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Bacterial Profile and their Antimicrobial Resistance Patterns from Body Fluids at Tikur Anbesa Specialized Hopital, Addis Ababa, Ethiopia

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Abstract

Background: Infections caused by multidrug resistant (MDR) bacteria remain a public health threat for patients and health care workers. There are scarcity of data on bacterial profiles and their drug susceptibility patterns from body fluids in Ethiopia. Hence, this study aimed at assessing bacterial profiles and their antimicrobial susceptibility patterns (AST) from body fluids at Tikur Anbessa Specialized Hospital (TASH), Addis Ababa, Ethiopia.

Methods: A cross sectional study was conducted from July 2015 to March 2016 by recruiting 384 study participants. Different body fluids were collected and cultured on Blood agar, MacConkey agar and chocolate agar then incubated aerobically and micro-aerobically. Moreover, gram staining, acid-fast staining (AFB) and White blood cell count (WBC) were performed for all collected body fluids sample. Bacterial identification was made using colony morphology, gram stain and biochemical tests. Antimicrobial susceptibility testing was performed on Muller-Hinton agar using disk diffusion method. Data was analyzed using SPSS version 20.

Results: Overall 14.1% (n=54/384) of the body fluids had bacterial growth. Most bacteria were isolated from Cerebrospinal Fluid (CSF) 57.4% (n=31/54) and pleural fluids 33.3% (n=18/54). Of all body fluids, primary gram stain yielded 10.7% (n=41/384) positive results. Majority of body fluids, 44.1% (n=173/384) had abnormal WBC count above 05 cells/mm^3 and 52.6% (n=91/173) of them had polymorphic features. Most frequent bacterial isolates were *K. pneumoniae* 16.7% (n=9/54), Coagulase negative Staphylococcus 15.0% (n=8/54) and Pseudomonas spp. 11.1% (n=6/54). Gram-negative and gram-positive bacteria showed highest resistance for Gentamycin (76%) and Erythromycin (59%) respectively. The MDR level recorded was 75.9% (n=41/54).

Conclusion: Significant numbers of bacteria with high MDR level were isolated from body fluids that call all health care workers and policy makers for concerted efforts for prudent antibiotic use, and limit the transmission of MDR bacteria in hospital and community settings. Regular monitoring of antimicrobial resistance patterns is essential.

Keywords: Bacterial isolates; Multidrug resistance; Pleural fluids; Synovial fluids

Introduction

Body fluids are important in transporting nutrients as well as waste products, regulating body temperature and assessing respiration process [1]. Generally, body fluids like cerebrospinal fluid (CSF), pleural, peritoneal, synovial and pericardial fluids are naturally free of microorganisms under normal circumstance [2]. However, under infectious condition of central nervous system, peritoneum, joints and other sterile sites, different types of bacteria, fungi, virus and parasites could present and change the physicochemical nature of the body fluids [1-3].

CSF is produced by ultra-filtration or secretion, and circulates through the ventricles and spinal cord and supply nutrients to brain cell and serve as a cushion [3]. Bacterial meningitis is a medical emergency that require urgent rational antibiotics therapy. An estimated 200,000 deaths occur worldwide per year and the mortality rate is higher in developing countries notably in Sub-Saharan Africa, with a range of 16-32% [4]. On the other hand, peritoneal effusions refers to the detectable and pathologic collection of fluid in the peritoneal cavity and it is the most common complication of cirrhosis and have been a challenge for hospitalized patients [5,6]. Ascites differentiate into transudative as result of systemic conditions such as cirrhosis, heart failure or nephrotic syndrome with low protein concentrations [7] or exudative effusions with high protein concentrations. Similarly, pleural effusions are because of excessive fluid accumulation in the pleural space that has transudative type and exudative type [7,8]. Pleural effusions in developed countries are due to malignancy while in developing countries it is mainly due to bacterial infections [9]. Joint fluid is a viscous substance that lubricates joints and many bacteria are responsible for the occurrence of arthritis [6] due to presence of bacterial cell wall fragments and bacterial DNA as indicated from experiments [10,11].

Different pathogenic bacteria like *Enterobacteriaceae*, *Streptococcus pneumoniae*, *Neisseria meningitides*, *Group B Streptococci*, *Listeria monocytogenes*, *Haemophilus influenzae*, *Staphylococcus aureus*, *Acinetobacter* and *Pseudomonas spp.* can invade the various organ and present in the body fluids [5,6,10-12]. Body fluids invaded by such

bacteria are characterized by having increased WBC count and protein concentration as well as decreased glucose concentration [9]. Bacterial invasion of body fluids cause severe diseases leading to morbidity and mortality unless identified early and treated [1,2]. Though isolation and identification of bacterial etiologies are critical for patient mangment [13] developing resistance against commonly used antibiotics are becoming a challenge for treatment success [14]. There are very limited data on the bacterial profiles and their antimicrobial susceptibility patterns from body fluids in Ethiopia in general and in this study site in particular. Hence, assessing bacterial profiles and their AST patterns from body fluids is very crucial to clinicians, microbiologist, and pharmacist and policy makers for proper diagnosis of different infections and for prudent antibiotic use. Therefore, this study aimed to assess the bacterial profiles and their AST patterns from various body fluids submitted to TASH bacteriology laboratory.

Materials and Methods

A prospective cross sectional study was conducted from July 2015 to March 2016 at TASH. It is the biggest referral hospital of Ethiopia, which is located in the capital city, Addis Ababa. A total of 384 study participants were recruited using convenient sampling technique. The sample size was calculated using a single population proportion formula by taking the value of P=0.5, with marginal error of 0.05 and 95% Confidence level. All patients, irrespective of age and sex, who gave body fluid samples for microbiological analysis, were included. Patients with history of antibiotics within the last two weeks, contaminated samples, incorrectly labeled and delayed body fluids for more than 2 hrs were excluded. Physicians, following aseptic procedures in sterile tubes, collected body fluids. Demographic information of patients and physical appearance of body fluids (clear, bloody/traumatic, cloudy and clear straw) was recorded using data collection sheet. Immediately after collection, all samples were taken to the bacteriology laboratory for bacteriological culture, primary gram stain, acid-fast stain, total white blood cell (WBC) and differential WBC count.

Microbiological isolation and identification

Collected body fluids (CSF, pleural, peritoneal and synovial fluids) were inoculated on blood agar (Oxoid, UK), chocolate agar (Oxoid, UK) and MacConkey agar (Oxoid, UK). Blood agar and MacConkey agar were incubated at 35-37C aerobically for 24 hours while chocolate agar were incubated at 35-37°C for 72 hours in a candle jar to provide 5-10% CO_2 concentration in order to give chance of growth for microareophilic fastidious bacteria. Plates were examined daily for the growth of bacteria and identifications of bacterial isolates were performed using colony morphology, gram stain from colonies (from culture plates) and conventional biochemical tests. Gram-positive bacteria were identified to species level using catalase, coagulase, latex agglutination test and Pastorex TM staph-plus (*Staphylococcus aureus* identification) test kits. For identification of gram-negative bacteria, different biochemical tests were used which were indole, triple sugar iron agar, citrate, urea, lysine decarboxylase (LDC) agar and motility. *K. puemoniae* (indole positive) and *K. oxytoca* (indole negative) differentiated based on their indole reaction. *Pseudomonas aeroginosa* differentiated from other *Pseudomonas species* using *Pseudomonas aeruginosa* Screen 80 tablet (Rosco, DK- 2630, and Denmark).

Cell count, gram stain and AFB staining of body fluids

After inoculation of all body fluids on culture media for bacterial isolation and identification purpose, body fluids samples were diluted and/or directly filled if it is clear and transparent in Improved Neubauer counting chamber and white blood cells were counted in all areas. Then, WBC was calculated per millimeter cube of body fluids as described in literature [15]. Similarly, on the sediment of body fluids three smears were made for differential WBC, gram staining and Acid fast staining methods following the standard procedures. Primary gram stain and acid fast staining were performed directly from collected specimens to compare their diagnostic importance with culture result since they are requested mostly as preliminary diagnostic tools until culture results obtained.

Antimicrobial drug susceptibility testing

Using Kirby-Bauer disk diffusion method, antimicrobial susceptibility testing performed for each pure isolates that was incubated at 35-37°C for 16-18 hours according to the Clinical and Laboratory Standards Institute (CLSI) [16]. Briefly, 3-5 pure colonies of bacteria were picked from blood agar for Gram positives bacteria, from MacConkey agar for Gram-negative bacteria and chocolate agar for late growers then emulsified in sterile nutrient broth using sterile wire loop. In order to make standardized inoculums size, the bacterial suspension was adjusted to 0.5 McFarland standard using Densitometer and the suspension was swabbed on to Muller-Hinton agar (dispensed on 100 mm plate). For *Streptococcus species,* Mueller Hinton agar supplemented with sheep blood were used.

As per the CLSI guideline, bacterial isolates were tested for ampicillin (10 µg, BD), amikacin (10 µg, Oxoid), Tobramycin (10 Oxoid), vancomycin (30 µg BD), ceftazidime (30 µg, BD), cefotaxime (30 µg, BD), ceftriaxone (30 µg, BD), chloramphenicol (30 µg, BD), clindamycin (2 µg, BD), gentamicin (10 µg, BD), TMP-SXT (1.25 µg +23.75 µg, BD), tetracycline (30 µg, BD), ciprofloxacin (5 µg, BD), penicillin (10 units, BBL), oxacillin (5 µg, BD), Rifampin (BD, 5 µg), Cefuroxime(30 µg BD) and erythromycin (15 µg, BD). Oxacillin (methicillin) susceptibility of *Staphylococcus aureus* and Coagulase negative Staphylococci was interpreted using 30 µg cefoxitin as a surrogate test for Multidrug resistant *Staphylococcus species*. The zone of inhibition was measured to the nearest millimeter and all bacterial isolates were classified as sensitive, intermediate and resistant.

Quality control and quality assurance

Standard Operating Procedures (SOPs) were strictly followed verifying that media meet expiration date and quality control parameters. Visual inspections of cracks on media or plastic petridishes, unequal fill, hemolysis, evidence of freezing, bubbles, and contamination was checked. Quality control was performed to check the quality of medium. Each new lot was checked before use by testing *Escherichia coli* ATCC 25922 and/or *Staphylococcus aureus* ATCC 25923 standard control strains. We have used known gram positive, gram negative and acid fast positive control slides to check the quality of the various staining methods.

Statistical analysis and interpretation

The data was entered, cleaned and analyzed using SPSS version 20. The descriptive statistics (mean, percentages or frequency) was calculated. The bi-variant logistic regression analysis was used to see the relation between different study variables. Those variables, which

showed statistical significant association from bi-variant logistic regression, were further analyzed using multiple logistic regressions. A P-value <0.05 was considered as statistically significant.

Ethical clearance

The Department Research and Ethics Review Committee (DREEC) of Medical Laboratory Science, School of Allied Health Sciences, College of Health Sciences, Addis Ababa University approved the study. Permission letter was also obtained from the study site. All information obtained from patients were kept confidential. Laboratory results were communicated to the requesting physicians on time.

Results

Socio-demographic characteristics

A total of 384 patient's body fluids were analyzed for isolation, identification and antimicrobial susceptibility testing. Of these body fluids, cerebrospinal fluids (CSF) was the most frequently encountered body fluids accounting 68.8% (n=264/384) followed by pleural fluid 76 (n=19.8%). The majority of body fluids were clear 61.7% (n=237/384) in terms of appearance while 19.3% (n=74/384) body fluids were turbid. Of 384 body fluids, 50.8% (n=195/384) of them were collected from female pateints and 50.8% (n=195/384) were collected from outpatient departments (Figure 1). The age range of study participants was between 24 days-86 years with mean and standard deviation of 15.1 ± 19.2 where 70.2% (n=271/384) of them were below 20 years.

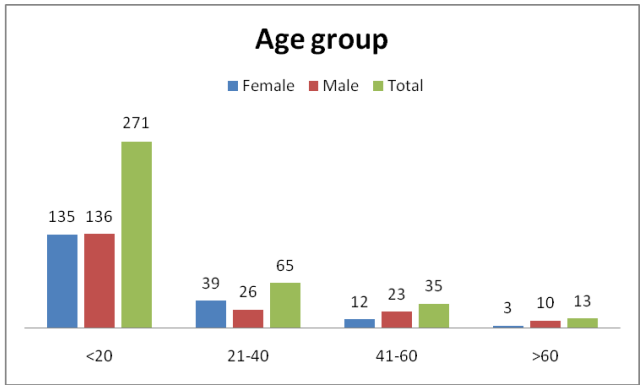

Figure 1: Age in year and sex frequency of pateints analsyed for bacteriology of body fluids at TASH from July 2015 to March, 2016.

Bacterial profiles of body fluids sample

Overall, 14.1% (n=54/384) of body fluids had bacterial growth. Of these, 62.2% (n=32/54) of culture positives were from female patients. However, there was no statistical significant difference between gender and culture results (OR=0.624, 95% CI=0.348-1.119, P value=0.114) (Table 1). Highest bacterial isolation was obtained in age group below 20 years old and statistically significant association was seen between age of patients and culture results (OR=1.022, 95% CI=1.009-1.036, P=0.001. In this study, 51.2% (n=28/54) culture positive findings were from admitted patients. Gram positive and Gram-negative isolates constitute 40.7% (n=22/54) and 59.3% (n=32/54) respectively with a gram positive to gram-negative ratio of 0.69:1. Most bacteria were isolated from CSF samples accounted 57.4% (n=31/54) followed by pleural fluids 33.3% (n=18/54) (Table 2). The frequent bacterial isolates were *K. pneumoniae* 16.7% (n=9/54) followed by Coagulase negative Staphylococcus 15.0% (n=8/54).

Variables	Culture results (n=38)		P-value	OR	95% CI
	Positive no. (%)	Negative no. (%)			
Gender					
Male	22 (40.7)	173(45.1)	0.114	0.624	0.348-1.119
Female	32 (59.3)	157(40.9)			
Age in years					
<20	27 (50.0)	244(63.5)	0.001	1.022	1.009-1.036
21-40	17 (31.5)	48(12.5)			
41-60	7 (13.0)	48(12.5)			
>61	3 (5.6)	48(12.5)			
Patient Type					
Inpatient	28 (59.9)	155(40.4)	0.05	0.651	0.424-1.001
Outnpatient	26 (48.1)	175(45.6)	0.015	1.839	1.126-3.002
Type of body fluids					
CSF	31 (57.4)	233(60.7)	0.0289	1.208	0.852-1.71
Pleural	18 (33.3)	58(15.1)			
Ascetic	3 (5.6)	31(80.7)			
Synovial	2 (3.7)	8(2.1)			
Appearances of fluids					
Turbid	43(79.6)	31(8.1)	0.000	1.903	1.378-2.628
Clear	6(11.1)	231(60.2)			
Clear-straw	4(7.4)	65(16.9)			
Trauma	1(1.9)	3(0.8)			
Total	54 (14.0)	330(85.9)			

Table 1: Association of gender, age, patient types, types of body fluids and appearance of body fluids nature with culture results at TASH from July 2015 to March 2016.

	Type of body fluids n (%)				
Isolation of bacteria	**CSF**	**Pleural fluids**	**Peritoneal fluids**	**Synovial fluids**	**Total**
Gram negative isolates					
K. pneumoniae	8(1.9)	1(0.2)	0(0)	0(0)	9(2.1)
Pseudomonas spp.	2(0.5)	4(.9)	0(0)	0(0)	6(1.4)
Acinetobacter spp.	4(0.9)	1(0.2)	0(0)	0(0)	5(1.2)
E. coli	0(0)	2(0.5)	1(0.2)	0(0)	3(0.7)
Citrobacter diversus	0(0)	0(0)	1(0.2)	0(0)	1(0.2)
K. rihinoscleris	0(0)	1(0.2)	0(0)	0(0)	1(0.2)
K. oxytoca	1(0.25)	1(0.25)	0(0)	0(0)	2(0.5)
Enterobacter cloacae	1(0.25)	1(0.25)	0(0)	0 (0)	2(0.5)
N. meningitides	1(0.2)	0(0)	0(0)	0 (0)	1(0.2)
P. aeruginosa	0(0)	1(0.2)	0(0)	0 (0)	1(0.2)
P. mirabilis	0(0)	1(0.2)	0(0)	0 (0)	1(0.2)
Total	17(53.1)	13(40.6)	2(6.3)	0 (00)	32(100)
Gram positive isolates					
Coagulase negative Staphylocoous (CONS)	8(1.9)	0(0)	0(0)	0 (0)	8(1.9)
S. aureus	1(0.2)	3(0.9)	0(0)	0 (0)	4(0.9)
S. pneumoniae	2(0.5)	1(0.2)	0(0)	1 (0.2)	4(0.9)
S.milleri	0(0)	0(0)	0(0)	2 (0.5)	2(0.5)
Enterococcus spp.	1(0.25)	1(0.25)	0(0)	0 (0)	2(0.5)
S. viridians	1(0.2)	0(0)	0(0)	0 (0)	1(0.2)
S. pyogenes	1(0.1)	0(0)	0 (0)	0 (0)	1(0.2)
Total	14(63.6)	5(22.7)	0(00)	3(13.6)	22(100)

Table 2: Frequency of bacteria isolated from different body fluids at TASH from July 2015 to March 2016.

Gram stain, acid fast stain (AFB), WBC count and appearance of body fluids

In this study, gram stain were performed directely from all body fluids sample and the yield was 10.7% (n=41/384) of which 56.1% (n=23/41) of the body fluids had gram-negative bacteria. Compared to culture positive results (n=54), gram stain yielded 75.9% (n=41/54) positive results while 24.1% (n=13/54) body fluids had neither gram positive nor gram-negative bacteria. Out of 384 body fluids, 45.1% (n=173/384) of it had relatively high number of white blood cell count (WBC) above 05 cells/mm^3 and of them 21.1% (n=92/384) of body fluids had a polymorphic differential WBC features. All body fluids, which were positive by culture, had WBC count of above 20 cells/mm^3 with dominant polymorphic differential features in 72.9% (n=39/54) of body fluids. About 79.6% (n=43/54) of culture positive body fluids were turbid in appearance and only 11.1% of body fluids (n=6/54) had clear appearance and yield positive cultures. There was significant association between appearance of body fluids and culture results (OR=1.64, 95% CI=1.903-1.378, P=0.00) (Table 3). Only two body fluids out of 384 were acid-fast positive but none of them was culture positive for any other bacteria.

Antimicrobial drug resistance pattern of bacteria isolated from body fluids

The antimicrobial drug resistance profiles of bacteria ranged from zero to 100%. The highest resistance rate for gram-negative isolates was recorded for gentamycin (65.6%), Ampicillin (62.5%), Ciprofloxacin (53.1%), Ceftriaxone (50%) and Tobramycin (50%) (Table 4). Similarly, gram-positive bacteria showed relatively higher resistance rate to Erythromycin (59%), Clindamycin (54%) and Trimethoprim-Sulphamethoxazole (50%) (Table 5). Of all bacterial isolates, 75.9% (n=41/54) of the bacterial isolates were multidrug resistant (MDR, a bacteria resistant for two or more drugs belonging to different classes of antibiotics). The level of MDR for gram positive and gram-negative isolates was found to be 72% (n=16/22) and 96% (n=29/32) respectively (Table 6). *K. pneumoniae* were the dominant isolates and 90% (n=8/9) of these isolates were MDR. From the gram positives isolates, CONS were dominant and all the isolates (100%, 8/8) were MDR.

Culture +ve (54)	**Gram stain**		**WBC**		**Body fluid appearance**			
	Gram bacteria	**Gram negative**	**Polymorphic**	**Monomorphic**	**Turbid**	**Clear**	**c/straw**	**Traumatic**
	18	23	39	15	43	6	4	1
Total	41*		54		54			

Table 3: Frequency of gram Stain, WBC count and sample appearance among the 54 culture positive body fluids at TASH from July 2015 to March 2016. *about 13 body fluids did not have both gram positive and gram negative bacteria.

Bacterial isolate (no.)	**AMP**	**GN**	**CTX**	**CRO**	**CTZ**	**CRX**	**TOB**	**CPR**	**AMK**	**CAF**	**RA**
K. pneumoniae (9)	5(55)	9(100)	4(44.4)	6(66.7)	4(44.4)	4(44.4)	4(44.4)	5(55.5)	2(22.2)	4(44.4)	NA

Acinetobacter (5)	3(60)	3(60)	2(40)	2(40)	4(80)	2(40)	3(60)	4(80)	4(80)	4(80)	NA
E. coli (3)	3(100)	1(33.3)	1(33.3)	1(33.3)	2(66.7)	1(33.3)	2(66.7)	2(66.7)	0(0)	0(0)	NA
Pseudomonas (3)	3(100)	3(100)	1 33.3)	1(33.3)	3(100)	3(100)	1(33.3)	1(33.3)	1(33.3)	1(33.3)	NA
P. aeruginosa (1)	0(0)	0(0)	1(100)	1(100)	0(0)	0(0)	0(0)	1(100)	0(0)	0(0)	NA
K. oxytoca (2)	2(100)	1(50)	0(0)	1(50)	0(0)	1(50)	2(100)	0(0)	1(50)	1(50)	NA
E. cloacae (2)	1(50)	2(100)	0(0)	1(50)	0(0)	0(0)	2(100)	1(50)	0(0)	0(0)	NA
C. divorce (1)	1(100)	1(100)	0(0)	1(100)	0(0)	0(0)	1(100)	0(0)	1(100)	0(0)	NA
K. rihinoscleris (1)	0(0)	1(100)	1(100)	1(100)	0(0)	0(0)	1(100)	1(100)	1(100)	1(10)	NA
P. mirabilis (1)	1(100)	1(100)	1(100)	1(100)	0(0)	0(0)	1(100)	1(100)	0(0)	0(0)	NA
N. meningitides (1)	1(100)	NA	0(0)	0(0)	NA	0(0)	NA	1(100)	NA	0(0)	1(100)
Total	20(62.5)	21(65.6)	13(40.6)	16(50)	11(34.4)	11(34.4)	16(50)	17(53.1)	10(31.3)	12(37.5)	1(3.1)

Note: NA: Not applicable; AMK: Amikacin; AMP: Ampicillin; SXT: Sulfamethoxazole-trimethoprim; CRO: Ceftriaxone; CTX: Cefotaxime; GN: Gentamycin; CTZ: Ceftazidime; CPR: Ciprofloxacin; TOB: Tobramycin; CAF: Chloramphenicol; RA: Rifampicin; R: Resistance.

Table 4: Antimicrobial resistance pattern of gram-negative bacterial isolates (n=32) from body fluids samples at TASH from July 2015 to March 2016.

Bacterial isolates, (no.)	**R**	**AM**	**P**	**CTX**	**CRO**	**CRX**	**CLN**	**ERY**	**VA**	**CPR**	**SXT**	**OX**
S. aureus (4)	R	2(50)	2(50)	2(50)	2(50)	2(50)	2(50)	2(50)	NA	1(25)	3(75)	2(50)
CONS (8)	R	5(64)	5(64)	3(38)	3(38)	4(50)	6(75)	6(75)	NA	4(50)	3(38)	3(38)
S. pneumoniae (4)	R	1(25)	1(25)	1(75)	1(75)	1(75)	2(50)	2(50)	1(25)	2(50)	2(50)	1(75)
Enterococcus spp (2)	R	1(50)	NA	NA	NA	NA	NA	NA	0(0)	NA	NA	NA
S. viridians (1)	R	1(50)	0(0)	1(50)	1(50)	1(50)	0(0)	1(50)	0(0)	0(0)	2(100)	1(50)
S. milleri (2)	R	1(100)	0(0)	0(0)	0(0)	NA	0(0)	0(0)	0(0)	1(100)	0(0)	0(0)
S. pyogenes (1)	R	1(100)	0(0)	0(0)	0(0)	NA	0(0)	0(0)	0(0)	1(100)	1(100)	1(100)
Total =22	R	10(45.4)	8(36.4)	7(31.8)	7(31.8)	8(36.4)	10 45.4)	11(50)	2(9)	10(46)	11(50)	9(36)

Note: NA: Not applicable; AM: Ampicillin; SXT: Trimethoprim–sulfamethoxazole; CRO: Ceftriaxone; CTX: Cefotaxime; CRX: Cefuroxime; VA: Vancomycin; P: Penicillin; CPR: Ciprofloxacin; OX: Oxacilin; ERY: Erythromycin; CLN: Clindamycin; R: Resistance.

Table 5: Antimicrobial resistance pattern of gram-positive bacterial isolates from body fluids samples at TASH from July 2015 to March 2016.

Multidrug resistance level of bacterial isolates							
Gram Negative	**No. tested**	**R1**	**R2**	**R3**	**R4**	**R>4**	**Total**
Pseudomonas spp	(6)	0	2	1	1	1	6
P. aeruginosa	(1)	0	0	1	0	0	1
E. coli	(3)	0	0	0	0	1	1
K. pneumoniae	(9)	0	0	0	4	5	9
Acinetobacter	(5)	0	0	0	2	3	5
P. mirabilis	(1)	0	0	0	0	1	1
K. oxytoca	(2)	1	0	0	0	1	2

Citrobacter diversus	(1)	0	1	0	0	0	1
K. rihinoscleris	(1)	0	0	0	0	1	1
Enterobacter cloacae	(2)	0	1	1	0	0	2
N. meningitides	(1)	0	0	1	0	0	1
Total	**32**	**1 (3.1)**	**4 (12.5)**	**4 (12.5)**	**7 (21.9)**	**13 (40.6)**	**29 (90.6)**
Gram positive isolates							
S. aureus	(4)	1	2	0	0	0	3
S. pneumoniae	(4)	1	0	1	0	0	2
Enterococcus spp	(2)	1	0	0	0	0	1
CoNS	(8)	0	0	2	2	4	8
S. viridians	(2)	0	0	0	0	0	0
S. milleri	(1)	0	0	1	0	0	1
S. pyogenes	(1)	0	0	1	0	0	1
Total	**22**	**3 (13.6)**	**2 (9)**	**5 (22.7)**	**2 (9)**	**4 (18.2)**	**16 (72.7)**
Note: R1: resistance to one drug; R2: resistance to two drugs; R3: resistance to three drugs; R4: resistance to four drugs; R>4: resistance to five or more drugs.							

Table 6: Multidrug resistance patterns for bacterial isolates from body fluids samples at TASH July 2015 to March 2016.

Discussion

The overall 14.1% (n=54/384) prevalence of bacteria from body fluids was relatively lower than the finding from Brazil study [17]. This might be due to over diagnosing, prior exposure to antibiotics and emergence of non-infectious conditions like malignancy. Coagulase negative staphylococcus and *Acinetobacter spp*. were the highest bacterial isolates in our findings that are in agreement with studies conducted in Iran, Korea and Brazil [1,17-19]. The detection of Coagulase negative Staphylococci & *Acinetobacter spp*. may be associated with a tendency of these pathogens to cause nosocomial infections, poor infection control practice in hospital, lack of standard facilities, poor sterilization of all gowns and equipment. *S. pneumoniae* and *N. meningitides* were the most common pathogens causing meningitis where our report is in agreement with previous studies from Namibia, Ethiopia, Iran, India and Nepal [9,12,14,20,21]. From CSF samples, *Haemophilus influenzae* was most frequently isolated bacteria from studies done in Namibia, Iran and Nepal [9,12,21] unlike in our study where no *Haemophilus species* was detected. This might be due to implementation of *Haemophilus* vaccine in routine EPI program in Ethiopia since some years back or difference in clinical conditions and the bacteriological culture system. *Pseudomomas* spp., *S. aureus* and *E. coli* were the commonest bacteria isolated from peritoneal fluids in our study, which is in agreement with studies done in Turkey, Brazil and Nepal [6,21,22]. It is known that gram-negative aerobic *Enterobacteriaceae* from the intestinal lumen can pass to mesenteric lymph nodes or other extra-intestinal sites across the intestinal-mucosal barrier and could appear in body fluids [23]. To the contrary, studies from India and Qatar reported that most frequent isolates of pleural fluids were *Streptococcus pneumoniae* [22,24].

S. pneumoniae showed 50% resistance level against penicillin in contrast to other studies done in Namibia, Ethiopia and Iran where 100% resistance level to penicillin were reported [1,12,14]. However, this big difference can explain in many ways like few strains isolated, change in the resistance profiles of the bacteria and difference in geographical nature of study populations. Similarly, *K. pneumoniae* showed lower resistance 55% to ampicillin compared to a study done in Iran that reported 87.5% resistance to ampicillin [6]. This could be the number of bacterial isolates and difference in the use of antibiotics in each country. The high level of MDR resistance (75%) in this study is in agreement with a previous study conducted in Gondar, Ethiopia [14]. This high MDR level may be due to inappropriate use of commonly prescribed antibiotics. Previous study in Ethiopia and in Iran reported high level MDR resistance among gram-negative bacteria that is similar to the current study [1,14].

Use of simple gram stain detect the presence of either gram positive or negative bacteria from 41 body fluids (10.7%) out of 384 total body fluids which is in agreement with a study conducted in India [25]. As compared to culture, gram stain missed only 3.4% (n=13/384) of body fluids, which were positive by culture. This means that, with such limitation, gram stain still provides valuable results as preliminary information until culture results reported. Especially, this is critical in area where culture facility is limited. Interestingly, 72.9% (n=39/54) body fluids, which were culture positive, had high WBC count (above 20 cells/mm^3) with dominant polymorphic differential features and majority of them were turbid (79.6%, n=43/54). From body fluids which were clear in appearance, 11.1% (n=6/54) of them were culture positive and statistically significant association was observed between body fluids appearance and culture yield (OR=1.64, 95% CI=1.903-1.378, P=0.00) (Table 3). Only two body fluids out of 384 were acid-fast positive but none of them was culture positive for any bacteria, which might be due to the possibility of disseminated tuberculosis infection. This very low number of AFB results from 384 body fluids might be due to unrelated clinical conditions to

tuberculosis and underscore the use of AFB stain to rule out tuberculosis infection.

Conclusion

Significant numbers of both gram negative and gram-positive bacteria were isolated from various body fluids samples. The high level of MDR strains observed urgently call for concerted and immediate attention of health care workers and policy makers for prudent antibiotic use and limits the transmission of MDR bacteria in the hospital and community settings. Moreover, regular monitoring of antimicrobial resistance patterns from all clinical samples including body fluids are essential and the antibiogram should be available to be used by clinicians and policy makers.

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Conflict of Intereset

The authors declare that they have no competing interests.

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