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Method for isolation and identification of anaerobes from excised lumbar discs

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Objective: To develop a method to isolate and identify anaerobes in lumbar disc herniation.

Methods: Subjects (n=101) who were confirmed for lumbar disc herniation and undergoing lumbar discectomy were included in this study. Skin scrapings, muscle biopsies and surgically removed disc material were aseptically collected and transferred into individual Robertson's cooked meat enrichment broth (RCM) using sterile forceps. The RCM enrichment broth was incubated for 48 hours at 37 °C. It was further sub cultured on blood agar, Bacteriods Bile Esculin agar and Brucella Blood agar and incubated at 37 °C anaerobically. Plates were read at day 2 and 7. Cultures positive for anaerobic incubation were subjected to aerotolerance test to confirm the presence of anaerobes. Gram stain, catalase test and RapID ANA II ID (remel, USA) kit were used for the identification of anaerobes. Skin scrapings and muscle biopsies were used as control to rule out contamination. This method was developed to isolate anaerobes in intervertebral disc tissue in Sri Lankan patients who are undergoing lumbar discectomy.

Results: Among the 101 patients, 6 disc cultures (6 %) were positive for anaerobes. Among the positive anaerobes *P.acnes* and *Gemella morbillorum* were identified. There were three disc cultures which could not be identified using RapID ANA II ID kit due to slow growth; but colonies resembled the appearance of Gram positive bacilli suggesting they could be *P.acnes*.

Conclusion: This method could be used to identify the presence of anaerobes.

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***In vitro* screening of the antibacterial and antifungal activities of fungal species isolated from soil**

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Objective: Isolation of antibiotic producing fungi from soil and evaluation of their antimicrobial properties.

Methods: Soil samples were collected from the Kelani river mouth (N6.978348, E79.870623) and twenty-nine fungal species were isolated and maintained on potato dextrose agar. Primary antibacterial test (disc method) was carried out to screen the antibiotic producing fungi. Two fungal species were identified as potential antimicrobial (antifungal and antibacterial) agents. Extraction of antimicrobial active compound from fungal species was done using methanol. The methanol evaporated sample was reconstituted with dimethyl sulfoxide (DMSO) and their antimicrobial activities were evaluated using Anti Bacterial Susceptibility Test (ABST) and Anti Fungal Susceptibility test (AFST) against human pathogenic Gram positive (*Staphylococcus aureus* and *Bacillus* sp.), Gram negative (*Salmonella typhi*, *Escherichia coli*) bacteria and fungi (*Candida albicans* and *Candida tropicalis*). Ciprofloxacin (antibacterial) and amphotericin B (antifungal) were used as positive controls. Minimum Inhibition Concentration (MIC) and Minimum Bactericidal Concentration (MBC) for crude extract were determined by broth microdilution assay using triphenyltetrazolium chloride (TTC).

Results: In primary antimicrobial test, higher antibacterial and antifungal activities of the fungal species 1 were recorded against *Bacillus* sp. (27.34±0.58 mm) and *C. tropicallis* (18.34±0.58 mm). In fungal species 2 antibacterial and antifungal activities were recorded against *S. aureus* (39.67±0.58 mm) and *C. albicans* (14.67±1.15mm) respectively compared to the other test organisms. In secondary antimicrobial test both fungal