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Original article

Examining the Necessity of Pleural Fluid Culture: A Microbiological Perspective

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Abstract

Background: The etiologic spectrum of pleural effusion depends on the geographic region and local incidence of various diseases which causes pleural effusion. In developing countries the prevalent causes are para pneumonia and tuberculosis Hence the study planned to know the prevalent organisms and their antimicrobial resistance pattern.

Methods: This two years prospective study was done in a rural tertiary care hospital. All pleural fluid samples were subjected to for direct microscopy. Simultaneously aerobic and anaerobic culture were put. In Suspected cases, mycobacterial culture and fungal culture were put. The remained sample was added to BHI media and subculture was done on next day on solid media.

Results: Out of 312 pleural fluid specimens, a total of 60 pleural fluid specimen shown growth of microorganism with a culture positivity (19.2%). Fifty seven specimens shown growth by aerobic culture, two by anaerobic culture and one fungal growth was observed. In the present study Gram positive bacterial isolates (31) outnumbered the Gram negative isolates (28) by very narrow number. Most common isolate found to be Staphyoccous aureus , Acinetobacter species and Streptococcus species . Anaerobic culture showed growth of peptostreptococci in two pleural fluid specimens. Sub culturing the pleural specimen from Brain Heart Infusion media (BHI) increased the aerobic bacterial isolation by 8.3%

Conclusion: Gram positive bacteria are progressively trailing down their foot hold in the pleural space to more threatening infections. Similarly, Gram negative bacilli isolates like Acinetobcater species and pseudomonas species having intrinsic drug resistance mechanisms are involved, alarms the careful use of higher antibiotics. We found sub culturing pleural specimen from Brain Heart Infusion media (BHI) to be helpful method than conventional direct plating and can be recommended where resources are minimum for better isolation.

Keywords: pleural fluid culture, gram negative bacilli, gram positive cocci, Aspergillus fumigatus

Introduction:

Imbalance between hydrostatic and oncotic pressure**,** increased capillary permeability and impaired lymphatic drainage are the reasons to cause collection of fluid in pleural space. Pleural effusion is an abnormal collection of fluid in pleural space. Infections of the pleural cavity continue to cause significant morbidity and mortality despite the improvement of antimicrobial therapy and the existence of multiple options for drainage of the infected space. These infections are mostly an extension of infection from pneumonia, lung , oral, retropharyngeal, paravertebral or skin abscess, mediastinal lymph nodes or external introduction of organisms due to trauma or surgery.[1] Empyema is a common complication and at times life – threatening. The etiologic spectrum of pleural effusion depends on the geographic region and local incidence of various diseases which causes pleural effusion. In developing countries the prevalent causes are tuberculosis and parapneumonia in adults. While in developed countries in adults cardiac failure, malignancy and pneumonia are commonest causes. [2] Commonest causes of pleural effusion in children are tuberculosis, dengue fever, heart failure, nephrotic syndrome, diaphragmatic abscess , uremia , rheumatic and rheumatoid diseases and pancreatitis.[3] In most

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diseases related to pleural effusion , the fluid analysis yields important diagnostic information, and in certain cases ,fluid analysis alone is enough for diagnosis. [4] Pleural fluid cultures are slow and can have false negative results because of small volume, previous antibiotic therapy, or unsatisfactory conditions of transport and storage, which can impair the viability of pathogens.[5]The bacteriology of pleural infection, is changing above and beyond. Though Streptococcus pneumoniae is considered to be commonest etiological agent in children and adult, Gram negative bacteria are also frequently isolated from the pleural fluid specimens. [6] There is lack of information regarding etiology of pleural effusion in India owing to few studies in India.

Material and method

Pleural fluid specimens (from any cause) received from in patient departments and outpatient departments from the Pravara Rural Hospital, Loni, at the Dept. of Microbiology were included in the study. Repeat sample from a patient was excluded. Single or mixed growth from one patient was included in study. All pleural fluid samples were subjected to Gram Staining and Ziehl Neelsen staining for direct microscopy.

Aerobic culture: All pleural fluid specimens were inoculated on Mac Conkey's and blood agar for aerobic culture..

Anaerobic culture: All pleural fluid specimens were inoculated in Robertson Cooked meat Medium (RCM) with a layer of liquid paraffin. Identification was done using available standard laboratory techniques.

Mycobacterial culture: In clinically suspected tubercular empyema cases pleural fluid was inoculated on Lowenstein- Jensen medium. Fungal culture : In clinically suspected fungal etiology, pleural fluid was inoculated on Sabouraud's Dextrose Agar with and without antibiotic. We also employed another method to maximize isolation, by adding remaining pleural fluid sample to Brain Heart Infusion (BHI) liquid media dispensed in sterile bulb and subsequently sub cultured on Mac Conkey's agar and blood agar the next day.The specimens were processed for isolation and identification was done using standard laboratory techniques. [7.8] Antibiotic susceptibility testing of bacterial isolates was done by Kirby Bauer Disc Diffusion method using standard protocol.[9]

Result

A total of 312 pleural fluid samples were received and processed during two years study period. In gender wise distribution of study population males (198) outnumbered females (114).Age wise distribution of study population showed most common age group as (61 yr -70yr) followed by (41 yr-50yr) and (31yr-40yr) age group . Only 12.1% study population was under 12 years as compared to above 12 years (87.8%) Direct microscopy of pleural fluid revealed pus cells and organisms in 42 specimens and only one specimen was found to be positive for acid fast bacilli by Zeihl Nelsen staining. Out of 312 pleural fluid specimens only 60 specimen showed growth (19.2%) while 252 (80.7%) pleural fluid specimens were sterile (isolates from BHI method). Out of 60 culture positive pleural fluid specimens 57 (95%) showed aerobic bacterial growth, two (3.33%) were positive for anaerobic culture and 1(1.6%) was positive for fungal culture. Anaerobic culture showed growth of Peptostrept-ococcus species in two of the specimen. The only fungal isolate found to be Aspergillus fumigatus. The result of comparison of direct plating and BHI methods of aerobic bacterial isolation showed overall increased number of isolates in second method by 8.3%.. By direct plating method 32 isolates were isolated as one specimen showed mixed growth, likewise by BHI method 59 isolates were isolated as two specimens showed mixed growth. As a whole by BHI method , 31 bacterial isolates were Gram positive (52.4%) and 28 were Gram negative (47.4%). Isolation by BHI method showed enhanced number of both Gram positive and Gram negative isolates than direct plating methods. Species wise distribution of bacterial isolates from study specimens showed Staph. aureus (22.4%) and Acinetobacter species (22.4%) as commonest isolate followed by Streptococcus species by BHI method.(Fig 6) Streptococcus species included Streptococcus pneumoniae(3), alpha haemolytic streptococci(4), beta hemolytic streptococci(3) and non haemolytic streptococci(2)

Discussion

Fluid accumulation in the pleural space indicate the disease. Many medical conditions that predispose to fluid accumulation by way of different mechanisms including increased pulmonary capillary pressure, decreased osmotic pressure. In the present study, gender wise distribution showed male dominance as

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males (63.5%) and females (36.5%).In our study, age wise distribution of study population revealed involvement of of elderly age group than younger population may be owing to its vulnerability to various outside environment and underlying conditions. Out of 312 pleural fluid specimens a total of 253(80.7%) were sterile. A total of 59 pleural fluid specimen shown growth of microorganism. Culture positivity in the present study found to be (19.3%).

A study by Sharma et al reported 15.7%, culture positivity by conventional method and Acinetobacter species as common isolate in their study. Whereas pleural fluid study by Lindstorm ST et al reported a higher culture positivity (40.6%). In contrast, Walshe et al in their study reported a lower culture positivity (3.5%).[12] The reason for the wide disparity in culture positivity rate may be attributed to differences in techniques, prior antibiotic use and prevalence of effusions caused by infective processes. A Ferrer et al study concluded that it is appropriate to inoculate all samples including non purulent pleural fluid to rule out infectious etiology in underlying condition such as neoplastic transudate.[8]JD Chalmers et al reported 18% culture positivity in their study with Sterptococcus milleri, as most common isolate.[13] A paediatric study by Maulik P. Saliya showed pleural fluid culture positivity as 11.76%. [2]

A total of 59 bacterial isolates were isolated by aerobic culture (by BHI method). In the present study Gram positive isolates (31)outnumbered the Gram negative isolates (28). Commonest isolates found to be Staphyoccous aureus (12) followed by Streptococci species and Acinetobacter sp.

We found sub culturing pleural specimen from BHI media to be helpful in association with direct plating on medias . Out of 312 pleural specimens inoculated aerobically growth was observed in only 31 (9.93%)direct inoculated plates while sub culturing from BHI could isolate organism in 57(18.26%) pleural fluid specimens. Hence improving culture positivity by 8.3% as compared to direct plating. In a one of the UK study by Sarah M Menzieset, use of blood culture bottle improved the culture positivity by 20.8% than direct plating. [14] Cholasseri et al study reported increased culture positivity by 8.4% using blood culture bottle in addition standard culture.[15] A study by Anusua Deb et al concluded, recovery of pathogens was maximum using enrichment by soyabean caesin digest broth and the difference was statistically significant for pleural fluids.[16]

In the present study there was no isolation of Mycobacteria but only one case we detected positive for M. tuberculosis in Ziehl Neelsen staining by direct microscopy. Pulak Raj and Rajesh khare study of pleural effusion in conjunction with tuberculosis observed that only two pleural fluid specimens out of 110 were positive for acid fast bacilli on Ziehl staining and only 8 pleural fluids were culture positive on Lowenstein's Jensen (LJ)medium stating low yield.[16] Only one fungal isolate found to be Aspergillus fumigatus in our study. Study by Shiaman chinko et al reported candida albicans as most common yeast while most common filamentous fungus isolate as Aspergillus species.[17] Anaerobic bacterial etiology is not uncommon in pleuro-pulmonary infections. Boyanova L. et al reported propiobacterium , bacteriods , prevotella and peptostreptococcus are common anaerobic isolate from pleural fluid.[18]

Though, unavailability of battery of tests for identification of anaerobic isolate , our study could isolate two isolates of peptostreptococcus species using Robertson's cooked meat broth (RCM). Wide etiological diversity was mentioned by G. Senol et al in their study from Turkey.[1]Jain Sonali et al in their retrospective study of pleural fluids quoted the absence of anaerobe isolate in their huge sample size (2219 pleural fluid specimens). Contributory factors could be inappropriate collection and transport of specimen and technical difficulty in growing anaerobes. Also polymicrobial etiology was observed in 16 cases (4%) in the same study.[19] .Anuradha De et al in their study from Mumbai reported various anaerobes from pleuro pulmonary infections among which 68.4% anaerobes were from pleural fluids.[20]

Comparison of various bacteria and viruses detection by pleural fluid culture and molecular method (PCR assay)was studied by Jose Maria and reported culture positivity only 10% by culture technique as compared to 68.3% by molecular assay indicating higher sensitivity in molecular assay.[21] We found methicillin resistance in staphylococcus aureus (MRSA) as 66.6% and carbepenenm resistance as in Gram negative isolates as 39.2%. All Enterococcus species isolates were vancomycin sensitive. Study by Jain Sonali et al in part of Delhi reported 79.3% MRSA and 35% MDR Gram negative isolates.[19] Study

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by Christian N. et al recommended cefuroxime plus metronidazole as empirical therapy for pleural empyema which covers the aerobic and anaerobic etiological agent.[6] Elie Azoulay et al studied use reagent strips for rapid diagnosis of infectious pleural effusions to categorize it as transudate and exudate based on pleural fluid protein.[22]

Conclusion:

Infectious etiology can be ruled out by culturing pleural fluid specimens which directs the proper management of patient clinically. It is natural that in conventional culture, bacteria may not survive if delayed inoculation is expected. Collection of body fluids like pleural fluids in enrichment media like BHI is an alternative in many laboratories which are not equipped with automated culture system especially in rural set up. Certainly this method will be useful in improving culture positivity and outcome.

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