Antimicrobial And Biofilm Profile Of Multidrug-Resistant Enterobacterales From Equine Veterinary Hospital Surfaces

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Introduction: Multidrug-resistant, ESBL-producing *Enterobacterales* pose One Health risks. As hospital surfaces act as reservoirs for nosocomial infections, environmental surveillance is vital to improve biosecurity and safeguard public health. **Objectives:** To evaluate biofilm, antimicrobial susceptibility, and resistance genes (beta-lactams, quinolones, quaternary ammonium) of ceftriaxone-resistant *Enterobacterales* from equine hospital surfaces. **Materials and Methods:** In 2024, 41 swabs were collected from surfaces at a surgery center in an teaching equine hospital, those being from: sinks, faucet, worktops, syringes, switches, doorknobs, hoses, doors, orotracheal tubes, squeeze bottles, mechanical ventilation circuit, container with sterile gauze, drawers, hobble, stethoscope, trash can lid, surgical center canvas, water fountain, medication box, fridge door, induction rubber, animal preparation rubber and surgical boxes. Those swabs were streak on MacConkey agar supplemented with ceftriaxone (4.0 ug/mL). The lactose-fermenting colonies were identified by MALDI-TOF and stored in 10% glycerol at -20°C for phenotypical and genothypical analysis. Biofilm production capacity was evaluated with microplate biofilm assay and the antimicrobial susceptibility profile was performed determining the minimum inhibitory concentration with the broth microdilution method. The MIC was determined for the following antimicrobials: amoxicillin/clavulanic acid (AMC), amikacin (AMI), aztreonam (ATM), cefepime (CPM), cefotaxime (CTX), cefoxitin (CFO), ceftazidime (CAZ), chloramphenicol (CLO), ciprofloxacin (CIP), colistin (COL), fosfomycin (FOS), gentamicin (GEN), meropenem (MPM), nalidixic acid (NAL), tetracycline (TET), trimethoprim/sulfamethoxazole (SUT). Genomic DNA of these strains were extracted and screened for beta-lactamases encoding genes (bla_{KPC}, $bla_{OXA\text{-}48\text{-}like}$, bla_{NDM}, $bla_{CTX\text{-}M}$ groups 1, 2, 8 and 9, bla_{TEM}, bla_{SHV}), plasmid mediated quinolone resistance (*qnrA*, *qnrB*, *qnrS*, *oqxB*, *oqxA*, *qepA*, *aac(6´)-Ib*) and disinfectant efflux-pump-encoding genes (*qacE*, *qacΔE1* and *sugE(c)*). **Results:** From these samples, 28 strains were isolated: 18 (64,2%) *Enterobacter hormaechei*, 8 (28,5%) *Escherichia coli* and 2 (7,1%) *Klebsiella pneumoniae* third-generation cephalosporin resistant strains. 12 (42.8%) isolates were strong biofilm producers, while 6 (21.4%) were moderate and 10 (35.7%) weak producers. 100% of the isolates were CIP, GEN and SUT resistant, followed by high resistance rates to ATM (92,8%; 26/28), TET (92,8%; 26/28), CTX (89,3%; 25/28), AMC (85,7; 24/28), AMI (85,7%; 24/28), and CPM (85,7%; 24/28). Most of the strains (96.4%; 27/28) were susceptible to COL, while none were resistant to MPM. All the isolates carried the *aac(6´)-Ib* and *qacΔE1* genes, while 92,8% (26/28) carried *qnrS* and *qnrA*, 89,2% (25/28). The ESBL-

encoding genes bla_{TEM} and bla_{SHV} were identified in 85,7% (24/28) of the studies isolates, while bla_{CTX-M} group 1 was found in 82,1% (23/28) of the samples. **Discussion:** The persistence of these isolates on hospital surfaces is explained by the high prevalence of ESBL-producing bacteria (89.2%) combined with strong biofilm formation and 100% *qacΔE1* carriage. This multi-resistance profile highlights critical cross-contamination risks. **Conclusion:** Hospital surfaces act as reservoirs for multidrug-resistant, biofilm-producing *Enterobacterales*. Environmental surveillance is essential to improve biosecurity and prevent surface-mediated infections.

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