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Original Article

Microbial and Antibiotic Susceptibility Profiles among Pleural Effusion Exudative Samples

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ABSTRACT

Background and Objectives: **Infection of pleural fluid is a common disease and because of antibiotic administration, the microbiology of this fluid has changed. The aim of this study was to determine the common bacteria and suitable antibiotics for treatment in pleural effusion (PE).**

Materials and Methods: **In this cross sectional study, 1210 samples with exudative features were cultured for possible growth of microbial pathogens and then examined for antibiotics sensitivity. Samples' characteristics were then analyzed according to the age and sex difference.**

Results: **Among 1210 exudative pleural effusions, 38.2% were obtained from females and 61.8% from males. Of 142 pleural fluid samples, 11.7% had a positive culture. Aerobic gram negative organism was the most common type among the other samples with a prevalence of 52% followed by aerobic gram positive (25.3%), non- aerobic gram negative (15.7%), non- aerobic gram positive (6.2%) and fungi (0.8%). *E. coli*, *Staphylococcus Aureus* and *Acinetobacter baumannii* were the most common types of organism among adult population.**

Conclusion: **Aerobic gram positive bacteria had the highest prevalence among the pathogens, and cephalosporins, aminopenicillins and β-lactams were the most effective antibiotics for their treatment.**

***Keywords*: Pleural Effusion, Bacterial Infection, Bacterial Sensitivity Test**

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Introduction

Pleural disorders usually manifest as pleural effusion (PE) (1). Inflammation of pleura leads to increasing the permeability of pleural vessels and fluid accumulation in the pleura. Pleural effusion is divided in two types of transudate and exudate based on the absolute lactic dehydrogenase (LDH) value, pleural fluid to serum LDH ratio, pleural fluid protein concentration and pleural fluid to serum protein ratio (2-4). Pleural infection which is a common problem, affects 60000 patients in USA and UK each year (4). It does not belong to any specific age groups but is more frequent in childhood and elderly (5-8). Based on the sex, it is two times more frequent in male and diabetes mellitus is another risk factor of the disease occurrence (5-8).

Evaluation of pleural fluid is usually done by Gram staining and culture. After administration of antibiotics for infected PE treatment, the microbiology of this fluid has changed. During 50 years ago, *Streptococcus pneumonia* was the most common organisms in parapneumonic effusion but today because of Pneumococal conjugate vaccine and penicillin usage the prevalence of this organism in positive cultures decreased to 10-15% (5, 7, 9). The prevalence of cultured pathogens is differs based on the infection of pleural fluid in the hospital or community. In hospital-acquired infections, *Staphylococcus aureus* specially methicillin resistant type, *Enterococcus, Enterobacteriaceae* and multi-drug resistant organisms are the most common pathogens but in community-acquired pleural infection, *Streptococcus milleri* group which is consist of *S. anginosus, S. constellatus* and *S. intermedius* are the most frequent organism and after that in order of prevalence, *Streptococcus Pneumoniae, Staphylococcus Aureus* and *Enterobacteriaceae* are common. In addition, up to 25% of community-acquired infection is due to anaerobic bacteria (5, 6, 8). For decreasing the morbidity and mortality of infected pleural effusion we should choose the appropriate antibiotics; therefore we need to know the bacteriology of pleural fluid and determine the most common pathogens in this liquid.

We aimed to analyze the PE to establish the common bacteria and suitable antibiotics for treatment.

Materials and Methods

Through a cross sectional study in Central Laboratory of Hematology and Microbiology in Imam Khomeini Hospital affiliated to Tehran University of Medical Sciences (TUMS) in Tehran, Iran, patients with diagnosis of pleural effusion undergoing thoracocentesis were included in this study between 2006 and 2012.

Plural tap was performed under sterile conditions with the patients in semi sitting position bending forward. Specimen were collected in a 20 ml tube and transferred to the laboratory for further hematological, biochemistry and microbiological evaluation with conducting microbiologic culture on the predetermined media for 72 hours. Effusion specimens with serous features were excluded from the study. Simultaneous measurements of blood samples were also performed in association with obtained pleural fluids.

The samples are studied and cultured based on standard instructions. The samples were first centrifuged so that slides were made of their sediments. The slides were then stained with Giemsa and Gram and examined for smearing. The sediment was cultured in three mediums – blood agar with sheep blood, chocolate agar and EMB. A portion of the samples was cultured in thioglycolate medium for enrichment. For non-aerobic culture, three gas-pack plates were used.

Identification

Upon receipt of specimen, within a maximum of one hour, the fluid sample was cultured on blood agar with sheep blood, chocolate agar and EMB

(Hi Media Co, India) plates separately. All plates were incubated at 37 °C for 24 hours. All bacteria growing on the culture media were identified by their biochemical reaction profile using Beckton & Dickinson and Mast Diagnostic Group UK identification products. In our study, we also accepted mixed cultures which do not reveal more than two predominant bacteria.

The isolated bacteria were inoculated on Mueller Hinton agar (Mast group Ltd, Merseyside, UK) and antimicrobial susceptibility testing was performed using disk diffusion method (as recommended by CLSI No: M2-A9) (7). The antibiotic disks were provided from Mast diagnostic group Ltd. The antibiotic panels were selected according to CLSI guidelines (as described by Clinical and Laboratory Standard Institute (CLSI) No: M100-S16) (8). The inhibition zone diameter was measured using a scaled ruler (antibiotic zone scale) and reported as resistant, intermediate and susceptible. Standard bacteria (ATCC) were used as control strains and the test results were only accepted when the inhibition zone diameters of the above mentioned control strains were within performance ranges (as described by CLSI No: M100-S16). In case of mixed bacteria, only the major and predominant pathogens were tested.

Demographic and primary clinical characteristics were extracted from patient's medical records and were registered in our data bank in association with other clinical and laboratory findings. Patients signed an informed consent before entering the study and the institutional review board of TUMS approved the study protocol.

Statistical Package for Social Sciences were used to analyze data of the current study with employment of student t-test for quantitative and Chi square test for qualitative variables while the values were considered statistically significant at a $P<0.05$.

Results

A total of 1210 exudative pleural effusion samples transferred to the central laboratory of our hospital were analyzed of which 463 samples belonged to females (38.2%) while the remainder 747 ones belonged to the male's patients (61.8%). Moreover, 24 samples were obtained from children (1.98%) defined as lower the age of 16 years old compared with 1186 samples taped from adults (97.02%) over the age of 16 years old. Demographic characteristics of patients' population showed in Table I.

Of these, 142 samples were positive in terms of microbiological culture (11.7%) of which 37 specimens belonged to females (7.9%) compared with 105 samples obtained from male patients (14.05%). Furthermore, three samples out of 24 ones in pediatric group results positive for microbiological culture (12.5%) while this proportion was as 139 positive cultures in adult group out of 1186 samples (11.72%). Type and antibiotic sensitivity of microorganisms are summarized in Table 2 and 3 according to sex and age groups. As the tables show, aerobic gram negative organism was the most common type among the others samples with a prevalence of 52% followed by aerobic gram positive (25.3%), non- aerobic gram negative (15.7%), non- aerobic gram positive (6.2%) and fungi (0.8%). On the other hand, *E. coli, S. aureus and A. baumannii* were the most common types of organism among adult population.

Table 1- Demographic characteristics of patients' population

	Groups	Count	Percent
Sex group	Male	747	61.8
	Female	463	38.2
Age group	Pediatrics	24	1.98
	Adults	1186	97.02
Positive culture	Pediatrics	3	12.5
	adults	139	11.72
	Male	105	14.05
	Female	37	7.9

Table 2- Bacteria Isolated from pleural effusion exudative samples based on age and sex

			Organisms and count
Age group	Adults		*Enterococcus* (8), *citrobacter freundii* (1), *Enterobacter aerogenes* (3), *Staphylococcus_epidermidis* (10), *Pseudomonas_aeruginosa* (7), *Alcaligenes-sp* (1), *Staphylococcus-Aureus* (28), *Acinetobacter baumannii* (24), *Streptococcus pneumonia* (12), *Nonhemolytic streptococcus* (1), *E.coli* (20), other(28)
	Pediatrics		*Staphylococcus haemolyticus* (1), *Staphylococcus_epidermidis* (1), *Citrobacter sp (1)*
Sex group	Adults	Male	*Enterococcus* (4), *Citrobacter freundii* (1), *Enterobacter aerogenes* (3), *Staphylococcus_epidermidis* (7), *Pseudomonas_aeruginosa* (6), *Alcaligenes-sp* (1), *Staphylococcus-Aureus* (23), *Acinetobacter baumannii* (19), *E. coli* (15), other(28)
		Female	*Enterococcus* (4), *Staphylococcus_epidermidis* (3), *Pseudomonas_aeruginosa* (1), *Staphylococcus-Aureus* (5), *Acinetobacter baumannii* (5), other (22)
	Pediatrics	Male	*Staphylococcus haemolyticus* (0), *Staphylococcus_epidermidis* (0),
		Female	*Staphylococcus haemolyticus* (1), *Staphylococcus_epidermidis* (1), *Citrobacter sp* (1)

Table 3- Antimicrobial susceptibility to the most frequent isolated microorganisms ,Isolated from pleural effusion exudative samples

Antibiotic Organism	Sensitive	Resistant
Enterococcus	Co Amoxiclav, Amoxicillin, mpicillin, sulbactam, Chloramphenicol, Tetracycline	Oxacillin, Imipenem, Clindamycin
citrobacter freundii	Gentamicin, Ciprofloxacin, ceftazidim, Ampicillin, Ceftriaxone, Imipenem	Cefixim, Co-trimoxazole
Enterobacter aerogenes	Ceftazidim, Cefixim, Ampicillin, Ampicillin-sulbactam, Imipenem, Gentamycin, Piperacillin_Tazobactam	Cefixim, Carbinicillin
Staphylococcus_epidermidis	Tetracycline, Chloramphenicol, Cephalotin, Rifampin	Gentamicin, cloxacillin
Pseudomonas_aeruginosa	Gentamicin, ciprofloxacin, Ampicillin, Piperacillin_tazobactam, Tobramycin	Ampicillin, Cefixim, Piperacillin, Cefepime, Clindamycin, Ampicillin
Alcaligenes-spp	Penicillin, Ciprofloxacin, Ticarcillin	Gentamicin, Ampicillin, Co-trimoxazole, Ceftriaxone, Carbinicillin, Imipenem

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Staphylococcus-Aureus	Vancomycin, Chloramphenicol, Imipenem, Cephalothin	Clindamycin, Penicillin, Ceftriaxone, Cloxacillin, Cephalexin
Acinetobacter baumannii	Gentamycin, Piperacillin-Tazobactam	Ampicillin, Cefepime, Ceftriaxone, Penicillin, Ceftizoxime, Cefixim, Piperacillin, Ampicillin_sulbactam, Amoxicillin, Co_amoxiclav, Ceftazidim, Ciprofloxacin
Klebsiella_pneumonia	Imipenem, Ampicillin_sulbactam, Piperacillin	Ampicillin, Ticarcillin, Co-trimoxazole
Streptococcus pneumonia	Amoxicillin, Vancomycin, Ampicillin, Cefazolin, Chloramphenicol, Tetracyclin, Rifampin, Clindamycin	Oxacillin, Co-trimoxazole
Proteus mirabilis	Gentamicin, Ciprpfloxacin, Imipenem, Pipracillin-Tazobactum, Ampicillin-Tazobactum	Cefazolin, ceftazidim, Cefixim, Ceftriaxone, Co_trimoxazole
Nonhemolytic streptococcus	Clindamycin, Amoxicillin, Chloramphenicole, Vancomycin, Erythromycin, Ciprofloxacin, Rifampin	Ampicillin
Citrobacter spp	Gentamicin, Ceftazidim, Ampicillin, Cefepime, Imipenem, Cefixim	Co-trimoxazole
Staphylococcus haemolyticus	Ampicillin, Cefazolin, Ceftriaxone, Vancomycin, Tetracyclin, Chloramphenicol	Penicillin, Oxacillin, Clindamycin, Co-trimoxazole
Stenotrophomonas maltophilia	Ciprofloxacin	Gentamicin, Ampicillin, cefixim, Piperacillin ,Co_trimoxazole, ceftazidim
beta-hemolytic streptococci	Oxacillin, Clindamycin, Vancomycin, Erythromycin, Rifampin, Teicoplanin	Co-trimoxazole
Streptococcus Group D	Vancomycin	Cefepime, penicillin, Clindamycin, Erythromycin
Viridans Streptococcus	Ciprofloxacin, Ampicillin, Chloramphenicol, Vancomycin, Erythromycin, Rifampin, Clindamycin, Teicoplanin	Co-trimoxazole
Pseudomonas sp	Ampicillin, Piperacillin-Tazobactam, Ciprofloxacin	Ampicillin, Imipenem, Ceftriaxone, Co-trimoxazole, Ceftazidim
Klebsiella_oxytoca	Ciprofloxacin, Ampicillin, Imipenem, Piperacillin, Cefotaxim	Ticarcillin, Ampicillin, Co-trimoxazole
Acinetobacter lwoffii	Tobramycin, Tazobactam	Cefixim, Piperacillin
Nonhemolytic streptococcus	Ampicillin, Clindamycin, Chloramphenicol, Vancomycin, Erythromycin, Ciprofloxacin, Ampicillin, Imipenem, Rifampin	Ampicillin
E.coli	Imipenem, Gentamicin, Piperacillin-Tazobactam, Tobramycin	Cefixim, Co-Amoxiclav, Cefotaxime, Penicillin, Carbinicillin
Streptococcus Pyogenes	Ampicillin, Chloramphenicol, Vancomycin, Erythromycin, Rifampin	Co-trimoxazole, Oxacillin

Discussion

Our study showed that 3 positive cultures out of 142 belonged to children including 12.5% of children lower the age of 16 years old. Ozol *et al.* found 40 positive culture samples among 107 children with parapneumonic pleural effusion. Among 1186 adult pleural samples, 39 cultures were positive belong to 11.7% of our samples. Jimenez *et al.* during their retrospective study on 259 patients with parapneumonic effusion demonstrated that 14 (5.4%) samples were positive for Gram stain and in another study 232 (54%) subjects out of 430 samples had positive cultures (8- 11). This low bacterial detection could be due to antibiotic therapy prior to pleural fluid culture. We found that aerobic gram positive bacteria with prevalence of 52% were the most common cultured pathogen, so *E. coli, S. Aureus* and *A. baumannii* were the most prevalent bacteria.

Bacteriology of pleural fluid infection is different among community or hospital- acquired pleural fluid infection. Based on other studies aerobic microorganisms especially gram positive type were most current particularly *Streptococci milleri, Streptococcus pneumonia* and *Staphylococcus aereus.* Other *streptococci, enterobacteria, Haemophilus influenzae, Pseudomonas* spp*, tuberculosis* and *Nocardia* were rarely seen in the positive pleural culture. Gram negative organisms like *Escherichia coli, Pseudomonas* spp*, Haemophilus influenzae*, and *Klebsiella* spp were in the second place of prevalence. Prevalence of anerobics pathogens was also increasing in positive pleural effusion cultures and their range was about 12%-34% (12-14), these organisms are cultured among community-acquired pleural fluid but in hospital acquired type, methicillin-resistant *Staphylococcus aureus*, other *staphylococci* and *enterobacteria* are common (14).

All of the patients with pleural infection should be treated with intravenous antibiotics as initial therapy. Antibiotic choosing is depending on various factors like blood and pleural fluid cultures and bacterial sensitivities. As determined in other studies, all the pathogens include of aerobics or anaerobic are resistant to penicillin but for treatment of *Pneumococcal* or *Streptococcus milleri* infection antibiotic treatment with β-lactams is recommended (15). In another study, the suitable treatment for aerobic pathogens of pleural fluid was intravenous aminopenicillin or second-generation cephalosporin (e.g. cefuroxime) and the proper antibiotics of anaerobic organisms like metronidazole or clindamycin. This treatment is covering the community acquired pleural fluid pathogens (16- 19), but in order to treat the hospital acquired organisms antibiotic should affect the Gram positive and Gram negative aerobic and anaerobic bacteria. Because of their highly antibiotic resistant characteristics, the choice of antibiotics include of third or fourth generation cephalosporin for example: ceftazidime and cefepime, carbapenems or antipseudomonal penicillins like piperacillin/tazobactam with metronidazole. Vancomycine, linezolide or other alternative antibiotics should be added for treatment of methicillin resistant *Staphylococcus aureus* (16-18). In our study the most frequent organisms were sensitive to β-lactams, vancomycine, aminoglycoside and cephalosporins.

Conclusion

Our study showed that due to high prevalence of aerobic gram positive bacteria, third and fourth generation of cephalosporin, aminopenicillins, β-lactams and vancomycine were the most effective antibiotics for pleural fluid infection treatment.

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