Emergence of $bla_{\text{CTX-M-15}}$, $qnrB1$ and $aac(6')$-$lb$-$cr$ resistance genes in *Pantoea agglomerans* and *Enterobacter cloacae* from Nigeria (sub-Saharan Africa)

Resistance of *Enterobacter* species to extended-spectrum cephalosporins is known to be mediated by hyperproduction of chromosomal AmpC $\beta$-lactamases. However, the additional expression of plasmid-encoded extended-spectrum $\beta$-lactamases (ESBLs) has become more prevalent worldwide in recent years (Ko et al., 2008). In Nigeria, ESBL production in *Enterobacter* species has been associated with TEM- and SHV-type ESBLs (Aibinu et al., 2003; Kasap et al., 2010). Other $\beta$-lactamase resistance determinants, conferring resistance to extended-spectrum cephalosporins, such as $bla_{\text{TEM}}$, $bla_{\text{OXA}}$, and $bla_{\text{CMY}}$, have recently been reported in Nigerian *Providencia* species strains (Aibinu et al., 2011). In addition, the worldwide spread of CTX-M-15 (Cantón & Coque, 2006) has reached Nigeria, having been identified in *Klebsiella* species and *Escherichia coli* (Soge et al., 2006; Olowe et al., 2010). There is no documented report yet on ESBL production mediated by $bla_{\text{CTX-M-15}}$ or the association of the spread of PMQR determinants in *Enterobacter* species from Nigeria. This study reports the phenotypic and genotypic characteristics of ten clinical isolates of *Enterobacter* species and one isolate of *Pantoea agglomerans* with respect to the occurrence of $bla_{\text{CTX-M}}$ and other resistance genes. The *Enterobacter* species, which consisted of *Enterobacter asburiae* (n=1), *Enterobacter aerogenes* (n=1) and *Enterobacter cloacae* (n=8), and the *Pantoea agglomerans* isolate represented 9.5% of all members of the *Enterobacteriaceae* isolated within a period of 6 months from October 2008 to March 2009 at Lagos University Teaching Hospital (LUTH), a tertiary hospital, in Nigeria. *Enterobacter agglomerans* was previously renamed *Pantoea agglomerans* to reflect its genetic distance from the genus *Enterobacter* (Sanders & Sanders, 1997).

Bacterial species identification was performed using the VITEK 2 system (VITEK2 GN-card; bioMérieux). Antimicrobial susceptibility testing was determined according to the guidelines of the Clinical and Laboratory Standards Institute (CLSI, 2010) by the broth microdilution method and VITEK2 AST-N13 card. The quality control strain used was *E. coli* ATCC 25922 (Oxoid). Etest strips containing ceftaxime in combination with clavulanic acid, and the double disc synergy tests (ESBL/AmpC ID D68C; Mast Group), were used for phenotypic detection and differentiation of both ESBL and AmpC production. Broth mate conjugation assays were performed as described by Pfeifer et al. (2009). Different ESBL genes ($bla_{\text{TEM}}$, $bla_{\text{SHV}}$, $bla_{\text{CTX-M}}$), PMQR genes ($qnr$, $aac(6')$-$lb$-$cr$) and class 1 and 2 integrons with tetracycline and trimethoprim resistance genes were detected by PCR as previously described (Ng et al., 2001; Frech et al., 2003; Bouallégue-Godet et al., 2005; Cano et al., 2009; Jin & Ling, 2009). All positive PCR products were sequenced using the ABI Prism 3100 genetic analyser (Applied Biosystems). Additionally, sequence analysis of the quinolone resistance determining region (QRDR) of genes gyrA and parC was performed (Cano et al., 2009). The epidemiological relationship between the 11 isolates was analysed by enterobacterial repetitive intergenic consensus (ERIC)-2 PCR (Versalovic et al., 1991).

Phenotypic analysis of the 11 isolates in this study revealed that two of the isolates (*Enterobacter cloacae* 213K and *P. agglomerans* 69K) were ESBL-producers. The ESBL gene $bla_{\text{CTX-M-15}}$ was identified in both isolates. *P. agglomerans* 69K was isolated from the blood culture of an adult male patient admitted for sepsis and diagnosed HIV type-1-positive on admission. The patient was treated empirically with ceftriaxone and was referred to another clinic for follow-up on HIV treatment. Several weeks later, the patient was rushed back to the emergency unit of LUTH and examination showed that he was dead on arrival.

Isolate *Enterobacter cloacae* 213K was recovered from the urine sample of an adult female patient attending the outpatient clinic of LUTH. She was diagnosed with a urinary tract infection and treated empirically with ceftriaxone. Two different urine cultures yielded, each time, two isolates (*E. coli* and *Enterobacter cloacae*), both harbouring the $bla_{\text{CTX-M-15}}$ gene. The two ESBL study isolates (*P. agglomerans* 69K and *Enterobacter cloacae* 213K) were multiply resistant to different antibiotics, including ampicillin, cefepime, cefoxitin, aztreonam, ceftazidime, cefotaxime, gentamicin, tobramycin, levofloxacin, ciprofloxacin, tetracycline and sulfmethoxazole/trimethoprim. Both isolates harboured the class 1 and 2 integrons. The identified gene cassettes within the class 1 integrons included aminoglycoside resistance genes [$aadA1$, $aph$ and $aac(6')$-$lb$-$Ib$], a sulfonamide resistance gene ($sul1$) and a chloramphenicol resistance gene ($catI$) in the *P. agglomerans* isolate (Table 1). The presence of the insertion sequence IS6ep1 upstream of the $bla_{\text{CTX-M-15}}$ gene was confirmed by PCR (Baraniak et al., 2002). Additionally, both isolates harboured the PMQR genes $qnrB1$ and $aac(6')$-$lb$-$cr$. The tetracycline resistance gene $tet(K)$, encoding an efflux pump, was identified in *P. agglomerans* 69K while *Enterobacter cloacae* 213K harboured $tet(A)$ and $tet(E)$ resistance determinants. By conjugation experiments, plasmids of >90 kbp were successfully transferred into *E. coli* J53 recipients. The *E. coli* J53 transconjugants
had a resistance pattern similar to that of the donor strain but remained susceptible to cefoxitin and showed MIC reduction for tetracycline; Ctx, cefotaxime.

They all harboured the class 1 integron. The class 2 integron was additionally found in 45% (n=4) of the isolates. Resistance to trimethoprim/sulfamethoxazole was associated with the presence of sul1 (100%) and either dfrA1 (72.7%) or dfrA14 (54.6%) or both genes (36.4%) (Table 1). The tet(A) and tet(E) genes were the predominant tet genes occurring. The strain typing by ERIC-2 PCR revealed distinct patterns, indicating heterogeneity of all Enterobacter species isolates.

We report in this study what we believe to be the first description of CTX-M-15 in P. agglomerans and Enterobacter cloacae isolates from Nigeria. This study showed a low occurrence of Enterobacter species in clinical infection during this study period (9.5%) and the rate of prevalence of ESBL production was 18.2% (n=2).

Unfortunately, it was not possible to determine whether the ESBL and PMQR genes in the isolates were hospital- or community-acquired because clinical data showed no record of previous hospital admission for the patients. The results of this study furthermore suggest that the association of blaCTX-M-15, PMQR determinants qnrB1 and aac(6’)-Ib-cr and other resistance genes in addition to mobile elements (ISEcp1, class 1 and 2 integrons) may facilitate the rapid dissemination of antimicrobial resistances into other Gram-negative bacteria in Nigeria, limiting the choice of antibiotic therapy.

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Table 1. Pantoea agglomerans and Enterobacter species antibiotic resistance phenotypes and determinants

<table>
<thead>
<tr>
<th>Species</th>
<th>Specimen (clinical condition)</th>
<th>Antibiotic resistance phenotype*</th>
<th>Trimethoprim and tetracycline resistance genes</th>
<th>ESBL, PMQR genes, integrons and resistance gene cassettes</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. aerogenes (28K)</td>
<td>High vaginal swab (coupious vaginal discharge)</td>
<td>Amp, Ams, Cef, Cfx, Tet</td>
<td>tet(A)</td>
<td>Class 1 integron, qacAE, sul1</td>
</tr>
<tr>
<td>E. asburiae (85K)</td>
<td>Urine (urinary tract infection)</td>
<td>Amp, Ams, Cfx, Sxt, Tet</td>
<td>dfrA1, dfrA14, tet(A)</td>
<td>Class 1 and 2 integrons, qacAE, sul1</td>
</tr>
<tr>
<td>E. cloacae (91b)</td>
<td>Catheter-tip</td>
<td>Amp, Ams, Cfx, Sxt, Tet</td>
<td>dfrA1, dfrA14, tet(E)</td>
<td>Class 1 integron, qacAE, sul1</td>
</tr>
<tr>
<td>E. cloacae (97K)</td>
<td>Urethral discharge</td>
<td>Amp, Ams, Cfx, Sxt, Tet</td>
<td>dfrA1, dfrA14, tet(E)</td>
<td>Class 1 and 2 integrons, qacAE, sul1</td>
</tr>
<tr>
<td>E. cloacae (60K)</td>
<td>Semen</td>
<td>Amp, Ams, Cfx, Sxt, Tet</td>
<td>dfrA1, dfrA14, tet(E)</td>
<td>Class 1 integron, qacAE, sul1</td>
</tr>
<tr>
<td>E. cloacae (54K)</td>
<td>Blood (sepsis)</td>
<td>Amp, Ams, Cfx, Sxt, Tet</td>
<td>dfrA1, dfrA14, tet(E)</td>
<td>Class 1 integron, sul1</td>
</tr>
<tr>
<td>E. cloacae (56K)</td>
<td>Blood (neonatal sepsis)</td>
<td>Amp, Ams, Cfx, Sxt, Tet</td>
<td>dfrA1, dfrA14, tet(E)</td>
<td>Class 1 integron, qacAE, sul1</td>
</tr>
<tr>
<td>E. cloacae (59K)</td>
<td>Catheter-tip</td>
<td>Amp, Ams, Cfx, Sxt, Tet</td>
<td>dfrA1, dfrA14, tet(E)</td>
<td>Class 1 and 2 integrons, qacAE, sul1</td>
</tr>
<tr>
<td>E. cloacae (64K)</td>
<td>Blood (neonatal sepsis)</td>
<td>Amp, Ams, Cfx, Sxt, Tet</td>
<td>dfrA1, dfrA14, tet(E)</td>
<td>Class 1 and 2 integrons, qacAE, sul1</td>
</tr>
<tr>
<td>E. cloacae (213K)</td>
<td>Urine (urinary tract infection)</td>
<td>Amp, Ams, Cef, Fep, Cet, Caz, Cip, Gen, Lev, Tob, Sxt, Tet, Ctx, Fox</td>
<td>dfrA14, tet(A), tet(E)</td>
<td>CTX-M-15, qnrB1, aac(6’)-Ib-cr, class 1 and 2 integrons, aph, aadA1, qacAE, sul1</td>
</tr>
<tr>
<td>Pantoea agglomerans (69K)</td>
<td>Blood (sepsis)</td>
<td>Amp, Ams, Cef, Fep, Caz, Cip, Gen, Pt, Tob, Sxt, Tet, Ctx, Fox</td>
<td>dfrA14, tet(K)</td>
<td>CTX-M-15, TEM-1, qnrB1, aac(6’)-Ib-cr, class 1 and 2 integron, aph, aadA1, cat1, qacAE, sul1</td>
</tr>
</tbody>
</table>

*Amp, Ampicillin; Ams, ampicillin/sulbactam; Azt, aztreonam; Cet, cephalothin; Cfx, cefazolin; Fep, cepfime; Caz, cefazidine; Cip, ciprofloxacin; Gen, gentamicin; Fox, cepoxitine; Pt, piperacillin/tazobactam; Tob, tobramycin; Sxt, trimethoprim/sulfamethoxazole; Lev, levofloxacin; Tet, tetracycline; Ctx, cefotaxime.


