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# Emergence of β-lactamases OXA-10, VEB-1 and CMY in *Providencia* spp. from Nigeria

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Sir,

Resistance to cephalosporins of the third and fourth generations in Enterobacteriaceae is an increasing problem worldwide. This resistance is mainly attributed to the production of extended-spectrum β-lactamases (ESBLs). In Nigeria SHV-, TEM- and CTX-M-type ESBLs have been reported in *Enterobacter* spp., *Klebsiella* spp. and *Escherichia coli*.[1–3] Besides these classical ESBLs, there are various other plasmid-mediated β-lactamases that are less common but which are regarded as emerging and increasing in frequency among the β-lactamase family. Here we report the emergence of OXA-10, VEB-1 and CMY β-lactamases and mobile genetic elements in three clinical isolates of *Providencia* spp. isolated between October 2008 and April 2009 in two tertiary hospitals in Nigeria.

Three strains of *Providencia* spp. were identified using the VITEK 2 GN card system following cultivation of clinical samples on MacConkey agar and blood agar base with 5% sheep blood (Oxoid, UK). Isolate *Providencia rettgeri* 58K was recovered in late 2008 from the catheter tip of a patient hospitalized at Lagos University Teaching Hospital (LUTH) in Lagos, Nigeria. The patient was notably hypertensive and was diagnosed with an exacerbation of chronic kidney disease, secondary to adult polycystic kidney disease. He had been catheterized for 19 days, with insertion of the catheter from the private hospital where he was hospitalized before his referral to LUTH. The patient was not febrile at any time and there was no infection documented. Isolate *Providencia stuartii* V1 was recovered from the gunshot wound of a patient admitted to the National Orthopaedic Hospital Igbobi (NOHI), Lagos, in early 2009, while isolate *P. stuartii* V2 was also recovered at NOHI from the wound swab of a patient who had had a motorcycle accident. The three patients had not received antibiotics prior to the isolation of the strains.

The three clinical strains of *Providencia* spp. were multiply resistant to different β-lactams, fluoroquinolones and aminoglycosides but remained susceptible to carbapenems using the VITEK 2 GN card system (Table 1). ESBL and AmpC production was confirmed using the double disc synergy tests (ESβL/AmpC test D68C; Mast Diagnostica GmbH, Reinfeld, Germany) and ESBL-Etest strips (bioMérieux, Nürtingen, Germany). By PCR and sequence analysis, β-lactamase genes $bla_{CMY-4}$, $bla_{TEM-1}$, $bla_{VEB-1}$ and $bla_{OXA-10}$ were identified in isolate *P. rettgeri* 58K. Isolate *P. stuartii* V2 harboured $bla_{CMY-41}$ and $bla_{TEM-52}$, while ESBL genes $bla_{VEB-1}$ and $bla_{OXA-10}$ were found in isolate *P. stuartii* V1. The $bla_{VEB-1}$ genes were located on *sul*-type class 1 integrons and IS*CR2* mobile elements. The $bla_{OXA-10}$ genes were also encoded on class 1 integrons. Furthermore, the plasmid-mediated quinolone resistance gene *qnrA1* was found in all three clinical isolates (Table 1). Transfer of all β-lactamase genes and gene *qnrA1* was successfully performed for the clinical isolates *P. rettgeri* 58K and *P. stuartii* V1 using broth mating assays with a sodium azide-resistant *E. coli* J53 recipient.[4]

In order to ascertain the potential transfer and acquisition of β-lactamase genes or resistant strains via the food chain, we investigated resistance determinants of *Providencia* spp. from faecal samples of apparently healthy farm animals. Farm animals serve as major sources of meat products for the population of Lagos. Between October 2008 and April 2009 we recovered 97 Enterobacteriaceae isolates from 115 faecal samples of different animals from three local farms. Using the VITEK 2 GN card system we identified seven *Providencia* spp. recovered from chickens ($n=6$) and pigs ($n=1$). Typing of human and animal *Providencia* spp. isolates by enterobacterial repetitive intergenic consensus PCR revealed different patterns, indicating no clonal relationship of these isolates. In contrast to the clinical strains, all animal isolates were susceptible to most of the antibiotics and none produced β-lactamases (Table 1). The variable regions of class 1 integrons in clinical and animal strains harboured the classic *qacE*Δ*-sul1* region and *dfrA* genes (*dfrA1*, *dfrA14* and *dfrA15*). The class 2 integron was additionally found in two isolates from chickens. Furthermore, the sequence of one *P. rettgeri* isolate from a chicken (156K) showed 99% identity with the integrating conjugative element ICE*Palban1* described in a *Providencia alcalifaciens* isolate from the USA (GenBank accession no. GQ463139). The absence of β-lactamase genes and quinolone resistance genes in the animal isolates suggested no correlation of horizontal transfer of these resistance genes between animal and human *Providencia* spp. strains. However, simultaneous occurrence of class 1 and 2 integrons in two isolates from chickens highlighted a possible variety of recombinatorial events among these genetic platforms according to Machado *et al.*[5]

This study presents the first report of *Providencia* spp. producing CMY-type and OXA-10 β-lactamases. To our knowledge, the only known documentation of a $bla_{CMY}$ gene in *Providencia* spp. is the submission of a $bla_{CMY-16}$ gene sequence from a *P. stuartii* isolate from Tunisia (GenBank accession no. FJ855437.1). Horizontal gene transfer has played a major role in the global spread of β-lactamases and Qnr determinants into various Gram-negative species. Furthermore, this is the first report of ICE*Palban1* in a *P. rettgeri* animal isolate. Though we only found a small number of *Providencia* spp. isolates, our data suggest that further population-based prevalence studies are needed in order to monitor the ability of clinical *Providencia* spp. to be a reservoir of different resistance genes.

The nucleotide sequences of some of the resistance genes in this study have been deposited in the GenBank nucleotide sequence database under accession numbers GU056840, GU056841, GU056843 and GU056844.

**Table 1.** Antimicrobial susceptibilities and resistance genes of 10 human and animal *Providencia* spp. isolates

| Species and isolate no. | Origin | Resistance phenotype | β-Lactamase genes | Other resistance genes | IntI1 | IntI2 | *qacEΔ1+sul1* | Further resistance gene cassettes and genes |
|---|---|---|---|---|---|---|---|---|
| *P. rettgeri* 58K | catheter tip | AMP, SAM, CTX, CAZ, PIP, TZP, CIP, LVX, TOB, GEN, TET, SXT, STR, FOX | $bla_{CMY-4}$, $bla_{VEB-1}$, $bla_{OXA-10}$, $bla_{TEM-1}$ | *qnrA1*, *tet*(A), *tet*(E), *tet*(L), | + | − | + | *dfrA1-aacA4-aadA1-aph* |
| *P. stuartii* V1 | wound swab | AMP, SAM, CTX, CAZ, PIP, TZP, CIP, LVX, TOB, GEN, TET, SXT, STR | $bla_{VEB-1}$, $bla_{OXA-10}$ | *qnrA1*, *tet*(A), *tet*(B), *tet*(D) | + | − | + | *dfrA15-aadA1-veb-1* |
| *P. stuartii* V2 | wound swab | AMP, SAM, CTX, CAZ, PIP, TZP, CIP, LVX, TOB, GEN, TET, SXT, STR, FOX | $bla_{CMY-41}$, $bla_{TEM-52}$ | *qnrA1*, *tet*(B), *tet*(C), *cat1* | + | − | + | *dfrA15-aph-cat* |
| *P. rettgeri* 139K | pig | AMP, SAM, SXT | − | − | + | − | − | *dfrA1* |
| *P. rettgeri* 156K | chicken | AMP, SAM, SXT, KAN | − | − | + | + | + | *dfrA1-aph* |
| *P. rettgeri* 164K | chicken | AMP, SAM, SXT, KAN, TET | − | *tet*(B) | + | − | + | *dfrA15-aadA2-aph* |
| *P. rettgeri* 167K | chicken | AMP, SAM, SXT, KAN, TET, STR | − | *tet*(B) | + | − | + | *dfrA14-aadA2-aadA1-aph* |
| *P. rettgeri* 172K | chicken | AMP, SAM, SXT, KAN, TET, STR | − | *tet*(B) | + | − | + | *aadA2-aadA1-aph* |
| *P. alcalifaciens* 144K | chicken | AMP, SAM, SXT, KAN, TET, STR | − | *tet*(A), *tet*(D) | + | + | + | *dfrA1- aadA1-aph* |
| *P. alcalifaciens* 154K | chicken | AMP, SAM, SXT, TET | − | *tet*(A) | + | − | + | *dfrA15* |

AMP, ampicillin; SAM, ampicillin/sulbactam; CTX, cefotaxime; CAZ, ceftazidime; PIP, piperacillin; TZP, piperacillin/tazobactam; CIP, ciprofloxacin; LVX, levofloxacin; TOB, tobramycin; GEN, gentamicin; TET, tetracycline; SXT, trimethoprim/sulfamethoxazole; STR, streptomycin; KAN, kanamycin; FOX, cefoxitin.

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## Transparency declarations

None to declare.

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# Pharmacokinetics of the raltegravir/maraviroc/etravirine combination

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