

Original Research Article

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An Aerobic Bacteriological Profile and Antibiogram of Various Body Fluids from a Tertiary Care Hospital in Telangana, India – A 5 Year Study

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ABSTRACT

Infection of sterile body fluid can lead to severe morbidity and mortality. Periodic surveillance and monitoring programmes are helpful for framing the antibiotic policy. The present study was undertaken to evaluate aerobic bacteriological profile along with their antibiogram from various sterile fluids over a period of five years. A total of 1708 body fluid samples were processed according to Standard guidelines and analysed in order to assess the changing trends in bacteriological profile and antibiogram. Out of 1708 body fluid samples 351 samples showed growth of organisms with an isolation rate of 20.55%. Isolates from different fluids were *E. coli* (27.92%), *Klebsiella* (22.22%), *Staphylococcus aureus* (17.66%), *Pseudomonas* species (12.53%), *Streptococcus pneumoniae* (5.41%), *Enterococcus* species (3.41%), *Streptococcus pyogenes* (3.41%), *Acinetobacter* species (2.27%), CONS (2.27%), *Proteus* species (1.7%) and *Citrobacter* species (1.13%). Gram negative isolates were mostly sensitive to carbapenems and aminoglycosides and gram positive isolates were highly sensitive to Vancomycin, aminoglycosides and fluoroquinolones. *Acinetobacter* and *Klebsiella* species were the most resistant pathogens. About 17.66% of *Staphylococcus aureus* were MRSA. So regular monitoring of prevalent pathogenic organisms and their sensitivities are essential as they help in formulating hospital antibiotic policy and aid the clinician in appropriate selection of antibiotic therapy, thereby preventing the indiscriminate use of unnecessary antibiotics and development of antibiotic resistance.

Keywords

Sterile body fluids,
Antimicrobial
resistance, Kirby-
Bauer disk diffusion
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Introduction

Sterile body fluid infections are associated with considerable morbidity and mortality and pose a substantial burden on health care system. Infection and antimicrobial resistance are of global concern in developing nations, including India. Sterile body fluid infections are a medical emergency and needs an early diagnosis and effective treatment knowledge

of prevalent strains along with their antimicrobial resistance pattern, prevention of emergence and dissemination of resistant organisms and their efficient management is critical for control of hospital infections.

So the present study was undertaken to evaluate the bacterial profile and their antibiotic susceptibility pattern from various body fluids in tertiary care hospital

Materials and Methods

Source of study

Patient samples sent to Microbiology central laboratory from different specialties of Inpatient Departments (IPDs) and Outpatient Departments (OPDs) of Mamata General Hospital, Khammam.

Study type and duration

A retrospective observational study was done between Jan 2013 to Dec 2017 in the Department of Microbiology.

Inclusion criteria

All sterile body fluids received for aerobic culture and sensitivity from different IPDs and OPDs irrespective of age and sex were included

Exclusion criteria

Blood samples, patient with history of antibiotics in the last 2 weeks, contaminated samples and samples received after 2 hrs of collection.

Sample processing

All the samples pleural fluid, ascitic fluid, CSF, pericardial fluid, synovial fluid and others (semen, hydrocele fluid, peritoneal dialysis fluid, antral wash, BAL) were processed in the laboratory using standard microbiological procedures. All the samples were subjected for gram stain and culture and sensitivity by standard methods (Betty, A *et al.*, 2007).

All the isolates were identified by standard biochemical tests and their antibiotic susceptibility testing was performed by Kirby Bauer's disk diffusion method and interpreted

as per Clinical and Laboratory Standards Institute (CLSI) guide lines (CLSI 2013). Routine antimicrobial sensitivity tests were put for the following antibiotics.

Drugs for Gram Positive Cocci (GPC)

The antibiotics tested for GPC were ampicillin (10mcg), amoxycillin (30mcg), amikain (10mcg), gentamycin (10mcg), ciprofloxacin (5mcg), cefipime (30mcg), tetracycline (30mcg), Erythromycin (15mcg), clindamycin (2mcg), vancomycin (30mcg), cefoxitin (30mcg), cotrimoxazole (1.25/23.75ug)

Drugs for Gram Negative Bacilli (GNB)

For gram negative bacilli ampicillin (10mcg), amoxycillin (30mcg), amikacin (10mcg), gentamycin (10mcg), ciprofloxacin (5mcg) cefotaxime (30mcg), ceftriaxone (30mcg), cefipime (30mcg), cotrimoxazole (1.25/23.75µg), Imipenim (10mcg).

Drugs for *Pseudomonas aeruginosa*

Antibiotics for *Pseudomonas* species were piperacillin/tazobactam (100/10mcg), cefipime (30mcg), amikacin (10mcg), gentamycin (10mcg), imipenem (10mcg) ciprofloxacin (5mcg), cefotaxime (30mcg), tobramycin (30mg) and Ceftriaxone (30mcg).

Results and Discussion

A total of 1708 samples were received during Jan 2013 to Dec 2017 from various departments. Out of total samples majority were pleural fluids (540) and least were pericardial fluid (48). Amongst 1708 samples 351 samples showed growth of organisms with an isolation rate of 20.55%. Growth pattern of various fluids is shown in Table 1.

Out of total 351 isolates 32.19% were gram positive cocci and 67.81% were gram negative

bacilli (Fig. 1). The isolates among gram positive were *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Streptococcus pyogenes*, *Enterococcus* species and Coagulase negative staphylococci (CONS) (Fig. 2). Of the 62 Coagulase positive staphylococci 8 (17.66%) were methicillin resistant *Staphylococcus aureus* (MRSA). Among gram negative bacilli the isolates were *Escherichia coli*, *Klebsiella* species, *Pseudomonas aeruginosa*, *Acinetobacter* species, *Proteus* species and *Citrobacter* species (Fig. 3).

In pleural fluid isolates *Klebsiella* species was most common bacteria followed by *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas* species, *Streptococcus pneumoniae*, *Streptococcus aureus*, *Pseudomonas* species, *Streptococcus pneumoniae*, *Streptococcus pyogenes* and CONS. Among the ascitic fluid isolates most common were gram negative bacilli, *E.coli* was the most common isolate followed by *Klebsiella* species and others. *Staphylococcus aureus* was common among gram positive isolates followed by *Enterococcus* species and CONS. Gram positive cocci were predominant in synovial fluid, whereas gram positive and gram negative isolates were equally found in CSF samples. Bacteriological profile of isolates in different body fluids is given in Table 2.

Antibiotic sensitivity pattern of gram negative bacilli is given in Table 3, they were mostly sensitive to carbapenems followed by Amikacin, Gentamicin and ciprofloxacin. *Acinetobacter* species was the most resistant bacilli followed by *Klebsiella* species. *Pseudomonas* species showed high resistance to cephalosporins and fluoroquinolones. They were highly sensitive to imipenem (98%), Tobramycin (95%) and Piperacillin/Tazobactam (90%). Table 4 shows the antibiotic sensitivity pattern of gram positive

cocci. Gram positive isolates were highly sensitive to Vancomycin followed by Amikacin, Gentamicin, Cefipime, Ciprofloxacin and Amoxicillin. They were least sensitive to Ampicillin.

Early detection and identification of microorganisms are crucial for appropriate management of infections of normally sterile body fluids. The present retrospective study was conducted at a tertiary care hospital, comprising 1708 various body fluids, with isolation rate of 20.55% which correlates with the studies done by Harshika *et al.*, (22%) and Mandira *et al.*, (18.36%) (Harshika *et al.*, 2018, Mandira *et al.*, 2018), and lower when compared to other studies by Sujatha *et al.*, (31%) and Rajni Sharma *et al.*, (30%) (Sujatha *et al.*, 2015, Sharma *et al.*, 2017). Several studies done on body fluid profile showed discordant results in the spectrum of pathogens causing infection which may be due to indiscriminate use of antibiotics, patient specific factors like surgical procedures, trauma or any other underlying conditions or by methodological factors such as proper specimen collection, transport and culture (Kasana *et al.*, 2015).

In our study, gram positive cocci isolation rate is 32.19% and 67.81% of growth is shown by gram negative bacilli, the most common being those belonging to Enterobacteriaceae family (194 isolates) followed by non-fermenter *Pseudomonas* (44 isolates). The overall predominant pathogens were *E.coli* (98), *Klebsiella*, (78), *Staphylococcus aureus* (62) and *Pseudomonas aeruginosa* (44).

Among various fluids highest isolation rate was seen in ascitic fluid (46.15%) followed by pleural fluids (30.5%) and others (14.8%) and least in pericardial fluid (0.56%). In case of pleural fluid and ascitic fluid gram negative bacilli were more compared to gram positive cocci showing similar result to the studies

done by Deb *et al.*, (2014) and Mandira *et al.*, (2018). This is in contrast to other studies where gram positive cocci accounted for maximum number of cases and *Staphylococcus aureus* (70%) was the most common pathogen isolated, followed by CONS and others (Kasana *et al.*, 2015). In this study among the pleural fluid isolates *Klebsiella* species (30.84%) was the most common followed by *E.coli* (19.62%), *Staphylococcus aureus* (17.75%) *Pseudomonas* species (14.95%), *Pneumococci* (8.41%), *Streptococcus pyogenes* (7.5%) and CONS (0.93%). Our results are in contrast to studies of Sujatha *et al.*, (2015) and Evans *et al.*, (2003). Our study supports the study done by Harshika *et al.*, which highlights the emergence of aerobic gram negative bacteria as the predominant pathogens in empyema. Studies by Sonali *et al.*, (88.4%) and Mohanty *et al.*, (86.4%) showed a similar high rate of isolation of GNB in pleural fluids (Sonali *et al.*, 2013, Mohanty *et al.*, 2017). Before the antibiotic era *S. pneumoniae* and *S. pyogenes* were most common bacteria accounted for most of the empyema cases. After the

widespread use of antibiotics, *Staphylococcus aureus* succeeded *S. pneumoniae* and *S.pyogenes* as the major cause of empyema. Staphylococcal empyema incidence has decreased after 1960, by the discovery of β -lactamase resistant semi-synthetic penicillins. Now empyema due to aerobic GNB as the predominant pathogen has increased markedly. Of the total 33 *Klebsiella* species one of the isolate was from PICU showing mixed culture with *Pneumococci* and other from elderly patient with *Candida* species. The isolation of GNB or multiple pathogens from pleural fluid is associated with poor prognosis and indicates a more aggressive antimicrobial chemotherapy. In ascitic fluid *E.coli* (40.13%) was the commonest isolate followed by *Klebsiella* (23.45%), *Staphylococcus aureus* (15.43%) *Pseudomonas* species (9.9%), *Enterococcus* species (4.93%), *Acinetobacter* species (1.85%), *Proteus* species (1.85%), *Citrobacter* species (1.23%) and CONS (1.23%) which is similar to studies done by Sujatha *et al.*, (2015) and Arroyo *et al.*, (2000).

Fig – 1 GROWTH PATTERN OF TOTAL ISOLATES (N =351)

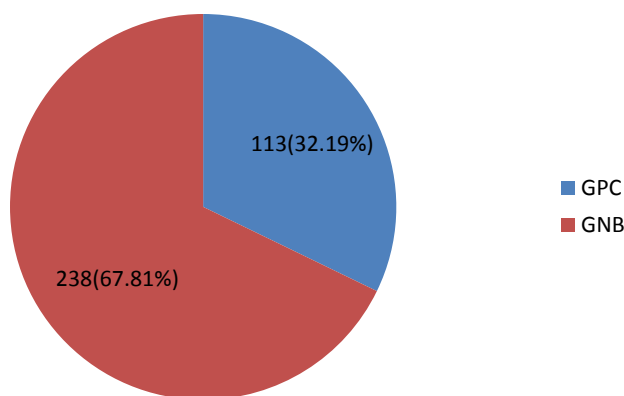


Fig – 2 GROWTH PATTERN OF GRAM POSITIVE COCCI (N=113)

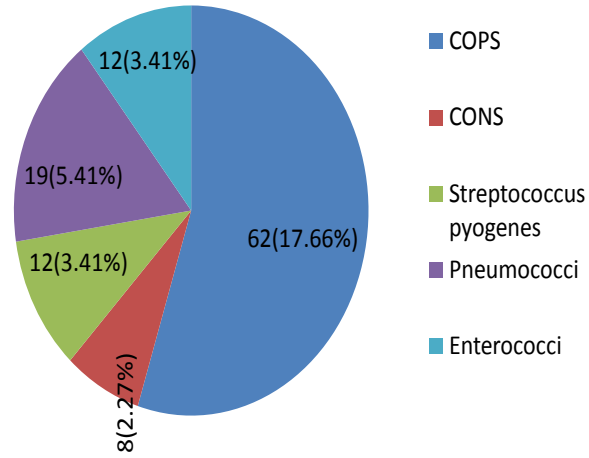


Fig – 3 GROWTH PATTERN OF GRAM NEGATIVE BACILLI (N=238)

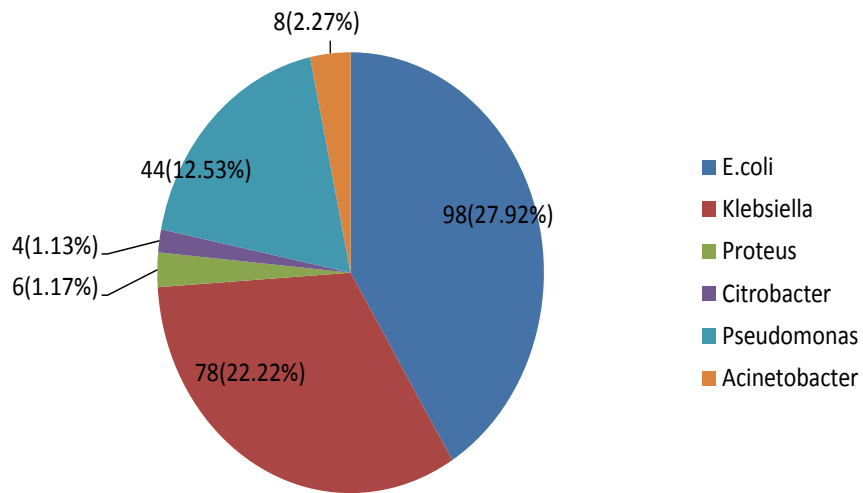


Table.1 Pattern of growth in various fluids

Sample	Total No	Growth (%)	No Growth (%)
Pleural fluid	540	107 (30.5)	433 (69.5)
Ascitic fluid	412	162 (46.15)	250 (53.85)
Synovial fluid	268	18 (5.12)	250 (94.88)
CSF	260	10 (2.84)	250 (97.16)
Pericardial fluid	48	2 (0.56)	46 (99.44)
Others	180	52 (14.8)	128 (85.2)
Total	1708	351	1357

Table.2 Bacteriological profile in different body fluids

Organism	Total No.	Pleural fluid (%)	Ascetic fluid (%)	Synovial fluid (%)	CSF (%)	Pericardial fluid (%)	Others (%)
Gram Positive cocci							
<i>Staphylococcus aureus</i>	62	19 (17.5)	25 (15.43)	8 (44.44)	2 (20)	-	8 (15.38)
CONS	8	1 (0.93)	2 (1.23)	3 (16.7)	-	-	2 (3.84)
<i>Streptococcus pyogenes</i>	12	8 (7.5)	-	2 (11.11)	-	-	2 (3.84)
Pneumococci	19	9 (8.41)	-	1 (5.55)	3 (30)	-	6 (11.53)
<i>Enterococcus species</i>	12	-	8 (4.93)	-	-	-	4 (7.69)
Gram Negative Bacilli							
E.coli	98	21 (19.62)	65 (40.13)	2 (11.11)	1 (10)	1 (50)	8 (15.38)
<i>Klebsiella species</i>	78	33 (30.84)	38 (23.45)	-	4 (40)	-	3 (5.76)
<i>Proteus species</i>	6	-	3 (1.85)	1 (5.55)	-	-	2 (3.84)
<i>Citrobacter species</i>	4	-	2 (1.23)	1 (5.55)	-	-	1 (1.92)
<i>Pseudomonas species</i>	44	16 (14.95)	6 (9.9)	-	-	-	12 (23.07)
<i>Acinetobacter species</i>	8	-	3 (1.85)	-	-	1 (50)	4 (7.69)
Total	351	107	162	18	10	2	52

Table.3 Antibiotic sensitivity pattern of gram negative bacilli

	E.Coli (%)	Klebsiella species (%)	Proteus species (%)	Citrobacter species (%)	Acinetobacter species (%)	Pseudomonas species (%)
Ampicillin	15	10	25	25	35	60
Amoxycillin	42	25	50	60	50	50
Amikacin	85	85	85	60	50	75
Gentamicin	75	75	80	65	45	68
Ciprofloxacin	65	65	82	45	40	65
Levofloxacin	62	55	95	50	45	55
Cefotaxime	35	42	60	25	20	55
Ceftriaxone	60	45	58	50	40	60
Cetipime	48	70	72	45	40	85
Cotromoxaryole	50	42	52	75	70	ND
Impienem	100	100	100	73	70	98
Piperacillin/ Tazyobactam	92	75	ND	50	70	90
Tobramycin	95	93	ND	ND	60	95

Table.4 Antibiotic sensitivity pattern of gram positive cocci

Drug	COPS (%)	CONS (%)	Streptococcus pyogenes (%)	Enterococcus species (%)	Streptococcus pneumoniae (%)
Ampicillin	28	35	25	15	50
Amoxicillin	87	75	89	80	65
Amikacin	100	82	90	75	67
Gentamicin	100	90	94	85	55
Ciprofloxacin	81	75	92	60	85
Caffeine	91	90	90	95	90
Cotromoxaryole	77	78	82	100	55
Erythromycin	68	70	79	100	83.3
Clindamycin	70	68	82	100	100
Cefoxitin	82	100	ND	ND	ND
Tetracycline	60	75	74	68	100
Vancomycin	100	100	100	100	100

On synovial fluid, studies done by Nutt *et al.*, (2010) and Ahmed *et al.*, (2010) and Rajani Sharma *et al.*, (2017) found *Staphylococcus aureus* as the most predominant isolate and our study correlates with them being *Staphylococcus aureus* the most common followed by CONS. As per the western

studies the relative incidence of meningitis caused by *H. influenzae*, *Neisseria meningitides* and *Listeria* is less in South East Asia. On the contrary gram negative bacilli such as *E. coli*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* are increasingly being reported in cases of meningitis

especially among elderly and in patients with cirrhosis, diabetes and malignancies (Mani R *et al.*, 2007, Tang LM *et al.*, 1999). Similar findings were observed in our study where *Klebsiella* species (40%) and *E. coli* (10%) were contributing 50% cases of meningitis followed by *S. pneumoniae* (30%) and *Staphylococcus aureus* (20%). Out of 48 pericardial fluid samples only 2 samples showed growth with *E. coli* (50%) and *Acinetobacter* species (50%) which is similar to study done by Mandira *et al.*, (2018). Bacterial infections of pericardium are relatively uncommon. Purulent pericarditis is almost exclusively seen as a secondary infection in the patients who are undergoing hemodialysis, thoracic surgery and / or with seriously underlying diseases such as AIDS. Among the other fluids (semen, hydrocele fluid, peritoneal dialysis fluid, antral wash etc) isolation rate was 28.88%. *Pseudomonas aeruginosa* (23.07%) is the predominant pathogen followed by *E.coli* (15.38%), *Staphylococcus aureus* (15.38%), *Pneumococci* (11.53%), *Acinetobacter* species and *Enterococcus* species 7.69% each.

Among the gram negative isolates *Acinetobacter* and *Klebsiella* species were the most resistant bacteria showing high degree of resistance to most of the antibiotics, which may be due to inappropriate use of commonly prescribed antibiotics (Mandira *et al.*, 2018). Being a tertiary care hospital most of the patients admitted here could have already exposed to antibiotics, which was also encountered in other studies (Vishalakshi *et al.*, 2016). *Acinetobacter* is an important public health problem for patients on broad spectrum antibiotics and requiring life support (Kempf *et al.*, 2012; Katragkou *et al.*, 2005). As most of our samples were from inpatients and ICU patients, highly resistant *Acinetobacter* are common in such groups. Other gram negative isolates showed increasing pattern of resistance to beta lactam

antibiotics, cephalosporins, fluoroquinolones and other 1st line of drugs but they were highly sensitive to carbapenems and aminoglycosides. *Pseudomonas* isolates were highly sensitive to imipenem (98%), Tobramycin (95%) and Piperacillin-tazobactam (90%). They showed good sensitivity to amikacin and cefipime, less sensitive to amoxicillin, levofloxacin and cefotaxime. Our results are correlating to Rajani Sharma *et al.*, (2017).

Gram positive isolates were 100% sensitive to Vancomycin. Total 62 *Staphylococcus aureus* were isolated, out of which 8 (17.66%) were MRSA. Other studies showed a bit higher isolation rate of about 28.57% and 38.5% (Mandira *et al.*, 2018). This variation might be because of variation in antibiotic usage and infection control practices in different places or variation in patient and clinical specimens. Except Ampicillin and Erythromycin MSSA was highly sensitive to almost all the antibiotics, whereas MRSA showed reduced susceptibility to clindamycin, ciprofloxacin and Tetracycline in addition to Ampicillin and Erythromycin (Kasana *et al.*, 2015). We had no VRSA or VISA isolates, this may be due to judicious and controlled use of Vancomycin in our hospital.

Streptococcus pneumoniae were highly sensitive to Tetracycline, Cefipime, Clindamycin, Ciprofloxacin and Erythromycin. *Streptococcus pyogenes* are highly sensitive to all the drugs except Ampicillin.

Enterococci have become increasingly important not only because of their ability to cause serious infections but also because of their increasing resistance to many antimicrobial agents. Enterococci are regarded as nosocomial pathogen and their infections are often refractory to treatment and the mortality is high. *Enterococcus*

isolates showed increased resistance to Ampicillin, Tetracycline and Ciprofloxacin and highly sensitive to Vancomycin and other drugs.

To conclude an overall increasing trend of drug resistance was observed in both gram negative and gram positive isolates, which warns for regular antibiotic surveillance studies. Microbiological profile and antibiotic surveillance studies help the clinicians for selection of most appropriate empiric antibiotic regimen and to prevent selective pressure as well as further development of resistance in these pathogens. Regular monitoring of prevalent pathogenic organisms and their sensitivity pattern help in formulating the hospital antibiotic policy, thereby preventing indiscriminate use of unnecessary antibiotics and the development of antibiotic resistance.

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