The Isolation of Multi-Drug Resistant Paenalcaligenes sp. UN24 from Bivalve Molluscs in Douglas Creek, Nigeria

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Background and purpose: Molluscs-associated infections represent a major health challenge in food production systems as well as a significant zoonosis problem globally. The development and transfer of multiple-antibiotic resistance genes in Gram-negative bacteria to human pathogens have been the cause of treatment failures with antimicrobials and a growing concern worldwide. This study sought to investigate the occurrence of multi-drug resistant Paenacaligenes sp. UN24 isolated from bivalve- molluscs in Douglas Creek, Nigeria.

Method: A total of 150 (80 clams and 70 oysters) were investigated for the presence of pathogenic bacteria using standard bacterial culture methods. Isolates were identified presumptively using phenotypic methods and confirmed by DNA sequencing of 16S rRNA gene. The organisms were screened for antibiotic resistance with 13 antibiotics namely; nalidixic acid, norfloxacin and ciprofloxacin, amikacin, cotrimoxazole, imipenem, aztreonam, ceftazidime, cefotaxime and cefpodoxime by the disk diffusion method.

Results: Seven (10%) and five (6.2%) oyster and clams respectively were positive for Paenalcaligenes sp. UN24. The mean heterotrophic bacterial counts in clams ranged from $8.04 \times 107 - 7.93 \times 103$(Cfu/g) for dry eason and $1.92 \times 107- 7.55 \times 105$(Cfu/g) for rainy season. The mean count in oysters ranged from $8.25 \times 107 - 6.58 \times 106$ (Cfu/g) in dry season and $2.55 \times 107- 3.11 \times 106$ (Cfu/g) in rainy season respectively. All of the Paenacaligenes sp. UN24 showed resistance to more than two antibiotic classes. The isolates were resistant to ceftriazone, ceftazidime, cefepime, imipenem, trimethoprim-sulfamethoxazole, chloramphenicol, ciprofloxacin, norfloxacin and nalidixic acid. The most effective antibiotics against the bacterium were amikacin, aztreonam, cefotaxime and cefpodoxime.

Conclusion: The Paenacaligenes sp. UN24 expressed multi-resistance (MDR) to antibiotics. The presence of MDR in the isolate is a threat to antibiotic treatment and is of public health concern.

Development and validation of different indirect ELISAs for MERS-CoV serological testing

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Introduction: Since 2012, MERS-CoV has caused up to 2,220 cases and 790 deaths in 27 countries with Saudi Arabia being the most affected country with ~83.1% of the cases and ~38.8% local death rate. Current serological assays such as microneutralization (MN), plaque reduction neutralization, immunofluorescence, protein microarray or pseudoparticle neutralization assays rely on handling of live MERS-CoV in high containment laboratories or need for expensive and special equipment and reagents and highly trained personnel which represent a technical hurdle for most laboratories in resource-limited MERS-CoV endemic countries. Here, we developed, compared and evaluated three different indirect ELISAs based on different MERS-CoV proteins.

Materials and Methods: MERS-CoV nucleocapsid protein (rN), spike (rS) ectodomain (amino acids 1-1297) and rS1 subunit (amino acids 1-725) proteins were expressed, purified and used for ELISA assay development. Assays were developed using checkerboard titration and finally validated with a total of 353 serum samples collected between 2014 and 2017 from high-risk groups (79 seropositive and 274 seronegative samples) in comparison with MN assay.

Results: Three different ELISAs were developed using MERS-CoV rN, rS1 and rS, and cut-off values were found to be 0.30 for rN-ELISA, 0.26 for rS1-ELISA, and 0.34 for rS-ELISA. Upon validation, both rS1 and rS ELISAs maintained high sensitivity and specificity (≥90%) across a wider range of OD values compared to rN-ELISA. Moreover, rS1- and rS-based ELISAs showed better agreement and correlation with MN assay in contrast to rN-ELISA.

Conclusions: Our data suggest that rS1-ELISA and/or rS-ELISA are more reliable than rN-ELISA as they showed higher sensitivity, specificity, agreement and correlation with MN assay. They could be used independently or in combination in large-scale and high-throughput seroepidemiological screening in MERS-CoV endemic regions as they do not require high containment laboratories and could be adapted easily by any lab.