enzymes and antimicrobial susceptibility of *Klebsiella* spp. isolated from abattoir

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ABSTRACT

Antimicrobial resistance in food chain is fast becoming a global phenomenon that is likely to pose grave health challenges to humans—owing to the emergence and spread of drug resistant bacteria in food-producing animals. This study investigated the occurrence of AmpC enzyme in *Klebsiella* spp. isolated from anal swabs of cow in a local abattoir in Abakaliki metropolis, Nigeria. A total of 40 anal swab samples collected from a local abattoir were bacteriologically analyzed for the presence of *Klebsiella* spp. They were identified using standard microbiology identification technique. Susceptibility studies were carried out using Kirby-Bauer disk diffusion method and AmpC enzyme was phenotypically detected using the Disk Approximation Method. A total of 17 isolates of *Klebsiella* spp. were recovered from the anal swab samples. The overall resistance rates of the isolated *Klebsiella* spp. to the tested antibiotics were notably high. The *Klebsiella* spp. were found to be highly resistant to cefoxitin (82.4%), ertapenem (100%), cloxacillin (100%), cefotaxime (70.6%) and ceftazidime (58.8%). Susceptibility of the tested *Klebsiella* spp. to the antibiotics was highest in imipenem (88.2%), meropenem (82.4%), gentamicin (82.4%) and ciprofloxacin (52.9 %). AmpC phenotypes were found in 35.3% isolates out of the 17 isolates of *Klebsiella* spp. used. Considering the increasing prevalence of AmpC production among Enterobacteriaceae isolates from the community, it is vital to establish and take the surveillance and monitoring of the emergence and spread of drug resistant bacteria in the community seriously.

INTRODUCTION

Antibiotics are frequently used in veterinary medicine especially in food-producing animals as prophylactic and growth promoting agents. Several studies have shown that the use of antimicrobial agents in animal husbandry has led to the emergence and spread of resistant bacteria through the food chain (Doi et al., 2010; Geser et al., 2011; Ejikeugwu et al., 2016a). The use of antibiotics as growth-promoting agents in farm animals is chiefly responsible for the emergence of drug-resistant bacteria in the community (Wegener, 2003; Doi et al., 2010). This continual use of antibiotics in the community may be contributing to the selection and spread of AmpC-producing Enterobacteriaceae in animals (Bradford, 2001; Paterson et al., 2005; Geser et al., 2011). AmpC β-
lactamases are clinically important cephalosporinases encoded on the chromosomes of many Enterobacteriaceae and a few other bacteria where they mediate resistance to the cephemycins (such as cefotetan and cefoxitin) and most penicillins (Jacoby and Munoz-Price, 2005; Hemalatha et al., 2007). However, AmpC enzymes can also be encoded by genes that are frequently located on mobile genetic elements such as plasmids, which have the ability to transfer horizontally within and between different bacterial species (Jacoby, 2009; Geser et al., 2011; Manoharan et al., 2012). Their ability to hydrolyze the cephemycins as well as oxyimino-β-lactams differentiates them from bacterial organisms that produce or harbor genes for extended spectrum beta-lactamases (ESBLs) – which have no activity on the cephemycins (Jacoby, 2009). Characteristically, AmpC β-lactamases are known to mask ESBL production in organisms harbouring both AmpC and ESBLs, and they are poorly inhibited by clavulanic acid – which have activity on ESBLs (Hemalatha et al., 2007). Zoonotic transmission of AmpC positive Klebsiella has been previously reported (Ewers et al., 2012; Schmeidel et al., 2014). In humans, irrational antibiotic usage has been established as a strong risk factor for colonization and infection due to AmpC-producing organisms (Cortes et al., 2010; García-Graells et al., 2010; Geser et al., 2011; Geser et al., 2012; Blaak et al., 2014). In food-producing animals, colonization with AmpC-producing bacteria must be considered a public health concern, since the transmission to humans cannot be ignored. AmpC-producing Enterobacteriaceae including Escherichia coli and Salmonella spp. have been isolated from farm animals in different countries inclusive of Nigeria (Geser et al., 2011; Ejikeugwu et al., 2016b). Food-producing animals have been suggested as the primary reservoir of zoonotic foodborne pathogens, including antimicrobial resistant bacteria (Geser et al., 2011). The presence of AmpC positive bacteria in faecal samples of food-producing animals represents a risk for carcass contamination at slaughter and subsequent contamination of retail meat products; and this contamination risk is high in developing countries where inadequate hygienic practices are employed in abattoirs. This study evaluated the occurrence of AmpC positive Klebsiella spp. from anal swabs of cow in Abakaliki metropolis, Nigeria.

MATERIALS AND METHODS

Sample collection and processing

The study area for this research was a local abattoir in Abakaliki metropolis of Ebonyi State, Southeastern Nigeria. A total of 40 anal swab samples were collected from cows awaiting slaughter in the local abattoir. Each of the anal swab samples were collected by inserting a sterile swab stick into the anal region of cow at a dept of about 2-3 cm, and then rotated at an angle of 360°C. The swab sticks were each returned into their containers and labeled. All the collected samples were transported to the laboratory of Applied Microbiology Department, Ebonyi State University, Abakaliki, Nigeria for bacteriological analysis.

Isolation of Klebsiella spp.

Each of the swab sticks containing the sample were dipped into test tubes containing 5 ml of freshly prepared nutrient broth (Oxoid, UK) and the test tubes were loosely covered with cotton wool, and incubated for 18-24 h at 30°C. Tubes showing turbidity after incubation were aseptically cultured onto freshly prepared Mac Conkey agar plates and eosin methylene blue (EMB) agar plates (Oxoid, UK), and these were incubated at 30°C for 18-24 h. Suspect colonies of Klebsiella spp. were sub-cultured onto freshly prepared EMB and MacConkey agar plates for the isolation of pure cultures of the organism. Klebsiella spp. produces mucoid colonies on MacConkey agar and mucoid colonies without metallic sheen on EMB agar. The isolated Klebsiella spp. was identified using standard microbiological identification techniques (Cheesbrough, 2006).

Antimicrobial susceptibility testing

Susceptibility test was performed using single antibiotic disks (Oxoid, UK), and antibiotic breakpoints was interpreted according to 2011 CLSI guidelines (CLSI, 2011). The antibiotic disks used include: amikacin (AK, 30 μg), cefoxitin (FOX, 30 μg), cefotaxime (CTX, 30 μg), gentamicin (CN, 30 μg), cloxacillin (OB, 10 μg), meropenem (MEM, 20 μg), ceftazidime (CAZ, 30 μg), ceftriaxone (CRO, 30 μg), ofloxacin (OFX, 5 μg), ertapenem (ETP, 10 μg) ciprofloxacin (CIP, 30 μg) and imipenem (IPM, 10 μg). Antimicrobial susceptibility testing was performed on Mueller-Hinton agar plates (Oxoid, UK), using overnight cultures of Klebsiella spp. (adjusted to 0.5 McFarland turbidity standards); and this was followed by incubation at 30°C for 18-24 h (Ejikeugwu et al., 2016a).

Screening for AmpC-enzyme production

To screen for AmpC enzymes, it is desirable to test all...
isolates with a combination of antibiotics that will allow for the detection of resistant mechanisms – since AmpC enzyme detection is usually difficult in organisms that also express ESBLs (which mask AmpC enzyme production). The isolated *Klebsiella* spp. isolates were screened for AmpC-enzyme production by testing for their susceptibility to cefoxitin, cefotetan, clavulanic acid, ceftazidime, ceftriaxone and cefotaxime (Oxoid, UK). Isolates that showed reduced susceptibility to the antimicrobial activity of the tested antibiotics especially to the cephamycins (cefoxitin and cefotetan) were suspected to produce AmpC-enzyme. Cefoxitin resistance were suspected in those *Klebsiella* spp. whose zone diameters were ≤ 18 mm; and these isolates were selected for phenotypic confirmation of AmpC enzyme production (El-Hady and Adel, 2015; Ejikeugwu et al., 2016b).

**Disk approximation test for detection of AmpC enzyme**

The suspect AmpC *Klebsiella* spp. isolates (adjusted to 0.5 McFarland turbidity standards) were aseptically inoculated on MH agar plates and a 30 µg cefoxitin disk was placed at the center of the inoculated MH agar plates (El-Hady and Adel, 2015). Then 30 µg ceftazidime disks, 10 µg imipenem disks, and 30 µg cefotaxime disks were each placed at a distance of 20 mm from the central disk (cefoxitin 30 µg). The MH plates were incubated at 30°C for 18-24 h. AmpC enzyme production was phenotypically confirmed in the test isolates when the isolates showed obvious blunting or flattening of the zones of inhibition between the ceftazidime, imipenem or cefotaxime disks adjacent to the cefoxitin disk. However, the absence of a distortion or flattening of the zone of inhibition around the cefoxitin disk and any of the inducing substrates (for example, imipenem) is indicative of a negative test result since cefoxitin was not inactivated by the inducing substrates (El-Hady and Adel, 2015).

**RESULTS AND DISCUSSION**

Table 1 show the frequency of *Klebsiella* spp. from the anal swab samples. Of the forty (40) anal swabs of cow bacteriologically analyzed in this study, a total of 17 *Klebsiella* isolates was isolated (Table 1). *Klebsiella* spp. is an important member of the Gram negative bacteria found in the Enterobacteriaceae family; and it is ubiquitously found in the gastrointestinal tract (GIT) of food-producing animals inclusive of humans (Brooks et al., 2004). Though the organism is also a normal flora in humans, human colonization with *Klebsiella* spp. from food-producing animal sources is possible via the food chain or through direct body contact with the animals or their carcasses in slaughter houses (abattoirs) especially when strict hygienic conditions are not followed in handling them. The antimicrobial susceptibility profile of the isolated *Klebsiella* spp. is shown in Table 2. The isolated *Klebsiella* spp. showed varying rates of susceptibility to the tested antibiotics.

The result obtained from the susceptibility studies showed that the *Klebsiella* spp. exhibited the highest susceptibility to imipenem (88.2%); and this was followed by meropenem (82.4%) and gentamicin (82.4%). However, the test *Klebsiella* spp. was found to be highly resistant to ofloxacin (100%), cloxacillin (100%), cefoxitin (82.4%) and cefotaxime (70.6%) (Table 2). This result is similar to the work of Akujobi et al. (2012) conducted in southwest Nigeria who reported 100% resistance in Enterobacteriaceae isolates particularly *E. coli* and *Klebsiella* spp. Other studies conducted by Amita and Rajesh (2007) and Adeleke et al. (2010) in Asia and Nigeria respectively have also reported that there is higher β-lactam drugs resistance in Enterobacteriaceae isolates particularly the *E. coli* and *Klebsiella* isolates from animal sources. The patterns of resistance in the *Klebsiella* spp. to the tested antimicrobial agents used in this study may be due to the widespread and lengthy use of antibiotics in animals for growth purposes. Previous studies have shown that the use of antimicrobial agents for animal production allow drug resistant microbes to emerge and spread in the community (Wegener, 2003; Winokur et al., 2000; Waters et al., 2011). The result of AmpC positive *Klebsiella* spp. is shown in Table 3. In this study, we screened 17 isolates of *Klebsiella* spp. for the production of AmpC enzymes using disk diffusion technique and the disk approximation of method. The result obtained from the screening test for AmpC enzyme production in the *Klebsiella* spp. isolates revealed that out of the 17 isolates screened, only 15 isolates showed reduced susceptibility to cefoxitin; and these were suspected to be AmpC producing *Klebsiella* spp. and were subjected to phenotypic confirmatory test using the disk approximation technique. AmpC enzyme was phenotypically detected in 6 (35.3%) isolates of the *Klebsiella* spp. out of the 17 *Klebsiella* spp. used in this study.

<table>
<thead>
<tr>
<th>Sample source</th>
<th>Number of sample</th>
<th>Number of <em>Klebsiella</em> spp.</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anal swabs of cow</td>
<td>40</td>
<td>17</td>
<td>42.5</td>
</tr>
</tbody>
</table>
study (Table 3). The incidence of AmpC enzyme production amongst the tested Klebsiella spp. used in this study is high. The prevalence of AmpC-producing Enterobacteriaceae including Klebsiella spp. from abattoir in this region is scarce. However, we have detected AmpC enzyme from non-enteric organisms particularly Pseudomonas aeruginosa from abattoir (Ejikeugwu et al., 2016b). This present study was undertaken to give impetus to the several report of multidrug resistant Gram negative bacteria from the community – which could be related to the many hospital visits in this region as a result of the emergence and spread of community acquired infections (Winokur et al., 2000; Waters et al., 2011). AmpC enzyme production in Gram negative bacteria portend serious health implications to total patient care and the efficacy of some available antimicrobial drugs because organisms harbouring genes that code for this enzyme and other multidrug resistance enzymes are less susceptible to many antimicrobials. According to Perez-Perez and Hanson (2002), plasmid-mediated AmpC enzyme producers are known to be resistant to multiple antibiotics; and this leaves few therapeutic options for the treatment of bacterial-related infections especially those caused by AmpC enzyme producers. Couidron (2005) also opined that plasmid-mediated AmpC β-lactamases pose a big challenge to infection control due to the fact that the AmpC gene can be expressed in larger amounts and has high transmissibility to other bacterial species in any environment. Antibiotic resistance is a serious and growing phenomenon in contemporary medicine and this has emerged as one of the pre-eminent public health concerns of the 21st century. Antimicrobial resistance in Enterobacteriaceae isolates from the community especially from food-producing animals as shown in this study has been reported worldwide; and increasing rates of resistance among Klebsiella spp. is a growing concern in both developed and developing countries (Aarestrup et al., 2000; Acar and Rostel, 2001; Heuer et al., 2006).

Conclusively, this study has demonstrated that antimicrobial resistant Klebsiella spp. abound in abattoir samples; and that these organisms harbour AmpC enzymes, that mediate bacterial resistance to some available beta-lactam antibiotics. The findings in this

### Table 2. Antimicrobial susceptibility of 17 isolates of Klebsiella spp.

<table>
<thead>
<tr>
<th>Antibiotic (µg)</th>
<th>Susceptible n (%)</th>
<th>Intermediate n (%)</th>
<th>Resistant n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CRO (30)</td>
<td>8 (47.1)</td>
<td>5 (29.4)</td>
<td>4 (23.5)</td>
</tr>
<tr>
<td>FOX (30)</td>
<td>3 (17.7)</td>
<td>0 (0.0)</td>
<td>14 (82.4)</td>
</tr>
<tr>
<td>IMP (10)</td>
<td>15 (88.2)</td>
<td>1 (5.9)</td>
<td>1 (5.9)</td>
</tr>
<tr>
<td>CAZ (30)</td>
<td>4 (23.5)</td>
<td>3 (17.6)</td>
<td>10 (58.8)</td>
</tr>
<tr>
<td>ETP (10)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>17 (100)</td>
</tr>
<tr>
<td>OB</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>17 (100)</td>
</tr>
<tr>
<td>OFX</td>
<td>13 (76.5)</td>
<td>2 (11.8)</td>
<td>2 (11.8)</td>
</tr>
<tr>
<td>CN</td>
<td>14 (82.4)</td>
<td>3 (17.6)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>CIP</td>
<td>9 (52.9)</td>
<td>4 (23.5)</td>
<td>4 (23.5)</td>
</tr>
<tr>
<td>AK</td>
<td>8 (47.1)</td>
<td>5 (29.4)</td>
<td>4 (23.5)</td>
</tr>
<tr>
<td>CTX</td>
<td>0 (0.0)</td>
<td>5 (29.4)</td>
<td>12 (70.6)</td>
</tr>
<tr>
<td>MEM</td>
<td>14 (82.4)</td>
<td>2 (11.8)</td>
<td>1 (5.9)</td>
</tr>
</tbody>
</table>

**Key:** AK, Amikacin; FOX, cefotixin; CTX, cefotaxime; CN, gentamicin; OB, cloxacillim; MEM, meropenem; CAZ, ceftazidine; CRO, ceftriaxone; OFX, ofloxacin; ETP, ertapenem; CIP, ciprofloxacin; IPM, imipenem.

### Table 3. Incidence of AmpC enzyme production in the Klebsiella spp.

<table>
<thead>
<tr>
<th>Isolate No.</th>
<th>Organism</th>
<th>Source</th>
<th>Phenotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>K2</td>
<td>Klebsiella</td>
<td>Abattoir</td>
<td>AmpC</td>
</tr>
<tr>
<td>K5</td>
<td>Klebsiella</td>
<td>Abattoir</td>
<td>AmpC</td>
</tr>
<tr>
<td>K7</td>
<td>Klebsiella</td>
<td>Abattoir</td>
<td>AmpC</td>
</tr>
<tr>
<td>K10</td>
<td>Klebsiella</td>
<td>Abattoir</td>
<td>AmpC</td>
</tr>
<tr>
<td>K11</td>
<td>Klebsiella</td>
<td>Abattoir</td>
<td>AmpC</td>
</tr>
<tr>
<td>K16</td>
<td>Klebsiella</td>
<td>Abattoir</td>
<td>AmpC</td>
</tr>
</tbody>
</table>
study show the necessity for continued monitoring and surveillance of the emergence and spread of antimicrobial resistant bacteria in the community especially from abattoir samples through thorough susceptibility studies. Such measures will help to curtail the spread of drug resistant bacteria in the community.

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