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Bacteriological profile and antibiotic sensitivity pattern in various body fluids –A retrospective study

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ABSTRACT

Introduction: Several bacteria like E.Coli, Klebsiella, Acinetobacter, Pseudomonas etc cause infections in various body fluids. Infections of the body fluids are mostly a medical emergency and may be life threatening if not managed timely. Very limited data are available about the antibiotic susceptibility of bacteria causing infection of body fluids. This retrospective study was undertaken between January to October 2019 in the Microbiology department of our diagnostic centre with the aim of identifying the bacteria causing infection of various body fluids and also to study their antibiotic susceptibility pattern.

Materials and Methods: This was a retrospective study carried out on 216 patients of all ages and both sexes registered for culture and sensitivity of different body fluids in the Microbiology department of our diagnostic centre between January to October 2019. The fluids included in the study were CSF, pleural, peritoneal, amniotic, ascitic, vitreous, synovial, aqueous, BAL fluid, bile fluid, semen, drain fluids from surgical sites, Endotracheal secretions and cystic fluids from breast, ovary and other sites.

Results: Male to female ratio was 2.17:1. The most common body fluid was cerebrospinal fluid (CSF) 31.02%, Klebsiella sps (22.9%) was the most frequently isolated bacteria, followed by Pseudomonas (21.3%), Acinetobacter (13.11%), Citrobacter (11.9%), E.Coli (8.19%), Stap. Aureus (8.19%), Enterococcus (4.91%), Stenotrophomonas (3.25), Candida sps (3.21%), Burkholderia and Proteus (1.63%) each. Gram positive bacteria were grown in 13.11% cases and 83.62% showed growth of Gram negative bacilli.

Conclusion: Regular monitoring and surveillance of organisms causing infection of body fluids is required for formulating an antibiotic and infection control policy so as to guide the clinicians in choosing appropriate antibiotics before a culture report is available thus preventing the development of antimicrobial resistance.

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1. Introduction

Body fluids play an important role in body functions by transporting nutrients as well as in regulating the temperature of the body, in respiratory processes as well as in transporting waste products of the body. Under normal circumstances, the body fluids like cerebrospinal fluid(CSF), peritoneal, pleural, pericardial and synovial fluids are sterile. They get infected when bacteria, fungi,

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parasites or viruses invade and change the physicochemical properties of these body fluids. CSF is produced by the choroid plexus and acts as a shock absorber to the brain. When it gets infected, it causes meningitis, which is a medical emergency and requires urgent medical attention. It is estimated that about 2 lakh people are affected by meningitis globally per year with resultant considerable morbidity and mortality. This is more pronounced in the developing countries where about 16-32% mortalities are attributed to meningitis.

Pleural, peritoneal, ascitic and synovial fluids may be exudative or transudative in nature depending upon the

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cause either infective or due to malignancy or any other organ specific disease. ^{5–9} In developing countries like India, antimicrobial resistance is a grave problem. Several bacteria like E.Coli, Klebsiella, Acinetobacter, Pseudomonas etc cause infections in various body fluids. WHO has emphasized on the study of emerging drug resistance and the strategies to control it. ^{10,11} Similarly, hospital infection control policies require continuous surveillance of antibiotic sensitivity pattern so that patients can be managed in better way by framing a hospital antibiotic policy. ¹² Infections of the body fluids are mostly a medical emergency and may be life threatening if not managed timely. ^{13,14} Very limited data are available about the antibiotic susceptibility of bacteria causing infection of various body fluids.

This retrospective study was undertaken between January to October 2019 in the microbiology department of our diagnostic centre with the aim of identifying the bacteria causing infection of various body fluids and also to study the antibiotic susceptibility pattern.

2. Materials and Methods

This was a retrospective study carried out on 216 patients of all ages and both sexes registered for culture and sensitivity of different body fluids in the microbiology department of our diagnostic centre between January to October 2019. The fluids included in the study were CSF, pleural, peritoneal, amniotic, ascitic, vitreous, synovial, aqueous, BAL fluid, bile fluid, semen, drain fluids from surgical sites, Endotracheal secretions and cystic fluids from breast, ovary and other sites.

The patients included in the study were divided into < 20, 21-40, 41-60,61-80 and > 80 years of age in both the sexes. Patients who had received antibiotics prior to culture and hospitalized patients were excluded from the study.

Samples were collected by following thorough aseptic techniques in sterile wide mouth containers and were plated on 5% sheep blood agar and Maconkey agar and incubated at 37° C for 18-24 hours. Isolated organism was identified by Gram's stain and colony morphology and further by biochemical tests. Antibiotic sensitivity was done on Vitec II (Biomerieux) according to CLSI guidelines by using AST N 280 for Gram negative bacteria and P628 for Gram positive bacteria. For Quality control, reference strains of Staph aureus (25923), E.coli (25922), Enterococcus faecalis (29212) and Pseudomonas aeruginosa (27853) were used.

3. Results

A total of 216 patients of all ages and both sexes coming to our diagnostic centre for different body fluids culture and sensitivity examination were included in the study.

Maximum patients 66/216(30.55%) were below 20 years of age with 23.6% males and 6.9% females, followed

by 61(28.24%) patients in 21-40 years of age, having 17.1% males and 11.1% females. This was followed by 51(23.61%) patients in 41-60 years age group with 14.4% males and 9.3% females. There were 35(16.2%) patients in 61-80 years age group with 12% males and 4.2% females. There were only three patients above 80 years of age, constituting 1.38% of the total patients, all of them being males (Table 1). The males outnumbered the females in all age groups with a male to female ratio of 2.17:1.

The most common body fluid was cerebrospinal fluid (CSF)31.02%, followed by pleural fluid (17.13%), semen (12.04%), synovial fluid (8.80%), drain fluid (5.56%), breast cyst fluid and ascitic fluid (5.09%), ET secretions(4.63%), BAL fluid (3.24%), vitreous fluid (2.78%), aqueous fluid and liver abscess fluid (1.39%), bile fluid, amniotic fluid, endometrial fluid and ovarian cyst fluid (0.46%) each (Table 2).

When the body fluids were analysed for bacterial growth, no growth was observed in 155(71.76%) patients while significant growth was observed in 61(28.24%) patients in different body fluids (Table 3).

Maximum growth was observed in CSF (34.4%), followed by ET secretions (16.3%), drain fluid (13.11%), BAL fluid (6.55%), aqueous fluid, ascitic fluid, breast cyst fluid and semen (4.91%) each, pleural fluid (3.27%), synovial fluid, vitreous fluid, ovarian cyst fluid and bile fluid (1.63%)each. No bacterial growth was observed in amniotic fluid, liver abscess fluid and endometrial fluid. (Table 3). Aqueous and vitreous fluids showed growth of Pseudomonas.

On analysis of different microorganisms isolated from body fluids, it was observed that Klebsiella sps (22.9%)was the most frequently isolated bacteria, followed by Pseudomonas (21.3%), Acinetobacter (13.11%), Citrobacter (11.9%), E.Coli (8.19%), Stap. Aureus (8.19%), Enterococcus (4.91%), Stenotrophomonas (3.25), Candida sps (3.21%), Burkholderia and Proteus (1.63%) each (Table 6).

Out of 61 fluids which showed bacterial growth, Gram positive bacteria were grown in 13.11% cases and 83.62% showed growth of Gram negative bacilli. 3.2% showed growth of Candida sps. Out of Gram positive bacteria 62.5% were Staph aureus and 37.5% were Enterococcus sps. Among the Gram negative bacilli, Klebsiella was the most frequent organism (27.45%), followed by Pseudomonas (25.4%), Acinetobacter (15.68%), Citrobacter (13.7%), E.Coli (9.8%), Stenotrophomonas (3.92%) and Burkholderia (1.96%) (Table 5).

3.1. Antibiotic susceptibility pattern

Burkholderia sps was sensitive to Meropenem and minocycline while showing intermediate resistance to Tigecycline. Stenotrophomonas was sensitive only to Trimethoprim/sulfamethaxazole. Citrobacter was sensitive towards

Table 1: Demographic distribution of different body fluids

S.	Comple True	< 20 yı	rs	21-40yrs	S	41 - 60	yrs	61-80 y	rs	>80 yr	rs
No.	Sample Type	M	\mathbf{F}	M	F	M	\mathbf{F}	M	F	\mathbf{M}	\mathbf{F}
1	Amniotic Fluid	0	0	0	0	0	1	0	0	0	0
2	Aqueous fluid	0	0	0	0	2	1	0	0	0	0
3	Ascitic Fluid	0	0	3	2	4	0	1	1	0	0
4	BAL Fluid	0	1	0	0	3	1	2	0	0	0
5	Bile Fluid	0	0	1	0	0	0	0	0	0	0
6	CSF	19	7	11	5	5	8	7	3	2	0
7	Drain fluid	0	0	3	4	1	2	2	0	0	0
8	E.T.Secretion	2	0	2	0	2	0	4	0	0	0
9	Endometrial Fluid	0	0	0	1	0	0	0	0	0	0
10	Liver Cyst Fluid	1	0	0	0	1	0	1	0	0	0
11	Ovarian Cyst Fluid	0	0	0	1	0	0	0	0	0	0
12	Pleural Fluid	4	7	5	6	5	2	4	3	1	0
13	Breast Cyst Fluid	1	0	2	2	3	3	0	0	0	0
14	Semen	20	0	6	0	0	0	0	0	0	0
15	Synovial Fluid	4	0	4	3	5	1	2	0	0	0
16	Vitreous fluid	0	0	0	0	0	1	3	2	0	0
	Total	51	15	37	24	31	20	26	9	3	0
	%	23.6%	6.9%	17.1%	11.1	%14.4%	9.3%	12.0%	4.2%	1.4%	0.0%

Table 2: Sample - wise distribution of different body fluid

S. No.	Sample Type	Total	%
1	Amiontic Fluid	1	0.46%
2	Aqueous fluid	3	1.39%
3	Ascitic Fluid	11	5.09%
4	BAL Fluid	7	3.24%
5	Bile Fluid	1	0.46%
6	CSF	67	31.02%
7	Drain fluid	12	5.56%
8	E.T. Secretion	10	4.63%
9	Endometrium Fluid	1	0.46%
10	Liver Cyst Fluid	3	1.39%
11	Ovarian Cyst Fluid	1	0.46%
12	Pleural Fluid	37	17.13%
13	Breast Cyst Fluid	11	5.09%
14	Semen	26	12.04%
15	Synovial Fluid	19	8.80%
16	Vitreous fluid	6	2.78%

Amikacin, Piperacillin/Tazobactum, Cefipime, Ertapenem, Meropenem, Gentamycin, Levofloxacillin, Norfloxacin, Tigecycline and Trimethoprim/sulphamethaxazole.

E.coli showed sensitivity to Amikacin, piperacillin/Tazobactum, Cefoperazone/Sulbactum,

Colistin, Ertapenem, Meropenem, Imipenem, Gentamycin, Nitrofurantoin, Tigecycline.

Proteus sps was sensitive to most of the drugs except ciprofloxacin, colistin, Imipenem, Nitrofurantoin, Tigecycline and Trimethoprim/sulfamethaxazole. Acinetobacter showed sensitivity to Amikacin, Cefipime, Colistin, Gentamycin, Imipenem, Meropenem.

Klebsiella sps was sensitive to Amikacin, Amoxy/clavulinic acid, Piperacillin/Tazobactum, Cefoperazone/Sulbactum, Ciprofloxacxillin, Colistin, Ertapenem, Meropenem, Imipenem, Gentamycin, Nitrofurantoin, Tigecycline and Trimethoprim/Sulfamethaxazole (Table 7).

OF the Gram positive bacteria, Staph aureus was sensitive to all antibiotics except Benzyl Penicillin. Enterococcus was resistant to Tetracycline and showed intermediate sensitivity to Erythromycin. It demonstrated high level synergy to Gentamycin (Table 4).

4. Discussion

Infection of body fluids results in significant morbidity and mortality. To add to it, the emergence of anti microbial resistance due to increased frequency of nosocomial

Table 3: Showing culture results in different body fluids

S. No.	Sample Type	No Growth	Growth	Total
1	Amiontic Fluid	1	0	1
2	Aqueous fluid	0	3	3
3	Ascitic Fluid	8	3	11
4	BAL Fluid	3	4	7
5	Bile Fluid	0	1	1
6	CSF	46	21	67
7	Drain fluid	4	8	12
8	E.T. Secretion	0	10	10
9	Endometrium Fluid	1	0	1
10	Liver Cyst Fluid	3	0	3
11	Ovarian Cyst Fluid	0	1	1
12	Pleural Fluid	35	2	37
13	Breast Cyst Fluid	8	3	11
14	Semen	23	3	26
15	Synovial Fluid	18	1	19
16	Vitreous fluid	5	1	6
	Total	155	61	216
	%	71.76%	28.24%	

Table 4: Antibiotic sensitivity patterns of gram positive organism

_	Enterococ	cus	S. aureus	
Drugs	MIC	Sensitive/ Resistant/ Intermediate	MIC	Sensitive/ Resistant/ Intermediate
Benzylpenicillin	8	Sensitive	0.25	Resistant
Cefotixin Screen	_	_	NEG	_
Ciprofloxacin	1	Sensitive	<=0.5	Sensitive
Clindamycin	_	_	0.25	Sensitive
Daptomycin	4	Sensitive	0.25	Sensitive
Erythromycin	2	Intermediate	0.5	Sensitive
Gentamicin	_	_	<=0.5	Sensitive
Gentamicin High Level (synergy)	SYN-S	Sensitive	_	_
Inducible Clindamycin Resistance	-	_	NEG	
Levofloxacin	1	Sensitive	0.5	Sensitive
Linezolid	2	Sensitive	2	Sensitive
Nitrofuratonion	_	_	<=16	Sensitive
Rifampicin	_	_	<=0.03	Sensitive
Teicoplanin	<=0.5	Sensitive	4	Sensitive
Tetracycline	>8	Resistant	<=1	Sensitive
Tigecycline	<=0.12	Sensitive	0.25	Sensitive
Trimethoprim/ Sulfamethoxazole	-	-	<=10	Sensitive
Vancomycin	2	Sensitive	_	_

 Table 5: Showing percentage of growth of gram positive & gram negative bacteria

Gram Positive Cocci	i (13.11%)	Gram Negative Bacilli (83.62%)	
S. aureus	62.50%	Klebsiella	27.45%
Enterococcus	37.50%	Pseudomonas	25.40%
		Stenotrophomonas maltophilia	3.92%
		Citrobacter	13.72%
		Acinetobacter	15.68%
		E.coli	9.80%
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S.	Sample Type	S.	Klebsiella	Klebsiella Pseudomonas S.	S.	Citrobacter	Enterococcu	Enterococcus AcinetobacterProteus	rProteus	E.coli	В.	Candida
No.		aureus			maltophilia						cepacia	
П	Amiontic Fluid	0	0	0	0	0	0	0	0	0	0	0
2	Aqueous fluid	0	0	3	0	0	0	0	0	0	0	0
3	Ascitic Fluid	0	1	0	0	0	1	0	0	1	0	0
4	BAL Fluid	0	1	0	0	0	0	2	0	0	0	1
5	Bile Fluid	0	0	0	0	0	1	0	0	0	0	0
9	CSF	3	4	3	2	7	0	1	0	1	0	0
7	Drain fluid	0	4	0	0	0	0	0	1	3	0	0
8	E.T.Secretion	П	2	3	0	0	0	3	0	0	0	1
6	Endometrium Fluid	0	0	0	0	0	0	0	0	0	0	0
10	Liver Cyst Fluid	0	0	0	0	0	0	0	0	0	0	0
11	Ovarian Cyst Fluid	0	0	1	0	0	0	0	0	0	0	0
12	Pleural Fluid	0	0	0	0	0	0	1	0	0	1	0
13	Breast Cyst Fluid	_	2	0	0	0	0	0	0	0	0	0
14	Semen	0	0	2	0	0	1	0	0	0	0	0
15	Synovial Fluid	0	0	0	0	0	0	1	0	0	0	0
16	Vitreous fluid	0	0	1	0	0	0	0	0	0	0	0
	Total	5 (8.19%)	14(22.9%) 13(13(21.3%)	2(3.27%)	7(11.47%)	3(4.91%)	8(13.11%)	1(1.63%)	5(8.19%)	1(1.63%)	2(3.27%)

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Drugs	Burkho cenacia	Burkholderia cenacia	Stenotroph maltophila	Stenotrophomonas Ci- maltophila	Citroba	trobacter	E.coli		Proteus		Acinetobacter	acter	Pseudomonas	ionas	Klebsiella	~
	MIC	S/R/I**	MIC	S/R/I**	MIC	S/R/I**	MIC	S/R/I**	MIC	S/R/I**	MIC	S/R/I**	MIC	S/R/I**	MIC	S/R/I**
Amikacin	1	1	ı	ı	8		<= 2		<= 5		1	ı	<= 5		<= 2	
Ampicillin	1	1	ı	ı	1	I	>16	R	<= 5		1	ı	ı	ı	>16	R
Amoxicillin/	1	1	1	1	>16	R	16	Ι	4		>= \ 4= \		ı	1	4	
Clavulanic Acid																
Piperacillin/	1	1	1	I	>= 4=		\ 4		> = >		I	ı	32	Ι	\ + = \	
Tazobactam					,		•		•							
Cefuroxime	1	I	1	ı	>32	R	32	R	\ = 1		1	ı	ı	1	8	* *
Cefuroxime		R	ı	I	>32	R	32	8	\ = 1		ı	I	I	I	∞	**
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Ciprofloxacin	2	R	I	I	ı	I	× ×	R	> 2	R	0.5		2	R	II	
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Colistin	I	I	I	1	× ^	×	0.5		%	~	8	×	<= 0.5		<= 0.25	
Ertapenem	I	I	1	1	<= 0.5		<pre>< 0.5</pre>		<= 0.5		I	I	I	I	<= 0.5	
Gentamicin	1	1	1	I	2		\ 		\ = 1		\ = 1		2		\ = 1	
Imipenem	I	ı	ı	I	*	R	<= 0.25		4	R	1		T		<= 0.25	
Levofloxacin	1	1	1	1			1	1	ı	1	ı	1	1	1	1	ı
Meropenem	4		I	I	1		<= 0.25		<= 0.25		-		-		<= 0.25	
Minocycline			1	ı	ı	ı	ı	1	ı	1	1	ı	ı	1	ı	1
Nitrofurantonin		I	I	I	128	R	 		128	R	I	I	I	I	32	
Norfloxacin		R	1	1			1	1	1	1	1	1	1	1	1	1
Tigecycline	4	Ι	1	I	-		<pre>< 0.5</pre>		4	R	<= 0.5		*	Ж	<= 0.5	
Trimethoprim/ – Sulfamethoxazole	- ole	I	<=20		> = 20		>160	24	>160	R	>160	~	I	I	<= 20	

infections and increase in immunocompromised patients has resulted in increase in the incidences of infection in various body fluids. 15 Patient demographics, rampant use of antibiotics, surgical procedures, trauma and any underlying medical conditions aggravate the infection. In our study we found that maximum patients (30.55%) were below 20 years of age with a overall male to female ratio of 2.17:1. Similar findings were observed in the study conducted by Ephrem T et al. ¹⁶ The most common body fluid in our study was CSF (31.02%). Our study correlates with the study by Firehiwot et al where CSF was the most common fluid with maximum isolates being from CSF accounting for 57.4% of the total isolates. ¹⁷ In the study by B. Vishalakshi et al, ascitic fluid was the most common (14.78%) with a culture positive rate of 14.78% and no growth in 85.22% fluids. 18 In our study we observed bacterial growth in 28.24% fluids and no growth in 71.75% fluids which is more or less similar to this study. In our study, the most common bacterial isolate was Klebsiella accounting for 27.45% of all Gram negative isolates while Staph aureus was the most common Gram positive bacterial isolate (13.11%). Our study is different from the study done in the USA and other parts of India where gram positive bacteria were more frequent. 18,19 In the study by Rajani Sharma et al in 2016, the isolation rate of organisms was 30% with E.Coli being the most common isolate (28.6%). ²⁰ In our study we found that Citrobacter and E.coli demonstrated resistance to many antibiotics while Acinetobacter and Klbsiella were sensitive to most of the antibiotics. Dimple K et al in their study reported 30% prevalence of MRSA.²¹ In our study we did not get any MRSA. Aqueous and vitreous fluids showed growth of Pseusdomonas in our study with a culture positive rate of 44.44%. In the study by Bhattacharjee et al in 2016, the culture positive rate in these fluids was 29.89% with Pseudomonas accounting for 26.45% of all isolates. ²² Our study correlates with the study by Lalita et al (53%)and Gupta et al (52.5%). ^{23,24} The pattern of antibiotic usage and demographic characteristics of the patient population as well as the techniques used for sample collection play a vital role in producing discordant results in studies done in different parts of the world. It may also be due to presence of anaerobic organisms, lack of enrichment techniques or a history of prior antibiotic usage. All these factors should be taken into consideration while interpreting the culture results.

5. Conclusion

Body fluids may be infected by both Gram positive and Gram negative bacteria. Regular monitoring and surveillance of prevalence of organisms causing infection of body fluids is required for formulating an antibiotic and infection control policy so as to guide the clinicians in choosing appropriate antibiotics before a culture report is available thus preventing the development of antimicrobial resistance.

6. Source of Funding

None.

7. Conflict of Interest

None

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