SLJID 2024 Vol 14 (Supplement):S15
DOI: https://doi.org/10.4038/sljid.v14i5.8793

Comparison of sheep blood and human blood-based media for the isolation and identification of selected pneumococcal strains

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Introduction and Objectives: Although the conventional culture using sheep blood supplementation is recommended for the identification of *Streptococcus pneumoniae,* developing countries use human blood as an alternative. Therefore, it is important to evaluate the impact of two different blood media on pneumococcal isolation and identification. This study aimed to compare the colony sizes, colony counts, antibiotic sensitivity (ABST) and minimum inhibitory concentration (MIC) of *S. pneumoniae* isolates on the two different blood media.

Methods: Twenty strains of *S. pneumoniae* (four strains each from the five commonest serotypes found in Sri Lanka; serotypes 19F, 6B, 6A, 14 and 23F) and *S. pneumoniae* ATCC 49619 were used. Colony sizes of the isolates were measured on SBA and HBA. Proportions of colonies ≥ 1 mm, colony counts in 0.5 McFarland Standard, 10^{-1}, 10^{-2}, 10^{-3}, and 10^{-4} dilutions were compared. Disc diffusion antibiotic sensitivity testing (ABST) was performed for erythromycin, tetracycline, and levofloxacin on the two types of blood-MHA. MIC for penicillin and cefotaxime was tested with micro broth dilution in the two types of blood-MHB.

Results: Draughtsman appearance and alpha hemolysis were obvious on SBA. Considering all isolates together, the mean number of colonies ≥ 1 mm was 8 (±9) on HBA and 23 (±10) on SBA. In higher dilutions (10^{-3}, 10^{-4}), mean number of colonies on SBA ($1.94x10^5$, $5.36x10^4$ CFU/ml) was higher than HBA ($1.78x10^5$, $4.18x10^4$ CFU/ml). Mean ABST zone diameters of tetracycline, erythromycin, levofloxacin on human blood-MHA were 24.5, 17.5, 25.2 mm and on sheep blood-MHA were 21.2, 12.7, and 23.5 mm. MIC50 and MIC90 for penicillin were similar in both media (2 and 4 µg/ml). MIC50, MIC90 for cefotaxime in human blood-MHB was 0.5 and 2µg/ml and in sheep blood-MHB was 0.75 and 2 µg/ml.

Conclusions: Since the typical colony characteristics were not seen, there is a possibility to misidentify pneumococci on HBA. Isolation of pneumococci on HBA is less when organisms are present in lower concentrations. Larger ABST zones on human blood-MHA may alter sensitivity interpretation. Therefore, human blood cannot be recommended for the isolation and identification of *S. pneumoniae,* and alternatives need to be sought for by countries like Sri Lanka.

***Keywords**: Streptococcus pneumoniae, sheep blood agar, human blood agar, isolation and identification*

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